Photo-Microbial Fuel Cells

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Kenneth Schneider
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Declaration of work done in conjunction with others

Ti$_2$O ceramics were initially fabricated within the group together with Doctor Rebecca Thorne and founded on her expertise in the field of ceramic replica processing routes. Ceramic manufacture was performed using the facilities of the Department of Mechanical Engineering at the University of Bath.

Ti$_2$AlC ceramics were produced and analysed in collaboration with Professor Christopher Rhys Bowen and his PhD student Tony Thomas from the Department of Mechanical Engineering at the University of Bath. Data on ceramic slip viscosity and compressive strength of Ti$_2$AlC ceramics was acquired by Tony Thomas. His optimisation of the manufacturing and sintering process resulted in the investigation of single and double coated ceramics with higher phase purity.

The influence of surface morphology and surface energy of flat anode materials in pMFC power generation was investigated in collaboration with the University of Cambridge’s Departments of Biochemistry, Materials Science and Metallurgy, Chemical Engineering and Biotechnology. Electrochemical polyaniline coating of fluorine doped tin oxide coated glass, AFM and contact angle analysis of all electrode materials were performed together with Doctor Rebecca Thorne at the University of Bath. Multichannel photo-microbial fuel cell experiments on all electrode materials were conducted by our collaborators at the University of Cambridge.

Scanning electron microscopy was performed with the help of Doctor John Mitchels in the Microscopy and Analysis Suite at the University of Bath.
Outline of the achievements within this thesis

Fundamental studies for the improvement of photo-microbial fuel cells (pMFCs) within this work comprised investigations into ceramic electrodes, toxicity of metal-organic frameworks (MOFs) and hot-pressing of air-cathode materials.

A novel type of macroporous electrode was fabricated from the conductive ceramic Ti₂AlC. Reticulated electrode shapes were achieved by employing the replica ceramic processing method on polyurethane foam templates. Cyclic voltammetry of these ceramics indicated that the application of potentials larger than 0.5 V with regard to a Ag/AgCl reference electrode results in the surface passivation of the electrode. Ti₂AlC remained conductive and sensitive to redox processes even after electrochemical maximisation of the surface passivation, which was shown electrochemically and with four terminal sensing. Application of macroporous Ti₂AlC ceramic electrodes in pMFCs with green algae and cyanobacteria resulted in higher power densities than achieved with conventional pMFC electrode materials, despite the larger surface area of the Ti₂AlC ceramic.

The effect of electrode surface roughness and hydrophobicity on pMFC power generation and on cell adhesion was examined using atomic force and confocal microscopy, contact angle measurements and long-term pMFC experiments. The high surface roughness and fractured structure of Ti₂AlC ceramic was beneficial for cell adhesion and resulted in higher pMFC power densities than achieved with materials such as reticulated vitrified carbon foam, fluorine doped tin oxide coated glass or indium tin oxide coated plastic.

Toxicity of the MOF MIL101 and its amine-modified version MIL-101(Cr)-NH₂ on green algae and cyanobacteria was assessed on the basis of both growth in liquid culture and by exclusion zones of agar colonies around MOF pellets. MOF MIL101 was found harmless in concentrations up to 480 mg L⁻¹ and MIL-101(Cr)-NH₂ did not exhibit toxic effects at a concentration of 167 mg L⁻¹.

Air-cathodes were produced from a range of carbon materials and ion-exchange membranes. Hot-pressing of Zorflex Activated Carbon Cloth FM10 with the proton-selective Nafion® 115 membrane provided the best bonding quality and pMFC performance.
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Glossary of terms

Latin symbols

[ ]  concentration at the electrode surface
[ ]°  standard concentration of 1 mol L⁻¹
[ ]_{bulk}  concentration in the bulk
[ox]  concentration of the oxidised form of a species
[red]  concentration of the reduced form of a species
°C  temperature unit degree Celsius
3N-BBM+V  Bold Basal medium with 3-fold nitrogen and vitamins modified
A  electrode surface area; or electric current unit ampere
a_{ox}  activity of oxidised species
a_{red}  activity of reduced species
Ac  electron acceptor
AFM  atomic force microscopy
Ag/AgCl  silver and silver chloride redox system
ATP  adenosine triphosphate
BEC  backscattered electron composition imaging
BES  bio-electrochemical system
c  concentration
C.vulgaris  Chlorella vulgaris
C_{dl}  double layer capacitance
CE  counter electrode
CEm  Chlorella emersonii
C-felt  carbon felt
C-paper  carbon paper
c_r  molar concentration of redox active species
CSo  Chlorella sorokiniana
CV  cyclic voltammogram
CVu  Chlorella vulgaris
Cytb_{6f}  cytochrome b_{6f}
D  diffusion coefficient
<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>die</td>
<td>forming mold used in combination with a press to compress and shape a powder into a pellet</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>Do</td>
<td>electron donor</td>
</tr>
<tr>
<td>DPI</td>
<td>Diphenyleneiodonium</td>
</tr>
<tr>
<td>D_r</td>
<td>diffusion coefficient of redox active species</td>
</tr>
<tr>
<td>E</td>
<td>electrode potential</td>
</tr>
<tr>
<td>E^*</td>
<td>standard electrode potential</td>
</tr>
<tr>
<td>E_1</td>
<td>first vertex potential in a cyclic voltammogram</td>
</tr>
<tr>
<td>E_2</td>
<td>second vertex potential in a cyclic voltammogram</td>
</tr>
<tr>
<td>EDX</td>
<td>energy dispersive x-ray spectroscopy</td>
</tr>
<tr>
<td>E_{equ}</td>
<td>potential at electrochemical equilibrium</td>
</tr>
<tr>
<td>E_{ox}</td>
<td>oxidation potential</td>
</tr>
<tr>
<td>E_{p,ox}</td>
<td>potential at which the peak oxidation current is achieved</td>
</tr>
<tr>
<td>E_{p,red}</td>
<td>potential at which the peak reduction current is achieved</td>
</tr>
<tr>
<td>E_{red}</td>
<td>reduction potential</td>
</tr>
<tr>
<td>ESEM</td>
<td>environmental scanning electron microscopy</td>
</tr>
<tr>
<td>F</td>
<td>Faraday constant</td>
</tr>
<tr>
<td>Fd</td>
<td>ferredoxin</td>
</tr>
<tr>
<td>FTO</td>
<td>fluorine doped tin oxide</td>
</tr>
<tr>
<td>FTO glass</td>
<td>fluorine doped tin oxide coated glass</td>
</tr>
<tr>
<td>g</td>
<td>weight unit gram</td>
</tr>
<tr>
<td>HOMO</td>
<td>highest occupied molecular orbital</td>
</tr>
<tr>
<td>i</td>
<td>electric current</td>
</tr>
<tr>
<td>i_0</td>
<td>exchange current at electrochemical equilibrium</td>
</tr>
<tr>
<td>I_c</td>
<td>compensation light intensity</td>
</tr>
<tr>
<td>i_{charging}</td>
<td>charging current</td>
</tr>
<tr>
<td>I_{ox}</td>
<td>oxidation current</td>
</tr>
<tr>
<td>I_{p,ox}</td>
<td>peak oxidation current</td>
</tr>
<tr>
<td>I_{p,red}</td>
<td>peak reduction current</td>
</tr>
<tr>
<td>I_{p,solution}</td>
<td>peak current of a redox active species in solution</td>
</tr>
<tr>
<td>I_{p,surface}</td>
<td>peak current of a surface immobilised redox active species</td>
</tr>
<tr>
<td>I_{red}</td>
<td>reduction current</td>
</tr>
<tr>
<td>I_s</td>
<td>saturation light intensity</td>
</tr>
<tr>
<td>ITO</td>
<td>indium tin oxide</td>
</tr>
</tbody>
</table>
**Glossary of terms**

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITO plastic</td>
<td>indium tin oxide coated polyethylene terephthalate sheet</td>
</tr>
<tr>
<td>$J_{\text{con}}$</td>
<td>particle flux due to natural and forced convection</td>
</tr>
<tr>
<td>$J_{\text{diff}}$</td>
<td>particle flux due to diffusion</td>
</tr>
<tr>
<td>$J_{\text{mig}}$</td>
<td>particle flux due to migration</td>
</tr>
<tr>
<td>K</td>
<td>temperature unit Kelvin</td>
</tr>
<tr>
<td>$k_{\text{ass}}$</td>
<td>rate constant of association</td>
</tr>
<tr>
<td>$k_{\text{diss}}$</td>
<td>rate constant of dissociation</td>
</tr>
<tr>
<td>$k_{\text{ox}}$</td>
<td>rate constant of oxidation</td>
</tr>
<tr>
<td>$k_{\text{red}}$</td>
<td>rate constant of reduction</td>
</tr>
<tr>
<td>L</td>
<td>volume unit litre</td>
</tr>
<tr>
<td>LHC</td>
<td>light harvesting complex</td>
</tr>
<tr>
<td>LUMO</td>
<td>lowest unoccupied molecular orbital</td>
</tr>
<tr>
<td>m</td>
<td>length unit metre</td>
</tr>
<tr>
<td>M</td>
<td>molar concentration unit mole per litre</td>
</tr>
<tr>
<td>mA</td>
<td>milliampere = $10^{-3}$ ampere</td>
</tr>
<tr>
<td>max</td>
<td>maximum</td>
</tr>
<tr>
<td>MFC</td>
<td>microbial fuel cell</td>
</tr>
<tr>
<td>MIL101</td>
<td>Materials of Institute Lavoisier 101</td>
</tr>
<tr>
<td>MIL-101(Cr)-NH$_2$</td>
<td>amine functionalised modification of the metal-organic framework</td>
</tr>
<tr>
<td>Milli-Q</td>
<td>ultrapure water grade with specific electrical resistance of 18.2 M$\Omega$cm at 25°C and no more than 50 mg L$^{-1}$ in total organic carbon</td>
</tr>
<tr>
<td>min</td>
<td>minimum</td>
</tr>
<tr>
<td>Mix</td>
<td>mixed culture of green algae and cyanobacteria</td>
</tr>
<tr>
<td>mm</td>
<td>millimetre = $10^{-3}$ metre</td>
</tr>
<tr>
<td>mM</td>
<td>millimole per litre = $10^{-3}$ mole per litre</td>
</tr>
<tr>
<td>mm/mm</td>
<td>compressive strain unit defined as ratio of change in length to original length</td>
</tr>
<tr>
<td>MOF</td>
<td>metal-organic framework</td>
</tr>
<tr>
<td>mol</td>
<td>amount of substance unit mole</td>
</tr>
<tr>
<td>mol L$^{-1}$</td>
<td>molar concentration unit mole per litre</td>
</tr>
<tr>
<td>MPa</td>
<td>mega Pascal = $10^6$ Pascal</td>
</tr>
<tr>
<td>mV</td>
<td>millivolt = $10^{-3}$ volt</td>
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<tr>
<td>mV s$^{-1}$</td>
<td>voltammetry scan rate unit millivolt per second</td>
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<tr>
<td>MZo</td>
<td><em>Muriella zofingiensis</em></td>
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<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------------</td>
<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td>n</td>
<td>number of electrons transferred per redox reaction</td>
</tr>
<tr>
<td>NADP</td>
<td>nicotinamide adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>Naftion® 115</td>
<td>Naftion® perfluorinated membrane</td>
</tr>
<tr>
<td>nm</td>
<td>nanometre = $10^{-9}$ metre</td>
</tr>
<tr>
<td>OCP</td>
<td>open circuit potential</td>
</tr>
<tr>
<td>Ox</td>
<td>oxidised species</td>
</tr>
<tr>
<td>Pa</td>
<td>pressure unit Pascal</td>
</tr>
<tr>
<td>PANI</td>
<td>polyaniline</td>
</tr>
<tr>
<td>PC</td>
<td>personal computer or plastocyanin</td>
</tr>
<tr>
<td>PEM</td>
<td>polyelectrolyte membrane</td>
</tr>
<tr>
<td>PGSTAT</td>
<td>potentiostat/galvanostat</td>
</tr>
<tr>
<td>pMFC</td>
<td>photo-microbial fuel cell</td>
</tr>
<tr>
<td>ppi</td>
<td>porosity unit pores per inch</td>
</tr>
<tr>
<td>PQ</td>
<td>plastoquinone</td>
</tr>
<tr>
<td>PSI</td>
<td>Photo-system one</td>
</tr>
<tr>
<td>PSII</td>
<td>Photo-system two</td>
</tr>
<tr>
<td>Pt</td>
<td>platinum</td>
</tr>
<tr>
<td>PTri</td>
<td><em>Phaeodactylum tricornutum</em></td>
</tr>
<tr>
<td>$Q_{\text{abs}}$</td>
<td>absolute charge transferred</td>
</tr>
<tr>
<td>$Q_{\text{dl}}$</td>
<td>amount of charge stored in the double layer</td>
</tr>
<tr>
<td>$Q_{\text{ox}}$</td>
<td>charge transferred to oxidise</td>
</tr>
<tr>
<td>$Q_{\text{ox,max}}$</td>
<td>maximum anodic charge transfer</td>
</tr>
<tr>
<td>$Q_{\text{red}}$</td>
<td>charge transferred to reduce</td>
</tr>
<tr>
<td>R</td>
<td>ideal gas constant or electrical resistance</td>
</tr>
<tr>
<td>$R^2$</td>
<td>coefficient of determination, $R$ squared</td>
</tr>
<tr>
<td>ramp</td>
<td>rate of temperature change with time</td>
</tr>
<tr>
<td>RE</td>
<td>reference electrode</td>
</tr>
<tr>
<td>Red</td>
<td>reduced species</td>
</tr>
<tr>
<td>redox</td>
<td>reduction-oxidation</td>
</tr>
<tr>
<td>ROI</td>
<td>region of interest</td>
</tr>
<tr>
<td>rpm</td>
<td>rotations per minute</td>
</tr>
<tr>
<td>$R_{\text{solution}}$</td>
<td>electrical resistance of the solution</td>
</tr>
<tr>
<td>RTP</td>
<td>room temperature of 298 ±5 K and pressure of approximately 100 kPa</td>
</tr>
<tr>
<td>RVC</td>
<td>reticulated vitreous carbon</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>S</td>
<td>conductivity unit Siemens</td>
</tr>
<tr>
<td>SCo</td>
<td><em>Synechococcus WH5701</em></td>
</tr>
<tr>
<td>SCy</td>
<td><em>Synechocystis PCC6803</em></td>
</tr>
<tr>
<td>SE</td>
<td>sensing electrode</td>
</tr>
<tr>
<td>SEM</td>
<td>scanning electron microscopy</td>
</tr>
<tr>
<td>SHE</td>
<td>standard hydrogen electrode</td>
</tr>
<tr>
<td>SMa</td>
<td><em>Spirulina maxima</em></td>
</tr>
<tr>
<td>SWV</td>
<td>square wave voltammetry</td>
</tr>
<tr>
<td>T</td>
<td>temperature</td>
</tr>
<tr>
<td>t</td>
<td>time</td>
</tr>
<tr>
<td>TPse</td>
<td><em>Thalassiosira pseudonana</em></td>
</tr>
<tr>
<td>V</td>
<td>electric potential unit volt</td>
</tr>
<tr>
<td>V s⁻¹</td>
<td>voltammetry scan rate unit volt per second</td>
</tr>
<tr>
<td>vol%</td>
<td>volume concentration in percent</td>
</tr>
<tr>
<td>W</td>
<td>power unit Watt</td>
</tr>
<tr>
<td>WE</td>
<td>working electrode</td>
</tr>
<tr>
<td>wrt</td>
<td>with regard to</td>
</tr>
<tr>
<td>wt%</td>
<td>mass fraction in percent</td>
</tr>
<tr>
<td>x</td>
<td>distance</td>
</tr>
<tr>
<td>XRD</td>
<td>x-ray diffraction crystallography</td>
</tr>
<tr>
<td>z</td>
<td>valency</td>
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<tr>
<td>Z</td>
<td>atomic number</td>
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<tr>
<td>za</td>
<td>charge of species a</td>
</tr>
<tr>
<td>ZACC FM10</td>
<td>Zorflex Activated Carbon Cloth FM10</td>
</tr>
<tr>
<td>ZACC FM30K</td>
<td>Zorflex Activated Carbon Cloth FM30K</td>
</tr>
<tr>
<td>zox</td>
<td>charge on the oxidised form of a species</td>
</tr>
<tr>
<td>zred</td>
<td>charge on the reduced form of a species</td>
</tr>
</tbody>
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Greek symbols

Γ  surface coverage
μ_{ox}  electrochemical potential of the oxidised form of a species
μ_{red}  electrochemical potential of the reduced form of a species
α  electron transfer coefficient
β  mass concentration
ϕ  potential of a certain phase
ϕ_{electrode}  electric potential of the electrode
ϕ_{int}  interface potential of an electrode
ϕ_{int,RE}  interface potential of the reference electrode
ϕ_{int,WE}  interface potential of the working electrode
ϕ_{solution}  electric potential of the solution
η  overpotential
μ_{a}  standard chemical potential of the species a
μ_{ox}  standard chemical potential of the oxidised form of a species
μ_{red}  standard chemical potential of the reduced form of a species
μ_{a}  chemical potential of the species a
μ_a  electrochemical potential of the species a
μ_{e^-}  chemical potential of the electrons exchanged per redox reaction
μ_{g}  microgram = 10^{-6} gram
μ_{ox}  chemical potential of the oxidised form of a species
μ_{products}  chemical potential of products
μ_{reactants}  chemical potential of reactants
μ_{red}  chemical potential of the reduced form of a species
ν  scan rate
ρ_{∞}  bulk resistivity of semi-infinite volumes
σ  volume concentration
Ω  electrical resistance unit ohm
Ω/sq  electrical sheet resistance, dimensionally equal to Ω
Ω_{cm}  specific electrical resistance unit ohm centimetre
Δμ^{°}  constant that equates to μ^{°}_{ox} - μ_{e^-} - μ^{°}_{red}
Chapter 1

*Introduction*
1. Introduction

1.1. Energy politics

Electricity generation from sunlight, commonly known as photovoltaic energy, has been the fastest growing renewable energy market, with an annual average market growth of 30% from 1999 to 2009.\[1\] This rapid development was driven by global political schemes for funding and support of renewable energy technologies resulting in an increase in total investment from USD 54 billion in 2004 to USD 260 billion in 2011.\[2\] The impact of political support for research and development became obvious through the financial crisis within this period. Annual funding growth rates of 22-51% in the years 2004 to 2007 plummeted to 1% in 2008, but recovered to 18% from 2009 to 2011. These figures include corporate as well as government funding and show that progress in renewable energy research is strongly dependent on public interest. The reason for this is due to the fact that renewable energy technologies are not yet economically competitive with conventional power plants. An analysis by Timilsina et al. showed that even if a damage cost of USD 100 per tonne of CO₂ released was considered, solar energy technologies would still remain uncompetitive with fossil fuel technologies.\[3\] However, the research progress in photovoltaics has resulted in a reduction of their capital costs by 25% and this reduction is projected to improve further, while the development of fossil fuel technologies is essentially stagnant. From an investment perspective, adding decentralised and versatile renewable energy generation assets to a portfolio of conventional energy generation translates into lower investment risk through portfolio diversification.\[4\] A lower investment risk further diminishes calculated capital costs and attracts corporate investors.\[5,6\] Additionally, a strong public interest in the move away from fossil fuels and towards renewable energies supports the additional funding for research, installation and adaptation of energy grids.

Such public demand occurred in response to the incident at the Japanese nuclear power plant Fukushima Daiichi in March 2011. This resulted in a fundamental shift in German energy politics, called the energy transition, and is exemplary for the emerging opportunities for renewable energies. In October 2010 the German governing party had passed a law to prolong the usage of the 17 German nuclear power plants.\[7\] A tsunami destroyed the nuclear power plant in Fukushima six months later, which caused in a nuclear meltdown and the release of radioactive
radiation through breaks in the reactor shell. The following public debate on the risks of nuclear power generation resulted in the amendment of the law, revoking the run time extension of all German nuclear power plants and constituted their shutdown until the year 2022.\cite{8} Consequently, $1.4\times10^{11}$ kWh in energy generation, which was 22.5% of the German energy mix at this time, have to be replaced with alternative resources.\cite{9} Increasing the fraction of fossil fuel derived energy to cover such a large energy production would lead to several contraventions against CO$_2$ reduction treaties and emission trading to avoid breaching such contracts would bring enormous costs with it. As a result, the German government passed a bill for the promotion of electricity generation from renewable energy resources that demands a continuous increase in renewable energy generation to at least 35% until 2020, 50% until 2030, 65% until 2040 and 80% until 2050, accompanied by all necessary funding required to achieve these targets.\cite{10} These ambitions were realised in the feed-in-tariff system that recompenses renewable energy generation costs with increasing state subsidies the smaller the input capacity is.\cite{11} The feed-in-tariff scheme has since been adapted by 75 countries across the world and is recognised as the most effective policy instrument to promote renewable energies.\cite{12} Additionally, electric utility providers have been mandated to purchase the complete output of grid connected renewable energy systems, thereby also supporting private investment of small producers. Evaluation of these subsidies should take into account that fossil fuel technologies already receive more than USD 500 billion in worldwide subsidies every year and that this is reflected in the prices per kWh, with which alternative technologies have to compete.\cite{13}

Energy generation from fossil fuel resources is also connected to a series of environmental, political and health problems, which ultimately lead to high costs and are usually not considered in economic calculations. More than 80% of the global energy demand was still covered by fossil fuels in 2010, of which coal, oil and gas contributed 34%, 40% and 26% respectively. Renewable energy supplied 13% and nuclear energy 7%. The 2012 report of the International Energy Agency warned that not more than one third of the known fossil fuel reserves can be consumed before 2050, in order to prevent a global temperature increase of more than 2 °C. The cost to achieve this goal becomes larger the longer the current consumption of fossil fuels continues and it is estimated to become impossible if actions are not taken by 2017.\cite{14} Furthermore, the uneven distribution of fossil fuel resources results in the dependency of resource poor nations on resource rich countries, which creates political tension regarding possession and transport rights.\cite{15,16} Exposure to fine particulate air pollution from fossil fuel combustion was identified as an important risk factor for cardiopulmonary mortality in regions with high energy demand.\cite{17}
The demand for more efficient renewable energy technologies is therefore high and is expected to increase further in the future. A study by the European Renewable Energy Council has projected that renewable energies could supply 50% of the global energy consumption by 2040 and that solar energy alone could cover 13%.\textsuperscript{[18]} Estimates by Shell International BV confirm these predictions and state a renewable energy fraction of up to 60% of the global energy demand, with 11% coming from solar energy, until 2050.\textsuperscript{[19]} Solar energy is expected to increasingly contribute to the global energy mix throughout the second half of this century, considering that technically useful solar irradiance was already 60-250 W m\textsuperscript{-2} on average across the world wide IMAGE-grid in 1997.\textsuperscript{[20]} Costs for such a transition were estimated as USD 1.4 to 5.1 trillion during the decade 2011 to 2020 and 1.5 to 7.2 trillion for the following decade.\textsuperscript{[21]} The higher figures are congruent with the goal to minimise global warming to 2 °C until 2050. Whilst such sums seem large, it should be taken into account that the average annual investment would still be lower than 1% of the world gross domestic product.

1.2. The carbon dioxide capture and utilisation project

The presented work investigates photo-microbial fuel cells (pMFCs) with a special focus on macroporous ceramic electrodes for enhanced biofilm formation. This technology employs microbial photosynthetic organisms to generate electricity and biomass from sunlight and carbon dioxide.\textsuperscript{[22,23]} Consequently, it combines the purposes of solar energy and carbon capture technologies into one device that utilises low cost materials and still offers the advantages of distributed energy generation. Contrary to conventional photovoltaic and solar thermal energy, pMFCs also improve energy security, since both electric power and biomass are generated in the dark as well as in the light. Photosynthetic organisms do not require high solar irradiance to operate at maximum efficiency, so the output is less dependent on weather conditions and enables the application of pMFC technology in regions with increased distance from the equator.

Biofuel production from feedstocks is controversial, since it competes with food crops for arable land and results in the tarnished reputation of biofuels in general. In contrast, microbial organisms are grown in tanks without the need for fertile soil and exhibit higher cell growth rates with a larger yield of usable oil per cell. Single cell cultures possess superior adaptability to harsh environments compared to multi-cellular organisms and thereby increase the flexibility in the application.\textsuperscript{[24]} More than 50% of the cells in a microbial culture can die or be harvested without detrimental consequences for the growth of the remaining culture, whilst a multi-cellular organism is usually lost in this case. Start-up time and yield of microbial fuel
cell systems benefit from this aspect, which reduces calculated capital costs for investors. Microbial derived fuels are therefore distinguished as third generation biofuels, where first generation biofuel is derived from food-feedstock and second generation from non-feedstock resources, such as oleaginous crops and lignocellulosic agriculture respectively. Combining photosynthetic microorganisms with a culture that feeds on their biomass and produces its own metabolic products provides further options for the synthesis of reduced carbon chemicals from CO$_2$ and for the generation of molecular hydrogen.

An additional advantage of utilising microbial organisms is the biological catalysis of carbon fixation. The alternatively used inorganic catalysts often require high operation temperatures and contain precious metals, which also involve large energy expenditure for mining and refinement. Inorganic catalyst substrates are modified to achieve large surface areas, for example with carbon nanotubes, thereby further decreasing the economical competitiveness through energy intensive processes.

Energy generation with pMFCs therefore tries to address multiple shortcomings of previous renewable power and carbon capture technologies. Whilst pMFCs have the potential to make a valuable contribution to the future energy mix, the current efficiency is comparably low. This mainly results from the poor understanding of electron transfer mechanisms between organism and electrode, as well as from the application of inefficient materials and electrode configurations. Membranes for the separation of anodic and cathodic compartments, for example, were just adopted from conventional fuel cells, although they are not optimised to work at a physiological pH range. Electrode surfaces have to be biocompatible and support cell adhesion, which limits the range of applicable materials. MFC technology is still fairly young and work is being conducted in all of these areas to improve the output and add a promising renewable power resource to the energy mix of tomorrow. The aim of this work was therefore to analyse biological and engineering characteristics of pMFCs to enhance future pMFC configurations. New reticulated ceramic electrodes were employed in mediator-free pMFCs and the biofilm support, as well as the power generation, were compared between differing electrode materials in long-term experiments. Initial investigations into the biocompatibility of the CO$_2$ capturing metal-organic framework MIL101 and its amine functionalised modification have been conducted.

Examinations in this study were performed on the basis of the primary pMFC outputs of electricity and biomass, without oxygen measurements. However, it should be noted that the potential output through refinement of the biomass is far greater. Typical photosynthetic microbial organisms used in pMFCs are green algae and cyanobacteria. The biofuel industry has already developed various processing
routes for these organisms to yield energy carriers and precursor chemicals. Figure 1 summarises the required inputs for pMFC operation, the primary outputs and the accessible refined products from the generated biomass.

Figure 1: Required inputs for pMFC operation and achievable outputs subdivided according to processing steps. Electricity is a primary output during the pMFC operation and can also be obtained from direct combustion of biomass. This energy can either be fed back into the system to minimise operational costs or transferred to an external consumer. Refinement of raw algae or cyanobacteria biomass yields energy carriers that can be transported with lower energy losses to distant energy consumers.

This study is part of a larger project on the “Nano-Integration of Metal-Organic Frameworks and Catalysis for the Uptake and Utilisation of CO₂” to discover an energy technology that is sustainable in its application as well as its production and is funded by the Engineering and Physical Sciences Research Council. An interdisciplinary team of engineers, chemists and biologists explored various methods of power generation and carbon capture to establish a basis for further development and to identify opportunities for joint ventures.
**1.3. Progress in microbial fuel cell research**

Solar power is on the rise with more installed capacity than any other renewable energy source in 2013.\(^{[34]}\) Solar thermal power is dominating the solar energy market contributing almost 70% to the solar power generation and the remaining 30% are almost completely provided by solar photovoltaic technologies. Electricity or biofuel derived from microbial photosynthetic organisms has been recognised as an emerging technology and was funded with USD 16.5 million in grants from the United States. Much more development is needed before microbial fuel cells can significantly contribute to the world wide produced 1.56 TW from renewable energies in 2013.\(^{[35]}\) However, the research field is growing rapidly with increasing diversity in its applications.\(^{[36,37]}\) Fundamental investigations are turning into sophisticated technologies, as shown on the basis of published patents displayed in figure 2 from a study by Yang et al.\(^{[38]}\)

![Figure 2](image)

**Figure 2:** The number of published patents on MFC technologies from 2001 to 2010 shows the transition from fundamental research to mature technologies in a study of Yang et al.\(^{[38]}\)

Initially unsustainable operational methods, such as exploiting soluble redox mediators to transport electrons between organisms and electrode, have been abandoned and research is now focusing on electrode design as well as more suitable separator materials.

Electrodes are tailored towards a macroporous structure that supports cell adhesion and maximises the conductive interface between biofilm and electrode. Carbon nanotube functionalised surfaces have been the predominant approach to enhance the electrode interface and also resulted in materials such as buckypaper and buckygel.\(^{[39-41]}\)

An alternative and less energy consuming strategy are osmium based redox hydrogels containing co-immobilised biocatalysts.\(^{[42,43]}\) Such hydrogel coatings can
be used to incorporate enzymes or whole cells and act as an electron mediating matrix, which effectively increases the conductive surface area. A further benefit from hydrogel matrices is the prolonged lifetime of exposed enzymes, which improves from several hours to days.

The trend in electrode design is toward hierarchical structures, where a macroporous scaffold is coated in a mesoporous substrate for the functionalisation with nano-materials. Macroporous scaffolds utilise the compartment volume with as much surface area as possible, and simultaneously ensure a minimum spacing between the functionalised materials for sufficiently high flow rates through the electrode. Mesoporous coatings on these scaffolds expand the options for functionalisation of the surface, allow the embedding of biocatalysts and further enlarge the surface area on the nanometre scale. Work on micrometer sized whole cells also benefits from surface modification on the nanometre scale, since it enables fine tuning of the surface hydrophilicity and of the conductive interface. Direct electron transfer of the bacterium *Shewanella oneidensis MR-1* was investigated on such a hierarchical electrode made from a fibrous carbon felt support, which was coated with carbon nanotube doped chitosan. Carbon based supports are generally the most applied macroporous structures, owing to the large variety of accessible geometries and comparatively simple modification routes, although metal foams have also been examined. Reticulated vitreous carbon (RVC) foam is amongst the most popular macroporous supports and exhibited promising results with a functional coating of multi-walled carbon nanotubes in a chitosan matrix.

Polymer electrolyte membranes are often used to increase the efficiency of MFCs, although their implementation generally involves a trade-off between coulombic efficiency and mass transport resistance in the MFC. Insufficient proton selectivity additionally causes the acidification of the anodic compartment and simultaneous pH increase in the cathodic compartment, which can result in non-physiological conditions in the whole MFC. Strik *et al.* have addressed this problem through the use of a reversible bio-electrode that acts as a cathode under illumination and as an anode in the darkness. However, the low ionic mobility in membranes remains a problem. The most frequently employed separator is the proton exchange membrane Nafion®, since its high selectivity was shown to result in the largest power outputs. MFCs are operated at physiological pH to minimise cell damage and prevent concentration polarisation from diminishing the power output. The ionic conductivity of Nafion® is minimal in this pH range, which effectively increases the internal resistance of the MFC and causes power losses. Identification of an efficient separator method between anodic and cathodic compartment is therefore a high priority in MFC research.
1 Introduction

The confirmation of direct electron transfer between microbial organisms and the electrode without the need for artificial electron mediators was a seminal discovery that fundamentally improved the sustainability of all MFC technologies. Several microbial electron transfer mechanisms have been suggested, from conductive pili to electron hopping between transmembrane proteins, which are elucidated in the theory section. Analysis of a single Shewanella oneidensis MR-1 pilus with nanofabricated electrodes and conducting probe atomic force microscopy showed a resistivity of only 1 Ω cm⁻¹. Applying a voltage of 100 mV resulted in an electron transport rate of 10⁶-10⁹ electrons per second. El-Naggar et al. compared these electron transfer rates with the specific respiration rate under cultivation conditions and found that the entire supply of respiratory electrons can be discharged to a terminal acceptor by a single bacterial pilus. The length of bacterial pili is in the range of nanometres, although the electrical conduction range can be extended into the micrometer scale through networks of pili between cells. These length scales illustrate the importance of biofilm formation on the electrode for an effective use of microbial exoelectrogenic activity. Furthermore, the electrical resistance of an external electricity consumer can no longer be adjusted according to the largest power generation alone, since the external resistance has also been shown to affect the structure of a biofilm. Currents obtained through direct electron transfer are comparatively low. Logan et al. therefore concluded that, for MFC technologies developed in the near future, exoelectrogenic bacteria are more suitable for energy recovery during wastewater treatment than for electricity generation.

However, El-Naggar et al. also found electrically conductive pili in the photosynthetic cyanobacterium Synechocystis PCC6803. Microorganisms that are both exoelectrogenic and photosynthetic drastically expand the field of applications, since carbon capture and solar energy utilisation improve the carbon neutrality and energy recovery of conventional MFC systems. A comprehensive overview of performance and efficiency of microbial solar cells (MSCs) has been published by Strik et al. and is shown in a modified form in figure 3. It also illustrates the undefined parameters of the respective MSCs, showing the importance of a more systematic characterisation procedure. Strik et al. conclude from this that the accurate analysis of carbon and electron fluxes, which determine the coulombic efficiency, represents one of the greatest experimental challenges in modern MFC research.
## Introduction

<table>
<thead>
<tr>
<th>MSC category</th>
<th>Photosynthetic organism</th>
<th>Electron donor</th>
<th>Microbial community</th>
<th>Operation time (days)</th>
<th>Current density ($\text{mA/cm}^2$)</th>
<th>Power density ($\text{mW/cm}^2$)</th>
<th>Coulombic efficiency (%)</th>
<th>Internal resistance (Ω)</th>
<th>Power conversion efficiency (%)</th>
<th>Electron acceptor (catalyst)</th>
</tr>
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<td>Rhodopseudomonas</td>
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<td>32</td>
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<td>4</td>
<td>87</td>
<td>525</td>
<td>O$_2$</td>
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<td>21</td>
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<td>14</td>
<td>22</td>
<td>19</td>
<td>1800</td>
<td>0.01</td>
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<td>111</td>
<td>11</td>
<td>10</td>
<td>750</td>
<td>0.01</td>
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<td>154</td>
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**Figure 3:** Summary of performance data on microbial solar cells (MSCs) by Strik et al. showing the power output of the various photosynthesis driven systems and lack of comparable data, especially regarding coulombic efficiency. [59]
1.4. References in introduction chapter

21. Edenhofer, O., et al., IPCC special report on renewable energy sources and climate change mitigation. 2011, IPCC.


Chapter 2

Theory
2. Theory

2.1. Mass transport in solution

Microbial fuel cells (MFCs) can be considered as electrochemical setups; containing both an anode, and a cathode, where at least one of them is in a liquid compartment.[1,2] From a practical perspective, the performance of a MFC is evaluated on the basis of how much electrical current can be generated through an external power consumer, which is often represented with a simple electrical resistor. The current is the flow of charge resulting from the injection of electrons into the external circuit at the anode and their subsequent discharge at the cathode. Injection and discharge are both caused by redox reactions at the electrodes and the slowest redox reaction determines the magnitude of the current. Mass transport is a crucial component of electrochemical analysis, since redox reactions can only occur following arrival of redox-active species at the electrode. As a result, the current can be limited by the mass transport of redox-active species. Especially in bio-electrochemical systems (BES), the mass transport types and rates become highly diverse and understanding them is considered an essential step for the correct interpretation of results and improvement of BES.[3] It has already been shown that understanding and optimising the mass transport conditions allows the coulombic efficiency of BES to be improved.[4,5] Furthermore, Beyenal et al. reported that biofilms are able to adapt to their external mass transport conditions by growing in different densities, thereby also changing their internal mass transport.[6]

Electrochemical methods are not limited to the study of charged analytes; however, a more comprehensive description of mass transport can be obtained by considering the motion characteristics of ionic species. The motion of ionic species is governed by extra charge related effects, in addition to the usual rules which apply to neutral species.

Mass transport is composed of three types of movements for ions in solution and these are diffusion, migration and convection. Each type of movement can be oriented towards a different direction and the input of all mass transport types has to be considered to determine the final mass transport direction. Assuming fast electron transfer kinetics at the electrode and a sufficient amount of redox-active species, the mass transport can become the current limiting factor.[7]
The random movement of diffusion occurs in solids, solutions and gases and is due to local concentration gradients being compensated through entropic forces. Adolf Fick conceptualised his empirical findings on diffusion into two laws, which were later confirmed by Albert Einstein’s derivations from thermodynamics. Although Fick’s laws of diffusion do not sufficiently describe the more complicated diffusion in inhomogeneous systems, this work does not include modelling of the complex mass transport in biofilms. The simplicity of Fick’s laws is therefore considered suitable for application.

Fick’s first law describes linear diffusion, \( J_{\text{diff}} \), as the flux of particles through a certain unit area as a function of the concentration gradient:

\[
J_{\text{diff}} = -D \cdot \frac{\partial c}{\partial x}
\]

Equation 1:

The change in concentration, \( c \), with distance, \( x \), is therefore the driving force behind the diffusion flux. Diffusion results from entropic forces and is therefore directed against the concentration gradient, which results in a negative diffusional flux. The diffusion coefficient \( D \) is a proportionality constant and describes the mobility of the species of interest. Equation 1 expresses diffusion with regard to concentration fluctuations in space; however, the concentration gradient changes over time as the diffusion flux affects the local concentration. This means that Fick’s first law only depicts a single moment in the process of diffusion. It should also be noted that Fick’s first law only describes the diffusion of a single species in a pure component system, for example a pure gas with discrepancies between local densities. This type of diffusion is called self diffusion.

In most cases, the species of interest is in a more complex environment, for example a binary system of solute in solvent. The concentration gradient of solute automatically entails a concentration gradient of the solvent in the opposite direction. Consequently, the diffusional flux of solute is accompanied by the oppositely directed flux of solvent, which is called mutual diffusion.

Fick’s second law is derived by applying the equation of mass conservation on a linear diffusion flux of particles. It considers both concentration changes over time and in space, which allows the intermediate stages of diffusion to be described:

\[
\frac{\partial c}{\partial t} = \frac{\partial}{\partial x} \left( D \cdot \frac{\partial c}{\partial x} \right) = D \cdot \frac{\partial^2 c}{\partial x^2}
\]

Equation 2:
An expression for 3-dimensional diffusion can be obtained from equation 2 by deriving it in three dimensions instead of one, through the use of the nabla operator $\nabla$:

$$\frac{\partial c}{\partial t} = \nabla(D \cdot \nabla c)$$

Migration is a type of mass transport which only affects charged particles under the effect of an external electric field, whilst diffusion concerns the mass transport of all particles according to concentration gradients.\textsuperscript{[11,12]} An ionic species experiences forces from the multiple electric fields of other charged particles additionally to the electric field of an electrode. The surrounding particles exhibit attractive or repulsive forces corresponding to their charge sign and result in a net force corresponding to the respective concentration. The situation becomes simple if a sufficiently strong external electric field is applied with an electrode, which makes the contributions of the surrounding ionic species negligible. In this case, the migration flux, $J_{\text{mig}}$, is proportional to both the gradient of the external potential $\phi$, and to the electrostatic potential energy of the ionic species:

**Equation 3:**

$$J_{\text{mig}} = -\frac{zF}{RT} \cdot D \cdot c \frac{\partial \phi(x)}{\partial x}$$

The electrostatic potential energy of an ion is determined by its valency $z$, and its position $x$ in the electric field. Diffusion coefficient and concentration of the ionic species affect the particle flux according to Fick’s laws. The Faraday constant $F$ relates the charge on a single ion to an amount of one mol of ions. Temperature and pressure dependence of the migration flux are expressed in the ideal gas constant $R$ and the temperature $T$. Electric field lines were approximated as linear on the scale of the ionic particle size in equation 3, but can be expressed in three dimensions by applying the nabla operator. The direction of the migration flux depends on the net charge of the electrode and of the species of interest. In this case, the equation was written for a positively charged electrode.

The third type of mass transport, convection, describes the movement of particles due to the action of a force, for example one kind of particles being carried away in the flow of another kind of particles.\textsuperscript{[13]} Hence, convection is only proportional to the concentration and velocity, $v$, of the particles:
2 Theory

Equation 4: \[ J_{\text{con}} = c \cdot v(x, y, z) \]

There are two kinds of convection, the natural convection and the forced convection. Natural convection is present in every solution or gas and is a result of temperature or density gradients. Forced convection is caused by external influences, for example a stirrer or a pump.

In summary, all three types of mass transport together result in a particle flux of:

Equation 5: \[ J = -D \cdot \frac{\partial c(x)}{\partial x} - \frac{zF}{RT} \cdot D \cdot c \cdot \frac{\partial \phi(x)}{\partial x} + c \cdot v(x) \]

With the particle flux in units of mol cm\(^{-2}\) s\(^{-1}\), the diffusion coefficient in cm\(^2\) s\(^{-1}\), the concentration in mol cm\(^{-3}\) and the particle velocity v in cm s\(^{-1}\).

Equation 5 is only expressed for linear particle flux; however, it can be extended for 3-dimensional calculations through application of the nabla operator.

Natural convection is difficult to predict and the simultaneous contribution of multiple types of mass transport complicates the calculations further; increasing the error in the results.\(^{[14]}\) In practice, techniques and measuring environments are therefore designed to reduce the mass transport contributions to diffusion and forced convection. Natural convection can be superimposed by forced convection with a well-defined hydrodynamic behaviour, which usually also makes mass transport due to diffusion negligible. Migration can be reduced by shielding the electrical field with high ionic strength solutions, which is elucidated in 2.2.9.

2.2. Electrochemistry

2.2.1. Types of electrode

An electrode is usually a chemically inert electron conductor which is in direct contact with an ionic conductor in the form of the electrolyte. Redox-active species in the electrolyte can exchange electrons with the electrode, if the electric potential between them provides sufficient driving force.\(^{[15]}\) There are three general types of electrodes, the working electrode (WE), the counter electrode (CE) and the reference electrode (RE).\(^{[16]}\) Investigations are performed on the interface of the WE by controlling its potential relative to the RE and by measuring the resulting current between the WE and CE, which is described in more detail in 2.2.8.
The potential at the interface between RE and surrounding electrolyte has to remain stable in order to conduct reliable measurements; however, the potential can only be measured, if a current passes and this in turn changes the potential.$^{[17]}$

Hence, the problem is that the measurement changes the system and the three electrode setup is designed to keep this effect to a minimum. A RE and a WE are connected over a large resistance, so that almost all current is passed between the CE and the WE. A RE is positioned close to the WE and includes a separate redox system with fast electrode kinetics, in order to adjust quickly to potential changes at the WE interface. The ion concentration within the RE has to remain constant to ensure a stable RE potential. This is realised by employing a large concentration of the potential determining ionic species, so that the small current flow through the RE only causes a negligible turnover. The RE redox system typically consists of the hardly soluble salt of a metal in equilibrium with a solution that contains the cations of this metal and the anions of the salt. The potential is determined by the concentration of metal ions in solution, which depends on the concentration of the anions and the solubility product of the hardly soluble salt. Thus, the potential difference between the WE and RE interface is initially sensed with the dissolved anions of the RE redox system. Silver chloride electrodes (Ag/AgCl), based on the redox reaction between the solids AgCl and Ag in a chloride containing solution, were the most frequently employed type of RE within this work.$^{[18]}$ The standard potential of this type of electrode is 200 mV wrt. the standard hydrogen electrode at 25 °C, and the high chloride concentration inside the electrode was maintained following storage in saturated KCl solution.$^{[19]}$

Almost all current is passed between the WE and the CE, apart from the negligible current needed for the potential measurement at the RE. The electrochemical properties of the CE, also called auxiliary electrode, do not influence the behaviour of the WE; however, it should not produce any electrolysis products, which could affect the environment of the WE, even if currents of high magnitude are passed.$^{[20]}$

Hence, the CE is usually made from a noble metal with a larger surface area than the WE, and is positioned with greater distance to the WE than the RE, as elaborated in 2.2.6. This was realised with a platinum net electrode in this work, if the size of the electrochemical cell allowed such a large electrode.

### 2.2.2. Potentiostat

Electrical quantities between electrodes can be controlled and measured with a potentiostat, which assures near ideal conditions of a potential measurement without current flow through the RE, and assists the current flow between the WE and CE, so that the external circuit resistance is almost zero.$^{[21]}$ This is achieved through a complex system of resistors and capacitors, which is designed for fast
switches between control amplifiers of 10 μs and less, while exhibiting small control deviations. Figure 4 illustrates a simplified potentiostat circuit connected to a three electrode electrochemical setup.

![Figure 4: Simplified circuit of a potentiostat](image)

The potentiostat measures the potential difference between WE and RE and controls it according to the amplified signal from the feedback circuit. Current is measured between WE and CE and computed as function of potential difference.

### 2.2.3. Electric double layer

The electric field of an electrode creates a region where the solution characteristics vary from the bulk solution due to the re-organisation of ions, and this environment is called an electric double layer.[22] The concentration differences between double layer and bulk solution are of particular importance for this work, since the response in voltammetric measurements is affected by the double layer thickness.[23] The electric double layer thickness is the Debye length $\kappa^{-1}$, which changes with ionic strength of the electrolyte and is usually in the range of nanometres.[24,25] Section 2.2.9 elaborates on the benefit of reducing the Debye length using a high ionic strength electrolyte.

The excess charge on an electrode is compensated with ions of the opposite charge in the electric double layer, which causes the potential between an ion and the electrode to drop with increasing distance to the electrode. As a result, the
organisation of ions in the electric double layer varies with distance from the electrode and this is the reason for its subdivision into several layers with differing properties. The number of layers that constitute the electric double layer changes between the multiple theories that model it according to the respective considerations. With relevance here is the Grahame model, which is a combination of the Helmholtz and the Gouy-Chapman model and is extended with an additional layer closest to the electrode surface.\footnote{26} This layer is called the inner Helmholtz layer and accounts for the few ionic species without solvation sheath, which are specifically adsorbed to the electrode surface, as shown on the example of non-solvated anions at a positively charged electrode surface in figure 5.

![Figure 5: The electric double layer according to the Grahame model is subdivided into: Red) inner Helmholtz layer, where not completely solvated ions are specifically adsorbed to the electrode surface; yellow) outer Helmholtz layer, which is a monolayer of solvated ions with a distance of their solvation sheath thickness to the electrode; as well as the double (green), reaction (blue) and diffusion (purple) layer, where thermal motion results in lower concentrations of ions.}

The specific adsorption to the electrode is not related to the charge of the ion, so that even ions with the same charge sign as the electrode can be bound to its surface.\footnote{27} The thickness of this layer (red) is defined by the distance of the plane through the centre of the specifically bound species to the electrode surface.

The Helmholtz model distinguishes the monolayer of solvated ions closest to the electrode (yellow outer Helmholtz layer) from the other solvated ions. The solvation sheath thickness determines the distance of the closest solvated ions to the electrode and the width of the outer Helmholtz layer is defined by the plane
through the centre of these ions. The inner and outer Helmholtz layer form a dense population of ions directly on the electrode surface, which causes the potential to drop linearly from the electrode surface to the outer Helmholtz layer. The Helmholtz layer differs in this aspect from the remaining layers, since the potential drops exponentially from the outer Helmholtz layer to the bulk solution. This is a result of thermal motion, which acts against the ordering forces of the electrical field, a concept introduced by Gouy and Chapman. Figure 6 illustrates the potential decline with distance from the electrode qualitatively (not to scale).

![Diagram of potential decline with distance from the electrode](image.png)

- \( \phi \): Galvani-Potential in the electrode
- \( \Delta \phi \): Galvani-Potential difference between electrode and bulk potential

**Figure 6**: The decline of the electrode potential \( \phi \) with distance from the electrode is linear within the Helmholtz layer and progresses exponentially outside the Helmholtz layer, where thermal motion decreases the ion concentration.

The exponential decay of the potential approaches the potential in the bulk solution. In principle, the potential decline with distance from the electrode surface is proportional to the net charge buildup of ionic species around the electrode. Hence, the potential drops rapidly in close distance to the electrode where the major amount of ions compensate the electrode charge. Accordingly, the interface between electrode and electrolyte is modelled as a capacitor and the capacitative charging of the electric double layer is often referred to as capacitative charging of the electrode.

The corresponding charging current of the electric double layer is also called non-Faradaic current because electrons are not transferred across the electrode.
solution interface.\[^{29}\] It must therefore be distinguished from the Faradaic current that results from redox reactions at the electrode in voltammetric experiments, where it represents the background (residual) current. The charging current is proportional to the electrode surface and to the rate at which the potential is changed, which is the reason for using small electrodes and slow sweep rates in voltammetric studies. This relation can be demonstrated expressing the charging current as a derivative of the capacitor equation with respect to time:

**Capacitor equation:**

\[ Q_{dl} = C_{dl} \cdot V \]

The amount of charge stored in the double layer, \( Q_{dl} \), depends on the double layer capacitance, \( C_{dl} \), and the applied potential \( V \). Deriving \( Q_{dl} \) with respect to time gives the charging current:

**Equation 6:**

\[ i_{charging} = \frac{\partial Q_{dl}}{\partial t} = C_{dl} \cdot \frac{\partial V}{\partial t} \]

The charging current is therefore proportional to the double layer capacitance, \( C_{dl} \), which is proportional to the electrode surface area. Additionally, the charging current is proportional to the change of potential with time, which is the sweep rate, also called scan rate, in voltammetric experiments.

Electrodes in MFCs are purposely produced with large surface areas in order to maximise the current generation per reactor volume. The voltammetric analysis of such electrodes in this work was therefore confronted with large background currents and experiments had to be performed with slow scan rates to minimise the ratio of charging to Faradaic current. Another effect of capacitative charging is the hysteresis between forward and backward scan in cyclic voltammetry. As it is illustrated in 2.2.10, the voltage ramp from one potential to another is followed by the reverse scan in a cyclic voltammetry experiment and the current flows into the opposite direction on the reverse scan. This means that a hysteresis cannot be avoided, but again can be reduced with slow scan rates and small electrode surface areas.

The underlying Grahame model was established for an ideal polarised electrode.\[^{30}\] This means that it describes the situation for an electrode at which no electron transfer reactions happen. The electrode is then operated in certain potential ranges, called double layer ranges. However, it can be assumed that the processes,
which are described by the Grahame model, occur at every electrode solution interface.

2.2.4. Nernst equation for electrochemical equilibrium

Electrochemical experiments often investigate a system by perturbing its electrochemical equilibrium with an overpotential and then measuring the response in the form of a current. The preceding equilibrium situation has to be well known, in order to evaluate such data.

Forward and reverse reaction occur with the same rate at the chemical equilibrium because the chemical potentials of reactants \( \mu_{\text{reactants}} \) and products \( \mu_{\text{products}} \) are the same.

Equation 7:
\[
\sum_{i=1}^{n} \mu_{\text{reactant},i} = \sum_{i=1}^{n} \mu_{\text{product},i}
\]

Where the chemical potential \( \mu_a \) of any species \( a \) is defined as:

Equation 8:
\[
\mu_a = \mu_a^0 + RT \cdot \ln \left[ \frac{[a]}{[a]^0} \right]
\]

With \( \mu_a^0 \) being the temperature and pressure dependent standard chemical potential and \([\ ]^0\) the standard concentration of 1 mol L\(^{-1}\). The energy term \( RT \) relates the molar energy of the given species \( a \) to the temperature \( T \) of the system through the ideal gas constant \( R \).

Similarly to this, the sum of the electrochemical potentials of the oxidised form of a substance \( \mu_{\text{ox}} \) and the electrons used to reduce it \( \mu_{\text{e}^-} \) are identical to the electrochemical potential of the reduced form \( \mu_{\text{red}} \) at the electrochemical equilibrium:

Equation 9:
\[
\sum_{i=1}^{n} \mu_{\text{ox},i} + \sum_{i=1}^{n} \mu_{\text{e}^-,i} = \sum_{i=1}^{n} \mu_{\text{red},i}
\]
The electrochemical potential of the species $a$ is the sum of its chemical potential $\mu_a$ and its electrical energy $z_a F \phi$:

**Equation 10:**

$$\bar{\mu}_a = \mu_a + z_a F \phi$$

Where the electrical energy term consists of the charge of species $a$, $z_a$, multiplied with both the charge on one mole of electrons, which is the Faraday constant $F$ and the potential of the phase the species $a$ exists in, $\phi$. Hence, the electrochemical potential is defined per mole as well as for constant temperature and pressure, in the same way as the chemical potential. The electrical energy term takes into consideration that the electrons are exchanged between two different phases, which possibly vary in electric potential, namely the electrode and the electrolyte.\(^{[32]}\)

This information is sufficient to derive an expression that describes the potential difference between the electrode and its surrounding electrolyte; for one half cell of the electrochemical system at an electrochemical equilibrium. Inserting equation 10 into equation 9 for one redox species that is in electrochemical equilibrium between its oxidised form with the charge $z_{ox}$ and its reduced form with the charge $z_{red}$ through exchange of $n$ electrons per redox process at the electrode gives:

$$\left(\mu_{ox} + z_{ox} F \phi_{solution}\right) + \left(\mu_{e^-} - n F \phi_{electrode}\right) = \left(\mu_{red} + z_{red} F \phi_{solution}\right)$$

Where $\phi_{solution}$ is the electric potential of the solution and $\phi_{electrode}$ the electric potential of the electrode. Rearrangement of this expression allows the combination of all contributions to the electric potential on one side and all chemical potential terms on the other side of the equation:

$$F \left[ n \phi_{electrode} - \phi_{solution} (z_{ox} - z_{red}) \right] = \mu_{ox} + \mu_{e^-} - \mu_{red}$$

The difference between the charge of the oxidised and reduced form of the redox species, $z_{ox} - z_{red}$, equates to the number of electrons, $n$, which are exchanged with the electrode during the redox reaction, so that the potential difference between electrode and solution can be expressed as\(^{[15]}\):

$$\phi_{electrode} - \phi_{solution} = \frac{\mu_{ox} + \mu_{e^-} - \mu_{red}}{nF}$$
Equation 8 is now inserted for the chemical potentials, in order to relate the above expression to the concentrations of the respective species, which gives:

\[ \phi_{\text{electrode}} - \phi_{\text{solution}} = \frac{\mu_{\text{ox}}^\circ + \mu_{e^-}^\circ - \mu_{\text{red}}^\circ}{nF} + \frac{RT}{nF} \ln \left( \frac{[\text{ox}]}{[\text{red}]} \right) \]

The chemical potential of electrons is not dependent on the electron density and can therefore be merged with the constants \( \mu_{\text{ox}}^\circ \) and \( \mu_{\text{red}}^\circ \) into a new constant \( \Delta \mu^\circ \) to give the Nernst equation for one half cell\(^{17}\):

**Equation 11:**

\[ \phi_{\text{electrode}} - \phi_{\text{solution}} = \frac{\Delta \mu^\circ}{nF} + \frac{RT}{nF} \ln \left( \frac{[\text{ox}]}{[\text{red}]} \right) \]

Although this expression works in theory to relate potential and analyte concentration, in practice, the potential drop \( \phi_{\text{electrode}} - \phi_{\text{solution}} \) of a single interface between electrode and solution (one half cell) cannot be measured. At least one more electrode with its particular electrode-solution interface potential is required to complete the electrical circuit and measure a potential difference. This second electrode, first mentioned in 2.2.1, is the reference electrode (RE). The WE and the RE are separated by solution with an electrical resistance \( R_{\text{solution}} \). This solution resistance contributes to the potential between both electrodes according to Ohm’s law (\( V = i \cdot R \)). The potential \( E \), measured at the WE, is therefore the sum of the interfacial potentials \( \phi_{\text{int}} \) of both WE and RE, plus the potential generation due to the solution resistance:

**Equation 12:**

\[ E = \phi_{\text{int,WE}} + \phi_{\text{int,RE}} + i \cdot R_{\text{solution}} \]

To prevent the RE potential drop from influencing the readings at the WE, the RE has to maintain a constant potential. The WE readings will not be affected by the constant potential of the RE, since a potential difference \( \phi_{\text{electrode}} - \phi_{\text{solution}} \) and not an absolute value is measured to determine concentrations. Therefore, by utilisation of a RE, the Nernst equation for one half cell (Equation 11) can be applied to describe the measured potential at the WE in the equilibrium state:
Theory

\[ E_{equ} = \left( \phi_{electrode} - \phi_{solution} \right)_{int,WE} + \phi_{int,RE} + i \cdot R_{solution} = \]

\[= \frac{\Delta \mu^o}{nF} + \phi_{int,RE} + \frac{RT}{nF} \ln \left( \frac{[ox]}{[red]} \right) + i \cdot R_{solution} \]

The term \( \Delta \mu^o /nF + \phi_{int,RE} \) is represented by the standard electrode potential \( E^o \) (also written \( E^{\circ} \)) which is determined relative to the standard hydrogen electrode (SHE).\(^{[17]}\) As elucidated further in 2.2.9, by the use of background electrolyte the term \( i \cdot R_{solution} \) becomes negligible. Finally, this gives the practically used Nernst equation to determine the potential at the WE with regard to the SHE:

**Equation 13:**

\[ E_{equ} = E^o + \frac{RT}{nF} \ln \left( \frac{[ox]}{[red]} \right) \]

Besides the work presented here, measurements at the electrochemical equilibrium also allow investigations into the solution pH, equilibrium constants, activities, solubility products, free energies, entropies and enthalpies.\(^{[7]}\)

### 2.2.5. Redox reaction at an electrode

All energy levels of electrons in a metallic electrode form an energy continuum known as the conduction band. At absolute zero temperature the energy maximum of the conduction band is the Fermi level. Electrons possess more energy at higher temperatures and under these conditions the Fermi level is defined as the energy level that has a 50 % probability of being occupied by an electron. The Fermi level is therefore proportional to the average electron energy. Fundamentally, the direction of electron flow at the interface between electrode and redox-active species is determined by the Fermi level of the electrode relative to the energies of LUMO (lowest unoccupied molecular orbital) and HOMO (highest occupied molecular orbital) of the redox-active species. The electrons flow into an energetically lower state, which means that the relative energy of the Fermi levels determines if a species is oxidised or reduced.\(^{[17]}\)

Electrochemical experiments within this work utilised a potentiostat, explained in 2.2.2, to control the Fermi level by adjusting the potential of the WE relative to the RE. The Fermi level of the WE should be higher in energy than the LUMO of the species in solution, if it should act as a cathode and vice versa if the oxidation of the dissolved species on the anode is required, as shown figure 7.
The redox-active species is contained in a salt solution, called the electrolyte. Electrode and electrolyte together form a half cell. An electrode attains a certain Fermi level according to the redox potential of its half cell, which can be controlled through the application of an overpotential. By convention, applying a negative overpotential to the electrode initiates a flow of electrons into it and this raises its Fermi level. A negative overpotential therefore causes the reduction of a redox-active species at this electrode, if it had been in an electrochemical equilibrium with this redox-active species before. Assuming the negative overpotential is not kept constant, the flow of electrons from the electrode to the electrolyte will decrease the Fermi level of the electrode again, while the Fermi level of the electrolyte rises until a new electrochemical equilibrium is reached.

A defined overpotential can also constantly be applied to the electrode, which holds the Fermi level of the electrode at a particular energy, whilst the Fermi level of the electrolyte changes. In this case, measuring the current flow as the Fermi level of the electrolyte rises can yield information on the redox potential of an electrolyte species and its concentration. The turnover of redox-active species can be calculated, if the chemical reaction equation is known, since the amount of charge that flows and the amount of redox reactions are proportional. Monitoring the current decline during the turnover indicates when the redox-active species has been completely converted to a certain oxidation state.
In practice, the various sources for current limitations have to be considered before the observed current changes can be interpreted. Chemical reactions might be necessary to form the redox-active species prior to its detection at the electrode, which means that the rate of the slowest chemical reaction can limit the current. Mass transport to and from the electrode can also limit the current. Both mass transport and chemical reaction rates influence concentration differences between the electrode surface and the bulk solution and have a great impact on the apparent electrode kinetics, which reflects in the calculated reaction rates and current densities.\[20\]

Figure 8 summarises the sources of current limitation for a general redox reaction at the electrode, involving the transfer of \( n \) electrons.

![Figure 8: Sources for current limitation of a general redox reaction include the rate of mass transfer to and from the electrode, the rates of required chemical reactions and the involved concentration differences between electrode surface and bulk solution.](image)

A redox-active species is detected and quantified according to the electron flow across the electrode interface. Assuming the reduction of an analyte, this means that the concentration of oxidised species in the vicinity of the electrode drops, while the concentration of the reduced form increases. If the electron transfer reaction is fast compared to the diffusion rate, then these concentration changes become especially strong in the electric double layer. The resulting concentration gradient of charged species in the electrode surface region contributes to the development of an overpotential.

In case that the mass transport into the electrode surface region is not limiting the current, then the Butler-Volmer equation describes how the measured current \( i \) differs from the current at zero overpotential \( i_0 \). The difference between bulk
concentration, $[\text{Red}]_{\text{bulk}}$, and electrode surface concentration, $[\text{Red}]_e$, of redox-active species is considered as ratios:

**Equation 14:**

$$i = i_0 \cdot \left( \frac{[\text{Red}]}{[\text{Red}]_{\text{bulk}}} \exp\left( \frac{(1-\alpha)F\eta}{RT} \right) - \frac{[\text{Ox}]}{[\text{Ox}]_{\text{bulk}}} \exp\left( \frac{(1-\alpha)F\eta}{RT} \right) \right)$$

The electron transfer kinetics for the redox reaction between the oxidised species, Ox, and reduced form, Red, are represented in the electron transfer coefficient $\alpha$. The overpotential, $\eta$, is thereby the difference between the equilibrium potential and the applied potential and $F$, $R$, $T$ describe the Faraday constant, the ideal gas constant and the temperature, respectively. More recent models have been found to provide more accurate transfer coefficients; however, the Butler-Volmer equation described the electrochemical systems adequately with simpler relations.

Additionally, the mass transport can limit the concentration of a species in the electrode surface region and therefore also limits the maximal achievable current. The current, which is obtained in such a situation, is called the diffusion limited current. The diffusion limited current is proportional to the mobility and the concentration of the ions. Regarding bio-electricity generation, the diffusion current can be seen as the optimal performance value, since reaching this value would mean that nothing else but the diffusion through the diffusion layer is limiting.

### 2.2.6. Consideration of auxiliary electrode processes

The reduction reaction at one electrode requires the oxidation at another electrode and vice versa, in order to provide the necessary electron flow. It is therefore necessary to take the redox reactions at both electrodes into consideration, in order to assure the correct setup and interpretation of electrochemical experiments. The WE can act as either anode or cathode, but in each case the CE has to fulfil the opposite role to enable the reaction at the WE. Thus, the crucial characteristic of a CE is that it does not limit the current to the WE, or affect its environment.

Assuming good mass transport conditions and electron transfer kinetics, the flow of electrons between the electrolyte and the electrode is limited by the surface of the electrode. Since the current flows between a WE and a CE with different surface areas, one of them will limit the current and cause the potential of this limiting electrode to increase. However, the measured and therefore controlled potential is
only the one at the WE. Hence, it should be assured that the current limitation occurs at the WE by using a WE with a much smaller surface area than the CE has. The rule of thumb for this is that the surface area of the CE should be at least 100 times greater than the surface area of the WE. Simple designs from inert and highly conductive noble metals, such as platinum, gold or glassy carbon further decrease the possibility of interference from the CE. In addition to this, it is important to confirm that the CE material does not catalyse electrolyte reactions in the applied potential range.

In the case that the surface area of the CE is insufficient, the rising potential at the CE would reduce the current. In the worst case the potential increase at the CE causes the current to flow in the opposite direction with respect to the desired direction. According to the setup, such a situation could cause material deposition on the CE instead of the WE, or electroactive organisms to lose all benefit from forming a biofilm on an electrode.[15]

2.2.7. Faradaic and charging current

Two types of current contribute to the overall measured current, Faradaic current due to redox processes at the electrode and non-Faradaic current (charging current) due to the double layer capacitance of the electrode, as elaborated in 2.2.3. Both types of current have to be distinguished in the evaluation of voltammetric experiments.

Charging current flows as soon as an electric field is applied to a system and whenever the electric field density changes resulting from a variation in potential. It is caused by the migration of ions in the solution according to the field force and is proportional to the surface area of the electrode and applied sweep rate.

The Faradaic current is dependent on the rate of the electron exchange at the electrode surface and occurs only if the potential of the electrode provides the required redox potential of the species in the electrolyte. The Faradaic current can be used to investigate the concentration, diffusion coefficient and redox potential of the redox-active species. If these quantities are known, then the Faradaic current can also serve to characterise the electrode, as shown on the example of the Randles-Sevcik equation in 2.2.10.

2.2.8. Two, three and four electrode mode

The commonly employed three electrode setup, shown in figure 9, comprises the WE, the CE and the RE.
The reaction of interest is examined at the WE by controlling its potential against an electrode of constant and well known potential, the RE. The current flows between CE and WE, thereby allowing the redox reaction at the WE to take place by coupling it to the respective reverse reaction. This separation of electrode functions allows analysis of the WE containing half cell independently from the changes that might occur at the CE, which greatly improves measurement accuracy in cases when the WE and CE environments differ, or when the WE itself is analysed.

If necessary, the CE can also fulfil the role of the RE as a pseudo RE in systems where the potential of the CE remains relatively stable. This approximation can only be done in systems with low current flow and over short measuring times.

On the contrary, an additional sensing electrode (SE) can be introduced to increase the voltage measurement accuracy by completely separating the voltage reading electrodes from current carrying electrodes, which is elaborated on in further detail in 2.2.12.

### 2.2.9. Purpose of electrolyte

Usually the electrolyte in a CV experiment consists of the redox active species of interest and additional salts as background electrolyte. There are four reasons to add this background electrolyte.

Firstly, the additional salts increase the conductivity of the electrolyte solution. As a result the resistance of the electrolyte solution is lowered and can be approximated as negligible compared to the other components of the system. The equation for the overall potential difference between WE and RE can then be simplified:
Equation 15: \[ \Delta V = \varphi_{\text{int}, \text{WE}} + \varphi_{\text{int}, \text{RE}} + i \cdot R_{\text{solution}} \approx \varphi_{\text{int}, \text{WE}} + \varphi_{\text{int}, \text{RE}} \]

\[ \approx 0 \]

Hence, without the increased conductivity of the electrolyte the experimental setup would become more complicated, since the distance between WE and RE has to be exactly defined to determine the contribution of the solution resistance to the potential difference. Moreover, if the solution consists of unknown substances, then their specific resistivities have to be determined separately beforehand. These additional measurement factors would then bring additional sources of error with them.

The high ionic strength of the background electrolyte shields the electrical field of the electrode. This electric field would otherwise cause three problems in the experiment, which are explained on the basis of a negatively charged WE, an aqueous solution of ferricyanide both with and without KCl background electrolyte, shown in figure 10.

![Figure 10: Purpose of the background electrolyte: a) Without electrolyte the electric double layer extends further into solution causing analyte molecules to orientate into a static layer around the electrode; b) in the presence of electrolyte the potential drops more quickly with distance due to charge compensation by the electrolyte.](image)

Molecules from the solution will orientate at the electrode surface according to charge compensation and stack up to form a static layer around the electrode,
called the electric double layer. The double layer, its subdivision and the respective characteristics were introduced in 2.2.3 in more detail. As long as the potential of the electrode is large enough, it will cause ions from the solution to orientate into the double layer. Therefore, the electric double layer thickness $\kappa^{-1}$, also known as the Debye length, depends on the range of the electric field from the electrode. Without background electrolyte the potential of the electrode drops less with distance than with the high ionic strength of the salts in the background electrolyte. Hence, one purpose of the electrolyte is to collapse the double layer.

The electron exchange between electrode and redox active species from the solution happens by tunnelling processes. The chance of a tunnelling process occurring decreases exponentially with distance. Consequently, for any redox process to happen at the electrode it is crucial that the redox active species can get close enough to the electrode surface. Collapsing the double layer, mediated by the background electrolyte, allows the redox active species to get within tunnelling distance to the electrode.

A more detailed description of the outer shell electron transfer process, without forming or breaking bonds, can be found in the Marcus Theory. This theory considers the importance of the solvent for the Gibbs Free Energy of activation for each electron transfer.$^{[35,36]}$ The underlying model splits the electron transfer up into the processes of association of electron donor and acceptor, the actual electron exchange between them, and their dissociation. The rate of association and dissociation of donor Do and acceptor Ac is described with diffusion constants $k_{\text{ass}}$ and $k_{\text{diss}}$, and the electron transfer by kinetic rate constants $k_{\text{red}}$ and $k_{\text{ox}}$.

$$\begin{align*}
Do + Ac & \xrightleftharpoons[k_{\text{diss}}^{-1}]^{k_{\text{ass}}^{-1}} [Do\cdots Ac] \xrightarrow[k_{\text{ox}}^{-1}]^{k_{\text{red}}^{-1}} [Do^+\cdots Ac^-] \xrightarrow[k_{\text{ass}}^{-1}]^{k_{\text{diss}}^{-1}} Do^+ + Ac^-
\end{align*}$$

The electron transfer may therefore be diffusion controlled, if the diffusion is slower than the electron exchange, or it is kinetically controlled in the inverse case.

The three types of movements for ions in solution, diffusion, migration and convection, were discussed in detail in 2.1. Each type of movement has to be accounted for in calculations. The purpose of adding background electrolyte in this context is to simplify the calculations for all the different particle movements. The background electrolyte reduces the influence of migration to the overall movement so much that any effects from this type of motion can be ignored. This is achieved through the shielding of the electrostatic field of the electrode by increasing the charge density of the solution with the salts of the background electrolyte. As a result, the potential of the electrode decreases more rapidly with distance and fewer ions are forced to migrate in a certain direction.
2.2.10. Cyclic voltammetry

In principle, potentiodynamic electrochemistry, or voltammetry, measures the current evolution, while the voltage is varied in a defined way.\textsuperscript{[20]} One type of voltammetry, known as cyclic voltammetry, was employed for the material and biofilm investigations within this work.\textsuperscript{[37,38]}

Measuring a cyclic voltammogram (CV) comprises three voltages that define the process; starting potential, first and second vertex potential. First and second vertex potential indicate the most positive and the most negative potential of the CV respectively. Hence, these set the voltage range in which the voltage is increased and decreased (cycled) continuously. Ideally, the starting potential does not cause any redox reactions, so that the current is zero, but it can also be set to match the first vertex potential.\textsuperscript{[32]} All three voltage values are determined in a preliminary CV measurement with the same electrode environments as in the intended experiment. The preliminary CV is conducted over a broad voltage range to identify disturbing redox reactions and a suitable starting potential. First and second vertex potentials are then fixed on values, which allow all essential redox reactions to happen, but exclude all the others. It is therefore crucial to optimise the system (electrodes, electrolyte, dissolved gases in the electrolyte) for each redox reaction of interest. The voltage change over time (sweep) for a general first and second vertex potential, $E_A$ and $E_B$, of a single CV scan is illustrated in figure 11.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure11.png}
\caption{Voltage sweep over time during a CV measurement: The voltage is swept from the first vertex potential $E_A$ to the second vertex potential $E_B$ and back with a defined sweep/scan rate. The starting potential is usually set in the range from $E_A$ to $E_B$ at potential that does not result in current flow.}
\end{figure}

Special equipment allows perfectly linear sweeps to be performed, but the sweep is normally realised in steps with small increments. Smaller steps result in a greater resemblance of the voltage change to a line, but it also increases the time for each single CV to be measured. Hence, smaller voltage steps increase the accuracy of a single CV, but diminish the time resolution of the whole measurement. Therefore, the general rule of thumb is that the potential steps should be smaller than a 15th
of the overall voltage difference which will be covered during the cyclic voltammogram.

Current resulting from the reduction of the electrolyte appears negative in the cyclic voltammogram and the oxidation current is positive, therefore, the nomenclature in Europe is defined according to that displayed in figure 12. However, the nomenclature found in publications from the United States of America is the reverse of this.

![Diagram of a cyclic voltammogram](image)

**Figure 12:** Typical current response of a reversible redox reaction during the potential cycling between the first vertex potential $E_1$ and second vertex potential $E_2$ shows an oxidation peak at $E_{p,ox}$ and a reduction peak at $E_{p,red}$ with a peak separation of $57/n$ mV, where $n$ is the number of electrons exchanged in each redox reaction.

The CV in figure 12 is an example for a single redox reaction with an oxidation potential $E_{p,ox}$ and the reduction potential $E_{p,red}$ at a stationary electrode. Typical features that define the CV shape are the peak separation $\Delta E_{pp}$, the peak magnitude $I_p$, and the ratio of charging to Faradaic current.\(^{39}\) The variation of these quantities is studied by recording a series of CVs at differing scan rates.

The peak separation reveals information on the reversibility of the redox reaction. A constant peak separation at varying scan rates indicates a reversible redox reaction with fast electron transfer kinetics. Oxidation and reduction peak of a reversible redox couple are separated by a potential difference given by:
Equation 16:
\[ \Delta E_{pp} = \frac{2.218RT}{nF} \]

Where \( n \) is the number of electrons transferred per redox reaction, \( R \) is the ideal gas constant, \( T \) is the temperature and \( F \) is the Faraday constant. This means that the expected peak separation of a reversible redox process is \( 57/n \) mV at 298 K.\(^{[40]}\)

Conversely, a non-reversible redox reaction is characterised by a scan rate dependent peak separation.

The magnitude of the peak current contains information on analyte characteristics, such as concentration \( c_R \), and diffusion coefficient \( D_R \), according to the Randles-Sevcik equation:

Equation 17:
\[ i_{p,solution} = 0.4463nFAC_R \sqrt{\frac{nFD_R}{RT}} \]

The peak current magnitude is also determined by the electrode surface area \( A \) and the scan rate \( \nu \). A redox-active substance of known concentration can therefore be utilised to identify the effective surface area of an electrode, which might differ from the nominal value due to roughness, or because of a broken seal around the conductor.\(^{[41]}\)

The hysteresis between the current response to the forward scan (\( E_1 \) to \( E_2 \)) and to the reverse scan (\( E_2 \) to \( E_1 \)) would also arise in the absence of a redox-active substance and results from the capacitative charging of the electric double layer, as explained in 2.2.3. Applying faster scan rates increases the hysteresis and diminishes the peak visibility, which is a particular problem in experiments on electrodes with large surface areas.

Furthermore, a peak arises in the cyclic voltammogram if the turnover rate of a redox species is faster than its mass transport to the electrode. As a result, the current magnitude decreases after reaching the peak current, despite the continuously increasing overpotential. Comparatively fast turnover of redox-active species gives rise to concentration differences between the bulk solution and the vicinity of the electrode, which define the diffusion layer around the electrode. The thickness of the diffusion layer \( \delta_{diff} \) is proportional to the scan rate \( \nu \), according to:
Equation 18: \[ \delta_{\text{diff}} \propto \sqrt{\frac{DRT}{vF}} \]

The diffusion layer thickness is approximately equal to the proportionality expression at the time when the peak current is observed. However, an exact value for \( \delta_{\text{diff}} \) depends on the tolerance range of the bulk concentration, since the concentration in the diffusion layer, \( c_0 \), approaches the bulk solution concentration, \( c_{\text{bulk}} \), asymptotically.\(^{32,42,43}\) Figure 13 illustrates the change in \( c_0 \) relative to \( c_{\text{bulk}} \) of the reduced form (blue) and the oxidised form (red) of a redox-active species as the potential sweep progresses.

Figure 13: Concentration profiles of the reduced (blue) and the oxidised (red) form of a species as well as the change in diffusion layer thickness \( D_x \) at various stages (purple circles) of a CV measurement: A) Small amounts have been oxidised, the diffusion layer is thin and the current is limited by electron transfer kinetics; B) the concentration of reduced form is depleting within the diffusion layer and the current gradient diminishes until a maximum is reached, where the mass transport to the electrode limits the current; C) the diffusion layer extends into the solution to \( D_x \) and thereby decreases the concentration gradient between electrode and bulk solution; D) Reduction current replenishes the concentration of reduced form at the electrode and a new double layer will form as soon as the current passes through zero.

Positive current at stage A of the voltammogram indicates oxidation and depletes the reduced form of the species as the concentration of the oxidised form rises. The current gradient between A and B is determined by the electron transfer kinetics, since sufficient reduced species is present. Increasing the overpotential beyond stage B results in a diminishing current gradient as the mass transport to the electrode starts to limit the turnover rate. The peak current is therefore known as
the mass transport limited current, or the diffusion limited current, if all other types of mass transport are assumed as negligible. This is often the case with stationary macro-electrodes in background electrolyte. Past stage B the concentration differences at the electrode start to extend into the bulk solution, which means that the diffusion layer thickness increases and the concentration gradient between bulk and electrode surface decreases. As elucidated in 2.1 on the basis of Fick’s laws, the concentration gradient is the driving force for the flux of redox species to the electrode in a diffusion controlled system and charge can only flow as fast as redox species reach the electrode. Hence, the lower current at stage C is a result of a lower flux of oxidisable species from the bulk to the electrode. Stage D shows how the reduction reaction during the reverse scan replenishes the concentration of reduced species at the electrode again. A new double layer will form as soon as the following forward scan starts the oxidation anew and repeats the process.

This current response is usually observed in voltammetry experiments on stationary electrodes, if the diameter is in the magnitude of millimetres and larger and if the scan rate is in the range of mV s$^{-1}$ and faster. The occurrence of a peak is useful for the identification and examination of certain characteristics of a redox-active species. However, the analysis of solution or electrode kinetics requires the current limitation to be reached with a better time resolution and lower charging current. This can be achieved through the minimisation of the electrode surface area, or with faster mass transport to the electrode. The smaller surface of micro-electrodes creates a spherical diffusion profile with improved mass transport to the electrode and enhances the detection limit due to negligible capacitive charging.$^{[44,45]}$ Alternatively, rotating disk electrodes generate a defined laminar flow perpendicular to the electrode surface, which allows the turnover characteristics in solution to be investigated using the Koutecky-Levich relation.$^{[46-48]}$ Both types of electrode allow the investigation of diffusion coefficients with a better time resolution than with macro-electrodes.

2.2.11. Polarisation and power curves

Polarisation and power curves were measured within this work in order to compare the cost and machinability of the electrode materials against the expected power generation in a pMFC. In addition to this, the data from such measurements provides the platform for comparisons between MFCs with varying configurations. Key values for publications in the MFC field are therefore the open circuit potential (OCP), maximum power density and optimal external resistance (also called optimal load), which characterise the practical use of a MFC as power generator.$^{[1,49]}$ Polarisation curves were either measured following intentional modifications of the
system, or for sporadic examinations of the long-term development of exoelectrogenic biofilms in pMFCs.

A polarisation curve measurement involves connecting the MFC in series with a variable external resistance and measuring the potential generation across it in the range from open circuit to short circuit conditions. The external resistance is kept constant until the potential reading indicates steady state conditions, which means that the potential has to remain stable within a certain tolerance for a period of time. Changing the resistance is usually followed by an asymptotic approach of the potential towards the steady state value and the stabilisation time can vary strongly depending on the magnitude of the external resistance, the biofilm composition and the MFC volume.

The biological component of a MFC also limits the maximum stabilisation times, since the biofilm adapts and evolves according to the mitosis rate of the implemented organisms. Furthermore, polarisation curve measurements demonstrate an abnormal stress factor for an exoelectrogenic biofilm, which could cause fluctuations and impaired representativeness due to unusual selection. Dwelling at high resistances prevents the organisms from utilising the electrode as a final electron acceptor, while remaining at low resistances compared to the standard operating conditions could cause depletion of reduced metabolites. Thus, the tolerance range for the potential change over time has to account for representative steady state outputs on a short time scale. Whilst complicating polarisation curve measurements, the presence of a biofilm has also been shown to yield toxicological information about its environment.

Current and power values corresponding to the respective external resistance are normalised by the surface area of the electrode, which is in contact with the power generating organism. The resulting current and power densities are plotted against the corresponding potentials in polarisation and power curves and the maximum power density determines the optimal external resistance.

The optimal load roughly matches the internal resistance of a MFC and is therefore a valuable tool to assess the impact of changes to the MFC configuration. During the MFC operation the internal resistance will change due to the developing biological system. The external resistance has a major impact on the direction of the biological evolution. Conversely, the resulting biofilm composition and structure affects the optimal external resistance. Consequently, the optimal conditions for a BES are not fixed, but are continuously changing, which requires constant reconfirmation of maximum power generation and optimal external resistance.
2.2.12. Four terminal sensing

Four terminal sensing, also known as 4-point probe or Kelvin sensing, is used in 6.3.3.8 to investigate the resistivity of Ti$_2$AlC without the interference of parasitic resistance from the measurement setup itself. The parasitic resistance is composed of:

1) Resistance from leads and probes, $R_{\text{Probe}}$
2) Constriction and surface contamination resistance at the probe to sample interface, $R_{\text{Contact}}$
3) Spreading resistance due to the current flowing along various paths between the probes, $R_{\text{spread}}$

Ti$_2$AlC has previously been reported as an excellent electrical conductor.\textsuperscript{[58]} Four terminal sensing was employed within this work, since parasitic resistance would have caused a significant error in the determination of Ti$_2$AlC resistivity in this case.

Figure 14 shows the contribution of the various parasitic resistances to the sample resistance $R_{\text{Sample}}$ and the two 4-point probe setups that were used to minimise their interference with the measurement.

![Diagram](image)

**Figure 14:** a) Parasitic resistances $R_{\text{Probe}}$, $R_{\text{Contact}}$, and $R_{\text{Spread}}$ interfering with the determination of the sample resistance $R_{\text{Sample}}$; b) Standard 4-terminal sensing setup with the voltage measuring SE and RE in between the force current carrying WE and CE to minimise lead resistance; c) Specialised 4-terminal sensing setup for the application of an external higher resolution voltmeter with its leads V1 and V2.

The reduction of parasitic resistance contribution to the voltage reading is based on the separation of current carrying and voltage sensing electrodes. For this purpose the voltage is measured with the two inside probes (figure 14b). In the case of extraordinary low sample resistivity, the voltage magnitude could become so low that an external high sensitivity voltmeter would have to be used. This required the SE to be connected with the WE and the RE with the CE to free the voltage sensing probes for the external voltmeter as shown in figure 14c.
2.3. Green algae and cyanobacteria as electron donors

2.3.1. Extracellular electron transport

Electricity generation in MFCs relies on the proton gradient between anode and cathode as well as the ability of organisms to utilise the anode as a final electron acceptor for reduced metabolites. The most common final electron acceptor in metabolic pathways is oxygen due to its high electronegativity. However, microbial organisms existed before oxygen was present in the atmosphere and developed several mechanisms to transfer electrons to other extracellular electron acceptors instead. These processes are commonly known as exoelectrogenic activity. The transfer mechanisms have been preserved in anaerobic organisms and are also functional in aerobic organisms alongside oxygen reduction, for example in cyanobacteria and green algae. This is an indication that extracellular electron transfer still provides a competitive advantage in nature and additional purposes, such as cell to cell communication, are debated.

The cell to cell electron transfer could also serve to extend the range of electron transfer to a final electron acceptor. An additional possibility is that anaerobic organisms use the more efficient oxygen reduction of aerobic organisms in a symbiotic relationship through intercellular electron transfer.

Two major classes of exoelectrogenic activity have been confirmed; the long range electron transfer with cell produced mediators and the shorter range conduction of electrons through the cell network of the biofilm matrix. Both types of electron transfer occur between cells as well as between cells and metal-ion containing substrates.

Short range electron transfer is realised by both cytochromes in the cell membrane and by conductive protein filaments, which extend out of the cell as pili. Both types of short range electron transfer have been shown to have large electron transfer rates.

A study by Bonanni et al. revealed that the electron transfer between cytochromes and at the interface between cytochromes and electrode is orders of magnitude faster than the intracellular electron transfer from acetate to the cytochromes. Hence, their results suggested that the current generation of a biofilm is limited by intracellular redox reactions and not by the cytochrome electron conduction.

Extracellular electron conduction of pili is currently explained with two contrasting models, on the basis of the conflicting results on the cultures Shewanella oneidensis and Geobacter sulfurreducens. The pili conductivity of the former organism
was explained with electron hopping along a series of cytochromes, whilst the second model proposes delocalised charge transfer along polymers. Contacting a *Geobacter sulfurreducens* biofilm with two gold electrodes across a non-conductive gap of 50 μm indicated the high pili conductivity of 5 mS cm⁻¹. As a result, the pili of exoelectrogenic organisms are also called microbial nanowires.

In addition to the described short and long range electron transfer mechanisms, *Shewanella putrefaciens CN32* has also been reported to incorporate Fe(III) and Mn(IV) minerals as an oxidant supply. The release of these minerals after reduction inside the cell and re-oxidation at an electrode could demonstrate an additional form of naturally mediated electron transfer in a MFC.

Contrary to exoelectrogenic organisms, there are also cultures which accept electrons from an electrode to facilitate the reduction of a terminal electron acceptor. These cultures are called electrotrophs and may be employed in the cathodic compartment of a MFC to enhance the potential generation between anode and cathode. However, this study only utilised exoelectrogenic activity in air-cathode pMFCs, in order to simplify the cathodic compartment for minimal interferences during anodic investigations.

### 2.3.2. Nutrient solutions

Growth media for liquid culture as well as agar plates are prepared from complex mixtures of sources for nitrogen, phosphorous, carbon, sulphur, minerals and in the case of green algae; vitamins. Vitamins are added after the remaining media ingredients have been mixed and sterilised, since certain vitamins are heat sensitive and would be denatured under standard autoclaving conditions of 121 °C and elevated pressure.

As a result of the nutrient providing salts, the ionic strength of growth media is usually high and benefits electrochemical investigations, since the addition of background electrolyte becomes unnecessary. This minimises the stress response of organisms to the environmental differences between standard culturing conditions and experiment, which improves the representativeness of the results. As elaborated in 2.3.4, extreme changes in the environmental conditions usually cause longer lag phases, which are characterised by stagnating growth and alternated metabolic activity to accommodate the cultures adaptation.

The compositions of the growth media utilised in this work were adjusted to the requirements of certain organisms and optimised according to growth curves. Culturing media with such defined ingredients are called synthetic media and are subdivided according to the purpose of the culturing process into supportive,
enriched, selective, or differential media.\textsuperscript{[75]} The growth media used within this work are supportive media, since these were designed to sustain the growth of a multitude of different organisms with a plethora of nutrients. Adding special ingredients to supportive media to promote the growth of sensitive cultures creates an enriched media type. Conversely, selective media only contain the necessary nutrients to facilitate the growth of a certain group of organisms, which can also be used as a technique to decrease the contamination risk of the pure culture. A growth medium can be selective and enriched simultaneously. Differential media show visual differences between varying types of organisms and often contain a coloured substrate.

\textbf{2.3.3. Culturing conditions}

Optimal growth conditions for high algae growth rates have been developed for biofuel and MFC applications.\textsuperscript{[76,77]} However, using artificial pulsed LED lighting for 16 hours per day to enhance algae growth rates also increases the energy input costs for the MFC operation and complicates the evaluation of photo-responses in pMFCs. The improved growth rates under flashing light effect might increase the pMFC power output sufficiently to generate a positive energy balance and are important for the upscaling of pMFC technologies, but a simpler algae growth and pMFC operating regime is often preferred for fundamental research on the laboratory scale.\textsuperscript{[78]}

Within this work, the lighting conditions were therefore simplified to diurnal 12 hour light and dark cycles using a constant white light source, in order to imitate natural light conditions. Interrupting the light cycles with dark periods allows for the respiratory metabolism of photosynthetic cultures, which is elucidated in 2.3.7. Liquid cultures are grown under constant agitation on an orbital shaker to prevent cells from precipitating and starving each other of nutrients. Additionally, a small fraction of parent culture is usually re-inoculated in fresh media in monthly intervals to maintain exponential culture growth and prevent the accumulation of metabolic waste products, which can inhibit culture growth and even become toxic in higher concentrations.

\textbf{2.3.4. Growth curve}

Growth curves are a measure of microbial population statistics and were used for toxicological investigations and for the determination of inoculation periods within this work. A culture is inoculated under an investigated condition and the nutrients are not replenished for the duration of the growth curve measurement, which is referred to as a batch culture.\textsuperscript{[79]} A growth curve results from the plot of cell density
against incubation time of the batch culture, which is the reason why concentration changes as consequence of nutrient replenishment have to be avoided. This limits the duration of growth curve experiments, since the metabolic activity exhausts the nutrients over time and causes increasing concentrations of waste products. These concentration changes also affect the growth of the culture in addition to the investigated incubation condition. Consequently, growth curve studies are performed relative to a reference culture, which is incubated under equal conditions apart from the examined factor. Variation in the results between equally incubated cultures might still occur, since every cell division involves a chance for random mutation, and doubling rates in microbial cultures are high. It is therefore crucial to confirm results with multiple repeats of an incubation condition.

A standard growth curve comprises the sequence of lag, exponential, stationary and death phase, which is illustrated in figure 15.

![Growth curve](image)

**Figure 15**: Growth curve showing stagnant growth due to adaptation during the lag phase (I), balanced growth during the exponential phase (II), stagnant growth as a result of nutrient limitation throughout the stationary phase (III) and exponentially declining cell numbers in response to nutrient shortage and toxin buildup in the death phase (IV).

Cells adapt to the new environment by synthesising new components following the inoculation, which results in a stagnant cell density during the lag phase (I). The duration of the lag phase therefore depends on the necessary adaptations and might not be visible, if a culture is frequently reinoculated in the same growth medium. An adapted culture in the presence of abundant nutrients exhibits balanced growth in regular doubling intervals during the exponential phase (II), which results in the least variations between the cells of the culture. The maximum health and equality between cells is also the reason for maintaining this growth phase with regular reinoculations in fresh media during standard culturing. Exponential growth changes into the stationary phase (III) in response to nutrient
limitation, high cell density, or due to the buildup of toxic waste products. Each of these environmental stimuli can cause this transition alone and results in a stagnant population density. Cells usually express starvation proteins during the stationary phase, which improve the viability under adverse conditions. Ultimately, one of the factors that initiated the stationary phase, or several of them, result in the decline of the cell density in the death phase (IV).

2.3.5. Cell counting

Culture growth is either determined directly according to the number of cells in a defined volume, from biomass, or can be estimated from the chlorophyll content, if the examined culture is photosynthetic. The latter two methods require calibration with known standards and involve sources of error during the sample preparation steps as well as error from the shifts in cell size and chlorophyll content corresponding to culture age and health. Within this work, cell densities were assessed directly from the number of cells, which allowed examination of cell size and morphology simultaneously, using the two methods haemocytometry and flow cytometry.

2.3.5.1. Haemocytometry

Haemocytometry is the original method of cell counting; it utilises a microscope to count the cells in the defined volume of a counting chamber, which is usually subdivided into squares. The counting grid of an improved Neubauer haemocytometer is shown in figure 16.

![Improved Neubauer haemocytometer grid](image)

Figure 16: Improved Neubauer haemocytometer grid with the red 1 mm² square containing a volume of 100 nL, the green 0.0625 mm² square with 6.25 nL, the yellow 0.04 mm² square with 4 nL and the blue 0.0025 mm² square with 0.25 nL.
An improved Neubauer haemocytometer has two grids for multiple counts; each consists of guiding lines, which lead to a centre of 5x5 squares. The combined volume of the centre squares is 100 nL, so it is crucial to achieve a representative sample within a relatively small volume, which makes multiple repeats of counts necessary. Squares around the centre are used to confirm the representativeness of the assessed sample in the middle. Initial counts are necessary to determine the required dilutions, since usual culture densities would cause an amount of \(10^4\) to \(10^8\) cells within the 100 nL square. Thus, overlap of cells and cell death during long counting times are prevented by diluting the sample to an order of magnitude of \(10^2\) cells on the centre grid, which is the main source of error in this technique.

2.3.5.2. Flow cytometry

Flow cytometry is a high throughput technique with substantially reduced error compared to haemocytometry due to larger sample sizes in the order of magnitude of \(10^4\) to \(10^5\) cells per sample and with rapid quantification, which improves the representativeness of the acquired data.\[^{84,85}\]

A micro-capillary is used to create a focused solution stream through an array of lasers with varying wavelengths, which only allows a single cell to pass through the laser beam at a time. Cells scatter the laser light according to size and morphology, which redirects the light past the obscuration bar and allows it to be detected and quantified for each laser. The scattering is distinguished into forward and side scatter, where the forward scatter is proportional to the cell size, whilst the side scatter depends on cell morphology and internal cell complexity in addition to its size. All data derived from the scattering intensity is measured relative to a calibration with pure cultures prior to the investigations, whilst the number of scattering events yields an absolute value of cell density without calibration. The calibration is also used to improve the visibility of changes in cell characteristics by limiting the detection ranges and through the application of filters.

An additional advantage of flow cytometry for this work is the inherent chlorophyll fluorescence of photosynthetic cultures. This property allows further improvement of the measurement accuracy through the application of filters according to the fluorescence wavelength of chlorophyll a and b. Changes in chlorophyll fluorescence intensity were used for toxicological investigations and made the implementation of specific fluorescence dyes and their side effects unnecessary.\[^{86,87}\]
2.3.6. Photosynthesis

The utilisation of photosynthetic organisms in pMFCs enables CO$_2$ fixation at concurrent electricity generation without the need for constant addition of organic molecules as food, which greatly enhances the pMFCs application area and reduced its operation cost.\cite{88} Photoautotrophic organisms harvest light in antenna systems and use it as a driving force to synthesise their food through the reduction of CO$_2$ with electrons from an initial water splitting step. The protons from this water splitting reaction create the pH gradient across the membrane in a pMFC, which is ultimately the driving force for the electric current generation. MFCs can also be created with heterotrophic organisms; however, these cannot reduce CO$_2$ and therefore must be supplied with reduced carbon sources, such as carbohydrates. This requirement restricts the application area to places where there is a continuous flow of required nutrients, for example waste water streams, or involves the frequent addition of carbon sources. Alternatively, heterotrophs can be employed as power generating cultures in combination with photoautotrophs, which serve as food source in a light utilising MFC. Consequently, the implementation of photoautotrophic organisms extends the range of MFC applications and requires the analysis of photosynthetic pathways for the optimisation of MFC technologies.

The photosynthetic apparatus within a cell is located in the chloroplasts, where the CO$_2$ fixation is initiated before it proceeds into reactions in the cytosol and in the mitochondria. The interplay of the reactions of interest and their respective location in the organelles is illustrated in a schematic by Kruse et al., which is given in figure 17.\cite{89}
Figure 17: Photosynthesis and carbon fixation pathways adapted from Kruse et al.\(^\text{(89)}\) a) Photoautotrophic CO\(_2\) conversion; b) The standard photosynthetic process in the thylakoid membrane with light energy indicated as yellow lightning bolts in photo-system one (PSI) and two (PSII) leads to the linear pathway with the steps 1-3. Large NADPH concentration induces the cyclic electron transport around PSI in step 4, which allows continuing ATP production with reduction NADP\(^+\). Anaerobic conditions with simultaneously inhibited oxidative phosphorylation result in the hydrogen production in step 5. The Mehler reaction in step 6 is also a regulatory pathway in response to high light intensities or large NADPH concentration. Step 7 decreases the pH gradient across the thylakoid membrane, which can become too large at high light intensities. The steps 4-7 all represent a less efficient use of light to protect the cell viability.

The difference in power generation between light on and off conditions (photoresponse) is an important investigative tool in pMFC research and it is therefore important to distinguish between light dependent and non-light dependent metabolic reactions. The light driven reactions of the photosynthetic process take place in the thylakoid membrane of the chloroplast, where the photo-systems one
(PSI) and two (PSII) are located. Each of them is connected to an extensive network of antenna proteins, called light harvesting complex (LHC), which facilitate the light absorption and thereby provide the necessary energy to drive the photo-systems. The photosynthetic process actually starts in PSII with the splitting of water and continues after a series of redox reactions in PSI, where additional light energy is utilised to reduce ferredoxin (Fd).

All metabolic processes in the chloroplasts are based upon electron transport reactions between redox active species along the potential gradient. Exceptions are only the two photo-systems themselves, where light energy can be utilised to drive reduction reactions against the potential gradient. While the direction of the electron transport is mainly predetermined, the involved redox species and the metabolic pathway may vary due to the environmental conditions around the cell. This is the starting point for the controlled production of preferred anabolic species, such as biofuels, in technologies centred on photosynthetic organisms.

The oxidation of water to oxygen in PSII is used to sequentially reduce plastoquinone (PQ), cytochrome b$_6$f (Cytb$_6$f) complex, plastocyanin (PC), which is then oxidised in PSI. These redox reactions are coupled to the transport of protons across the thylakoid membrane, which creates a gradient from the thylakoid lumen to the cytosol. This gradient ultimately drives the synthesis of the metabolically vital molecule adenosine triphosphate (ATP). The comparatively high reductive potential of the reduced species in PSII is thereby used more effectively in a chain of redox processes between reaction partners with similar reduction and oxidation potentials. The most efficient use of energy is achieved with the sequential reduction of Fd in PSI and nicotinamide adenine dinucleotide phosphate from its oxidised form NADP$^+$ to NADPH, which is represented in the steps 1-3 in figure 17. The molecules ATP and NADH transport the energy obtained from the light and are used in the Calvin Cycle to bind CO$_2$ and to convert it into C$_3$ and C$_6$ sugars.

The alternative regulation steps, 4-7 in figure 17, result in a less efficient use of light energy, which affects the pMFC power output. In particular regulation responses following the creation of excessively large pH gradients under extreme light intensity have to be accounted for in the setup of experiments and in the engineering of pMFCs.

There is a limit to the useful light intensity for photosynthesis. However, the antenna proteins are capable of absorbing light beyond this limit and this can cause damage to the photosynthetic apparatus of the cell. The origin of the limit to useful light intensity is the dead time after a photon is absorbed by the system. In this period of 1 to 15 milliseconds the system resets itself before it is ready to assimilate the energy of another photon. However, the light harvesting through charge separation in the reaction centres occurs within pico- to nanosecond intervals.\[90\] As
a result, every photosynthetic organism absorbs excess energy, if the light intensity is greater than needed to drive photosynthesis at its maximum rate.

As described by Carvalho et al., this bio-photonic aspect sets certain challenges for the engineering of photo-bioreactors and suitable photosynthetic species.\[91]\] Irradiating a photosynthetic culture with a wavelength that is sufficient to excite the LHC results in differing photosynthetic rates according to the light intensity. This is shown qualitatively in figure 18.

All light energy in region I is utilised in photosynthesis and the photosynthetic rate is only limited by the compensation light intensity $I_C$ and the saturation light intensity $I_S$. At $I_C$ the photosynthetic uptake of carbon dioxide equals its respiratory output. The photosynthetic rate is often measured either in the overall growth of photosynthesis related biomass or in the changes of CO$_2$ and O$_2$ concentration. These values do not change at the compensation light intensity and the photosynthetic rate is therefore set as 0, although photosynthesis is taking place.

The regime of region I changes at the saturation light intensity $I_S$, which is sufficient to drive photosynthesis at its maximum rate. The magnitude of $I_S$ is temperature dependent, since it is determined by the rate of the involved chemical processes that reset the photosystems after the absorption of a photon. Further increase of the light intensity in regime II only results in the dissipation of excess light energy in the form of heat and fluorescence to protect the organism from damage.\[92]\]
An additional factor for the consideration of light utilisation in pMFCs is the mutual shading of cells in dense photosynthetic cultures. Cells with overdeveloped antenna systems cause the light intensity to drop rapidly with depth in the illuminated culture. This limits the effectively used volume in a device and with it the output of bioelectricity and biofuel. The light penetration depth can be increased with elevated light intensity within regime II, but results in photo-inhibition at larger light intensities, which is denoted with \( I_n \) in figure 18. Consequently, the photosynthetic rate of the culture diminishes in regime III, since the high light intensities damage the LHC by photo-oxidation, which causes cell death. Whilst quantum yield measurements of Mitra et al. indicate that the initial photon conversion efficiency is better than 82 \%, Carvalho et al. state that irradiating a culture with full sunlight may lead to a waste of 50-80 \% of the absorbed photons due to photo-inhibition of the culture layer close to the light source and mutual shading of the more distant layer.\[^{[93]}\] Hence, mixing of the culture with high flow rates and adjustment of cell density or reactor design is essential for the effective use of photosynthesis.

An alternative approach is to minimise the light absorption of individual cells with genetic engineering methods to truncate the size of the antenna system.\[^{[94]}\] A six to seven times greater photosynthetic productivity and a two to three times larger yield of oxygen production has already been reported for the green microalga species *Dunaliella salina* with truncated antenna systems.\[^{[95]}\]

It has been verified that *Chlorella vulgaris* can adapt to high light intensities and irradiation duration by decreasing its chlorophyll a content and increasing its \( \beta \)-carotene levels, which leads to greater growth rates under high irradiance conditions.\[^{[96]}\] This capability is favourable for the application in BES, since it allows employment of higher light intensities and consequently, larger pMFC volumes. *Chlorella vulgaris* has therefore been a frequently employed model organism for non-genetically modified green algae within this study.

According to the endosymbiotic theory, cyanobacteria are considered to be the prokaryotic predecessors of eukaryotic algae.\[^{[97]}\] They are also capable of oxygenic photosynthesis and represent a simpler structured model organism with higher growth rates than usually seen with microalgae.\[^{[98]}\] Hence, investigations were conducted on cyanobacteria in parallel to green algae.

A second point of interest for the technological utilisation of photosynthesis is the bypass pathway that leads to the formation of molecular hydrogen, shown as step 5 in figure 17. In this process hydrogenases catalyse the reduction of protons to molecular hydrogen using the electrons from the reduced ferredoxin, which decreases the light energy conversion efficiency, since this sequence of reactions excludes the formation of NADPH. Thus, hydrogen is only formed in an organism...
under environmental stress from an anaerobic and sulphur depleted environment after 10-15 hours.\textsuperscript{99,100} Oxygen acts as an inhibitor for the proton reducing hydrogenases, so the oxygen levels in the cell must be lowered before the hydrogen evolution becomes possible. Depriving the organism of sulphur reduces the activity of the oxygen evolving PSII, in order to promote the anaerobic conditions.\textsuperscript{101}

The required oxygen depletion of the system for the efficient and safe evolution of hydrogen presents another technical challenge for the setup of a carbon capture device. Additionally, oxygen is necessary for the constantly conducted respiratory metabolism of the organism, making its withdrawal over a longer time period lethal for the cell functions. Other metabolic pathways besides the reduction of NADP\textsuperscript+ compete with the hydrogen formation for electrons, namely the cyclic electron transport back into the electron transport chain and the reduction of O\textsubscript{2} to H\textsubscript{2}O (step 4 and 6 in figure 17).

Research on controlling photosynthetic pathways to access highly efficient synthesis of energy carriers with microbial organisms is crucial to the development trends of pMFC technologies. Comparatively high growth rates with correspondingly large metabolic turnover and simplicity of the genome make algae and cyanobacteria promising targets of this research.

\textbf{2.3.7. Respiration in photosynthetic organisms}

An essential aspect for the understanding of pMFC function and data evaluation is that photosynthetic organisms also perform light independent respiration.\textsuperscript{102} Although light and carbon dioxide can be considered as the fuels for photoautotrophic organisms and photosynthesis as the machinery to convert this fuel, the metabolism of the photosynthetic cell still needs to be sustained in times of low light intensity. The ATP production through respiration is continuously running in the organism, but with lower rate as the light intensity increases.\textsuperscript{103}

Respiration also involves the transport of electrons along the potential gradient in many redox steps to translocate protons through a membrane and drive the ATP synthase with the resulting proton gradient. The main difference with regard to this work is the electron source for this process. Whereas photosynthesis uses light energy to drive the endergonic oxidation reaction of water and eventually the production of reduced carbon compounds, respiration releases electrons into the electron transport chain through the exergonic oxidation of carbohydrates to CO\textsubscript{2}.\textsuperscript{104} Respiration and photosynthesis are particularly closely coupled in cyanobacteria, since these organisms use the same electron mediators in both processes. Moreover, the indications of an existing respiratory mechanism in the
photosynthetic organelles of unicellular photosynthetic organisms emphasises the importance of its consideration for pMFC applications.\textsuperscript{[105]}

### 2.3.8. Biofilm formation

The accumulation of free floating exoelectrogenic cells in dense biofilms on the electrodes of a MFC has been shown to improve power output.\textsuperscript{[106]} In addition to a higher density of power generating cells, biofilms allow direct electron transfer between organism and electrode without the necessity for electron mediators, which is elucidated in 2.3.1.\textsuperscript{[107,108]} Cells within a biofilm have less access to dissolved nutrients than in free floating state\textsuperscript{[6]}. However, a microbial community organised in a biofilm has increased resistance to harmful chemicals and improved viability in extreme temperatures or under harsh shear flow. Thus, the MFC operating conditions have to be adjusted to favour biofilm formation despite this disadvantage, which requires adverse conditions, or the necessity for symbiosis at close distances.\textsuperscript{[109,110]} Furthermore, the electrode surface hydrophobicity and roughness become crucial factors for cell adhesion and therefore also for the development of MFC specific electrodes.\textsuperscript{[111]} Electrode surface qualities have even proven to be a useful tool for the selective formation of exoelectrogenic biofilms. Functionalisation of electrode surfaces with positively charged aryl diazonium salts was shown to enhance MFC power output in this manner.\textsuperscript{[112]}

The cell adhesion to a surface is assisted by an initial preconditioning step, where cell debris and other ambient molecules form a film on the surface. Cells can adsorb to this substratum, but readily detach again until a minimum number of cells adsorb within a certain area at the same time. This is detected by each single cell by means of intercellular communication based on secreted signalling molecules, referred to as quorum sensing.\textsuperscript{[113]} High concentrations of these signalling molecules initiate the synthesis of extracellular polymeric substances (EPS), which improve the adhesion conditions, while the cells change to a flatter shape by spreading out on the surface. The EPS matrix consists of non-conductive proteins, glycoproteins, glycolipids, polysaccharides and DNA, which can be incorporated by neighbouring cells and causes an enhanced gene transfer across the biofilm. Adaptation processes are therefore equally as enhanced as the resistance of cells to adverse environmental conditions.

### 2.3.9. Photo-response measurements

The current or voltage generation in pMFCs under differing light conditions is a common characterisation method to gain insight into the exoelectrogenic activity of a photosynthetic organism.\textsuperscript{[114]} It can be measured continuously across the external
resistance, without disturbing the system as a result of the measurement itself. Measuring the photo-response in combination with defined modifications to the environment of the photosynthetic organism can help to identify supporting or detrimental factors in the operation method. However, the consecutive determination of possible light catalysed reactions of each pMFC component is necessary before the photo-response of the employed culture can be analysed.

2.4. Microbial Fuel Cell Engineering

2.4.1. Function and configurations of photo-microbial fuel cells

All MFCs can either be operated in continuous mode with a constant flow between MFC chamber and a reservoir, or in batch mode with occasional modifications to an otherwise unchanged solution, which is contained within the MFC. Continuous mode operation requires more energy input, due to pumping and maintenance of stock solutions, but has been shown to improve power output compared to batch mode through optimisation of nutrient replenishment and removal of toxic side products.\cite{115}

Maximisation of the light exposed surface area and the necessity to maintain the light intensity as consistent as possible throughout the culture volume results in flat and thin pMFC geometries.\cite{116} This is a fundamental difference between pMFC design and MFC design, since the latter employ non-photosynthetic, heterotrophic electron donors and are therefore focussed on configurations with minimal mass transport distances and maximum electrode surfaces areas.\cite{117} The studies in this work exclusively utilised pMFCs, in order to benefit from the advantages entailing the implementation of photoautotrophic electron donors, as elucidated in 2.3.6. Consequently, the numerous adaptations of the versatile MFC technology for heterotrophs are not described here.

Figure 19 shows the essential processes during the light operation of a pMFC on an air-cathode pMFC design, which has also been used for the investigations within this study.
Figure 19: Air-cathode pMFC operating principle in light: I) water splitting by photosynthetic organisms releases electrons (reduced metabolites) and protons (lowers pH); II) direct electron transfer to the anode; III) artificial electron mediators assist in electron transfer to the electrode; IVa) increasing pH gradient causes diffusion of protons through the proton exchange membrane (PEM), which corresponds to a flow of positive charge into the cathodic compartment; IVb) the flow of positive charge is the driving force for electric current through an external resistance; V) electrons reduce oxygen to water in the presence of protons.

As elaborated in 2.3.6, the splitting of water during the photosynthetic process (denoted as I) releases protons (H\(^{+}\)) and reduced metabolites (shown as electrons e\(^{-}\)) from the cell (represented by an algae cell). Equally, protons and electrons are released as a result of the respiratory metabolism in the dark, but from the oxidation of carbohydrates instead of water.

Exoelectrogenic organisms in the anodic compartment can inject electrons either directly into the anode (II), or artificial electron mediators are employed to enhance the transport of electrons (III).\(^{[118]}\) Direct electron transfer can occur via one or multiple of the mechanisms, which were described in 2.3.1. Alternatively, reversible redox systems are employed as electron mediators and are represented here by the redox mediator couple ferricyanide (Fe\(^{3+}\)) and ferrocyanide (Fe\(^{2+}\)). However, the use of artificial redox mediators has been largely abandoned due to the low efficiency of mediated electron transfer, the requirement of regular mediator addition and environmental concerns about the large scale use of these substances. Mediated electron transfer is therefore only used as an investigative tool.\(^{[119]}\)

In an air-cathode pMFC design only the anodic compartment contains a liquid phase. Hence, the anodic and cathodic compartments have to be separated by a watertight, but proton permeable membrane.\(^{[115]}\) The pH decline in the anodic
compartment creates a proton gradient to the cathodic compartment and acts as
the driving force for the diffusion of protons through the proton exchange
membrane (PEM), denoted as process IVA in figure 19. This is equivalent to the flow
of positive charge into the cathodic compartment, which cannot be compensated
with anions from the anodic compartment due to the selective membrane. Charge
neutrality is therefore the driving force for the flow of electrons through the
external electricity consumer, which is illustrated as a simple resistance (IVb). The
proton gradient across the PEM is maintained by the recombination of protons with
oxygen and electrons to water at the cathode (V), while further protons are
constantly released into the anodic compartment by the microbial metabolism.

The air-cathode design represents one of the simplest pMFCs, which is beneficial
for fundamental investigations into electrode materials as well as organisms in the
anodic compartment. Material and operation expenditures are minimal in this
design and allow for cost-effective bulk production of pMFCs, which makes it
suitable for screening of cultures and further improves the confidence in results
with an increasing number of replicas. Liumstra et al. demonstrated a practical air-
cathode pMFC design for the investigation on exoelectrogenic activity, which
employed an anode chamber for US$0.60 and a cathode assembly for $60.00.\textsuperscript{120} Such low costs for the anodic compartment were achieved by using an inexpensive
coating on the chamber base as anode, which consisted of a mixture of polypyrrole
and conductive carbon adhesive with a carbon fibre lead. The air-cathode consisted
of an Ultrex membrane pressed onto platinated carbon fibre cloth with a titanium
ring contact, which leaves room for further improvements on cost efficiency,
considering that the titanium ring constituted more than a third of the cathode
price.

Higher performance orientated pMFC designs employ electrotrophs in an additional
liquid compartment on the cathodic side to enhance the pMFC potential
generation.\textsuperscript{121} Phototrophic oxygen generation can also be used to support the
cathodic reaction, since the respiration of heterotrophic bacteria would otherwise
deplete the oxygen in the cathodic compartment, or require an additional energy
investment for artificial oxygenation. However, the phototrophs only oxygenate the
solution during illumination, while the anodic reaction is continuously running and
requires a high oxygen concentration at the cathode at all times. Cathodic
applications of phototrophs are therefore rather supplementary to the utilisation of
electrotrophs and do not avoid oxygen limitation as well as air-cathodes.

Photosynthetic carbohydrate generation can assist the utilisation of heterotrophs
on each electrode as food source.\textsuperscript{122,123} In this setup the phototrophs are grown in
an external chemostat and are only pumped into the MFC during short feeding
periods, which means that the actual MFC geometry does not need to be optimised
Theory

for high light intensities within the electrolyte. Furthermore, the separation of photosynthetic culture and electrode compartment allows the MFC to be operated under anaerobic conditions, which expands the selection of exoelectrogenic organisms and is a requirement for microbial hydrogen production. Such a configuration is therefore promising for high revenues, but also requires careful control of growth rates and solution composition, which is not ideal for fundamental investigations.

It is crucial for the electricity generation in a MFC that the charge imbalance created by the proton release in the anode chamber is not compensated by the movement of any other positively charged species to the cathode. Only protons recombine with electrons and oxygen to water at the cathode; however, cations such as $K^+$, $Na^+$, $Ca^{2+}$ and $Mg^{2+}$ are always present in biological systems to maintain metabolic activity. Restoration of charge neutrality by the movement of these species to the cathode would not support the reduction reaction and therefore diminish the driving force for electron flow through the external circuit. Furthermore, the accumulation of protons in the anode chamber causes acidification, which further decreases MFC power generation as a result of biocatalyst deactivation and denaturation. Consequently, membranes are used to restrict the mass transport between anode and cathode chamber and are more effective the higher their selectivity for protons is. Furthermore, the use of separators allows operation of the anode and cathode in different, reaction optimised environments.

A drawback of using separators is that the mass transport of protons is limited by slow diffusion through the membrane and that other cationic species can also compete with the protons for the anionic functional groups within the membrane. Blocking of these functional groups with competing cations makes them unavailable for proton conduction and further slows the mass transport of protons into the cathode chamber. Although membranes are often used to increase pMFC power output, they are not necessary, if the mass transport is slow compared to the distance between the electrodes. A good example of this is the sediment pMFC design, which is utilised with microbial organisms as well as with vascular plants. These pMFCs generate electric potential between photosynthetic organisms on the anode above the sediment and heterotrophic bacteria in the sediment. Thus, this technology benefits from low material costs and simple handling, since photosynthetic organisms are used in their natural environments. Sediment pMFCs are therefore attractive for decentralised use in private households and in growing areas of aquatic crops such as rice fields. The power generation from sediment pMFCs is comparatively low and the nature of this technology does not allow modifications to the operational conditions. Hence, such pMFCs are rather a tool for on-site energy recovery rather than for investigations on electrode materials and exoelectrogenic organisms.
2 Theory

2.4.2. Replica method for porous ceramic electrodes

This work investigated macroporous ceramic anodes with reticulated geometry in pMFCs to assess whether biofilm formation on the electrode could be promoted by offering an electrode as a beneficial growth substrate. The intricate structure reduced the shear flow intensity within the electrode and ensured that the pMFC volume was optimally used with a large electrode surface area. Large pores were preferred to micropores in order to avoid clogging from the growing biofilm, since this would have caused the nutrient starvation of organisms in the electrode interior. Consequently, a biomimetic electrode shape inspired by coral shapes was chosen to facilitate biofilm growth on the electrode in strong shear flow conditions.\textsuperscript{[130]}

The difficulties of machining metal electrodes in such shapes were avoided by using cellular ceramic electrodes, fabricated through the replica method. Ceramic shapes are more adaptable, since the viscous ceramic precursor, called ceramic slip, enables the use of templates and gas releasing chemical reactions to determine the geometry after the sintering. Studart et al. merged the numerous processing options for cellular ceramics into three method categories; replica, sacrificial and direct foaming, as shown in figure 20.
The replica method provides the most open structures and was therefore chosen as the processing route for biofilm substrates. This technique utilises a template with the desired shape, which is coated in ceramic slip and is ultimately thermally decomposed during sintering. For this reason, the template should decompose slowly at comparatively low temperatures during the sintering, which allows gaseous products to escape before the ceramic is sintered. Polyurethane foam was employed to produce macroporous ceramics in this study, since it is fully inert at room temperature and decomposes to carbon monoxide and small amounts of nitrous oxides as well as hydrogen cyanide from 110 to 170 °C. Sintering of ceramics is always preceded by a setting/drying step of the ceramic slip to prevent excessive evaporation from destroying the unsintered ceramic structure. Accordingly, the setting temperature has to be below the thermal composition temperature of the template.
A drawback of the replica method is the low mechanical strength of the resulting ceramics as a result of cracks, which form during the thermal decomposition of the template. This problem is addressed with thicker coatings, which requires fine tuning of the slip viscosity, since a uniform strut coating becomes more difficult as slip viscosity and sample volumes increase.\textsuperscript{[134]} Low slip viscosity creates a uniform, but thin coating that results in a highly fractured structure, while a highly viscous slip can clog pores and does not penetrate well into the template volume before it sets, as shown by ceramics resulting from slips with increasing viscosity from figure 21a to c.

![Figure 21](image)

**Figure 21**: Optical photographs of cellular Ti$_2$AlC ceramics resulting from slips with decreasing water content from a to c. The correspondingly higher slip viscosity improved mechanical strength of the ceramic; however, increasingly viscous slips penetrated less into the template volume before setting, which caused the clogging of the exterior pores and low coating thickness around the interior struts. Biofilms growing inside an electrode, as shown in c, would have suffered from nutrient starvation and insufficient removal of dead cell material, followed by toxin buildup.

The clogging of the template pores decreases the expected pMFC power generation, since it reduces electrode surface area, light penetration into the electrode and mass transport through the pMFC. Consequently, biofilm formation inside an electrode structure, such as the one shown in figure 21c, would be limited by low replenishment of nutrients and the accumulation of toxins.

A successful approach to achieve thicker and more uniform coatings is the application of multiple coating steps with gradually decreasing slip viscosities. The initial slip viscosity for a multiple coating procedure is lower than in a process with a single coating step and only serves to aid the enhanced retention of the following coatings. Such a procedure involves fine tuning of the slip viscosity of each coating step and requires a setting period following each coating, but it has been shown to result in improved mechanical strength and allows the pore diameter to be controlled by the number of coatings.\textsuperscript{[135]}

Alternative processing routes for cellular ceramics are the sacrificial template and direct foaming method. The sacrificial template method creates a ceramic imprint of the template, which distinguishes it from the positive template morphology that is achieved with the replica method. As a result, ceramics with pore diameters
below a micrometer become accessible and the issue of insufficient template impregnation is resolved by submersing the template in the slip container before setting. However, both of these advantages are not beneficial for the desired open and macroporous structures of pMFC anodes. The same reasoning resulted in the dismissal of the direct foaming method, where pores are formed by means of gas releasing chemical reactions before the ceramic slip sets. Direct foaming does not use a template that could damage the ceramic during its thermal decomposition and similar pore sizes are possible throughout the whole sample, irrespective of its size. However, the disadvantage stems from the necessity for the bubbles to be stabilised before the ceramic sets, which is often achieved with surfactants and becomes increasingly difficult the larger the desired pore diameter is.

2.4.3. TiO$_2$ and Ti$_2$AlC ceramic

The replica method was applied to the non-conductive TiO$_2$ ceramic and to the inherently conductive Ti$_2$AlC ceramic for the pMFC studies in this work. Optimisation of the slip composition and sintering conditions for TiO$_2$ ceramic has already been investigated by Thorne et al., who used the light reflective properties of TiO$_2$ particles to enhance light penetration into the electrode. Their results showed that the replica method can be employed as a useful tool to yield reticulated electrodes with defined porosity, although the TiO$_2$ ceramics required coating in conductive fluorine doped tin oxide by chemical vapour deposition to become conductive.

Ti$_2$AlC ceramic was therefore chosen as the main focus in this work to develop the research on macroporous ceramic electrodes further. It is part of a whole group of conductive ceramics, first discovered in powder form by Nowotny et al. in 1971, and later called MAX phases by Barsoum et al., where M stands for an early transition metal, A for an A-group metal and X for either carbon or nitrogen. The stoichiometry of MAX phases follows the general formula $M_{n+1}AX_{\text{iv}}$ as illustrated in figure 22.
Figure 22: Stoichiometry and composition of known MAX phase ceramics.

Ti$_2$AlC ceramic is part of the sub-group of 211-MAX phase ceramics and is the thermodynamically most stable compound amongst the Ti-Al-C systems.\cite{139} In the broader field of MAX phases it is amongst the lightest and most corrosion resistant ceramics.\cite{140} Additionally, the Ti$_2$AlC ceramic is suitable for self-propagating high temperature synthesis, due to the exothermic reactions of titanium with carbon and aluminium, which ensures high turnover efficiency and reduces the energy costs associated with sintering.\cite{141}

However, sintering of Ti$_2$AlC has to be conducted in the absence of oxygen, since the ceramic would otherwise decompose to form the thermodynamically more stable metal oxides and carbon monoxide, as described by Wang et al.\cite{142}

Equation 19:

$$4 \text{Ti}_2\text{AlC} + 13 \text{O}_2 \xrightarrow{T \geq 1000 \degree C} \text{8TiO}_2 + 2\text{Al}_2\text{O}_3 + 4\text{CO (g)}$$

The sintering is therefore performed in an argon stream to continuously remove oxygen, whilst the argon flow rate is kept low to retain heat within the oven.

Dwelling time in certain temperature ranges and ceramic slip composition determine the content of Ti$_2$AlC phase in the final ceramic.\cite{143} The Ti$_2$AlC synthesis requires the formation of the precursors TiC and TiAl$_3$ at lower temperatures, but has to avoid the competing reactions at higher temperatures, as depicted by the simplified reactions in figure 23.
Figure 23: Ti$_2$AlC synthesis requires temperatures of 1200 to 1300 °C and the formation of the precursors TiC and TiAl$_3$ at lower temperatures for a complete conversion. The Ti$_2$AlC phase yield can be reduced by competing reactions at temperatures above 1350 °C, increasing contents of carbon in the ceramic slip, or insufficient formation of TiAl at higher temperatures.

The general reaction equation for the synthesis of Ti$_2$AlC ceramic is therefore:

Equation 20:

$$(2 + X)\text{Ti} + (1 + X)\text{Al} + C \xrightarrow{T \geq 1200 \degree C} X\text{TiAl} + \text{Ti}_2\text{AlC}$$

The precursors TiC and TiAl$_3$ readily form at comparatively low temperatures, but the abundance and spatial distribution are determined by the relative contents of the elements in the ceramic slip$^{[144]}$. TiAl$_3$ reacts with additional Ti to TiAl at higher temperatures, which then reacts with TiC to form the Ti$_2$AlC ceramic. The optimal temperature range for a complete conversion to Ti$_2$AlC ceramic is 1200 to 1300 °C; however, competing reactions also take place at these temperatures and produce impurities in the ceramic. High concentrations of carbon in the ceramic slip can result in a high TiC content, which then forms Ti$_3$AlC$_2$ with TiAl instead of the 211 MAX phase$^{[145]}$. Additionally, unreacted carbon converts Ti$_2$AlC to Ti$_3$AlC$_2$.$^{[146]}$ Both reactions become dominating at temperatures higher than 1350 °C. Thus, the carbon content in the ceramic slip and the sintering temperature are limited to the required levels.

2.4.4. Evaluation of the microbial fuel cell efficiency

Certain parameters have been established to assess the power generation efficiency of a microbial fuel and the purpose of a MFC usually determines which of
the parameters is used to describe the system. All performance parameters depend on the critical factors: substrate availability to the employed organisms, the substrate turnover rate of the organism, the coulombic efficiency and the internal resistance of the MFC.

Substrate availability is commonly ensured during laboratory scale experiments and is first investigated in field tests following the identification of all other factors. The substrate turnover is crucial to the power generation; however, it cannot be used as a simple parameter to describe the pMFC efficiency, since it is subjected to the complex interplay of the various metabolic pathways in an organism.

The coulombic efficiency is the ratio of charge that reaches the external circuit to the charge that would be obtained from the complete oxidation of the substrate. It is therefore a more general parameter than the substrate turnover and allows assessment of a system without the necessity to understand all of the involved metabolic processes. Coulombic efficiency is essentially the efficiency of charge recovery from the substrate and represents the ideal utilisation of the substrate with a value of 100 %. Assuming a non-limiting cathode reaction, a coulombic efficiency below 100 % can result from both the incomplete oxidation of the substrate due to anabolic metabolism and the oxidation of the substrate or electron mediating molecules by electron acceptors in the anolyte. The current through the external circuit can also be limited by ion transport through the separator membrane and processes in the cathodic compartment, such as oxygen and mass transport limitation, which are elucidated on the basis of the various pMFC configurations in 2.4.1.

Coulombic efficiency has developed into the standard assessment value for the efficiency of a MFC, since its generality allows the comparison between different types of MFCs, independent of the employed organisms, materials or configurations. However, such a generalisation also makes this parameter less useful for the analysis of specific MFC characteristics, including the identification of improvements and drawbacks of a certain configuration. For this reason, several more parameters are still reported to focus on the advances in particular application areas.

Limitations to the power generation are summarised in the internal resistance of a MFC. Consequently, internal resistance contributes to the coulombic efficiency and it is a parameter that is often used for the problem analysis of a MFC configuration. In addition to the incomplete utilisation of a substrate for electricity generation, it also comprises ohmic and activation losses, retarded mass transfer, and concentration polarisation. Ohmic losses arise from electrical resistance and poor contacts, whereas activation losses result from the energy waste involved in
charge transfer between electron donor and acceptor. Activation losses can be minimised by employing catalysts and by reducing the mass transfer resistance. Insufficient mass transfer results in concentration polarisation, if the turnover rate cannot be met with adequate substrate supply. This leads to the formation of overpotentials, which increases the required energy for charge transfer and therefore causes elevated activation losses. Such overpotentials are also called activation overpotentials. Reasons for hindered mass transfer in MFCs are often the substrate and electron mediator transport through dense biofilms as well as the slow and unspecific ion-conduction through separator membranes. Analysis of the internal resistance not only helps to improve the MFC configuration, it also assists in the identification of the optimal external resistance, which is the resistance of the external energy consumer that facilitates the maximum power generation of the MFC.

An alternative assessment parameter to the coulombic efficiency is the energy efficiency, which is defined as the ratio of power produced by the MFC to the heat energy that could be obtained from the combustion of the added substrates. Energy efficiency therefore relates the output of the MFC to the simplest and often least energy efficient method of utilising resources. It is a practically orientated parameter that compares MFC technologies to alternative energy recovery methods. Additionally, it simplifies the consideration of the energy investment into fabrication and operation of the various systems, in order to estimate the expected revenue. However, it should be noted that energy efficiency does not consider any purpose of a technology beyond the energy generation, for example CO₂ capture, wastewater treatment and reduction of harmful emissions in the case of MFC systems.

MFCs for bio-electricity generation are evaluated on the basis of current and power density. These parameters are closer to the actual raw data that originates from the measurement of potential generation across a known external resistance. They therefore contain smaller error and less assumptions about the processes within the MFC. The resulting current and power calculations are normalised to the anode surface area, or in special cases to either the cathode surface area, or MFC volume to allow comparison between different MFCs. Anode surface area is the common basis for the current and power normalisation as the biological processes on the anode are required, whereas the cathode can have multiple configurations, as elaborated in 2.4.1. Normalisation per reactor volume is performed if the anode surface area is difficult to determine without large errors, for example when granules are used. Power generation is normalised per cathode area, in case the cathodic processes are assumed to be limiting the MFC output, or in general in cathode research. Current and power density are insightful efficiency
parameters, if electrodes are investigated in similar MFC configurations and are therefore mainly used to assess the efficiency of pMFCs in this work.

2.4.5. Metal-organic frameworks for \( \text{CO}_2 \) capture

This work investigated the metal-organic framework (MOF) MIL101 and its amine modification MIL-101(Cr)-\( \text{NH}_2 \) regarding their potential for application in pMFCs.\[^{[156]}\] MOFs are mesoporous modular structures composed of metal containing secondary building units, which are connected with organic linkers. These organic linkers are the fundamental difference to zeolites and enable MOFs with much larger pore size and enhanced diffusion characteristics.\[^{[157]}\] The reticulated cage structure of MOFs exhibits promising properties for gas storage, catalysis, filtering, molecular recognition for biomedical imaging and electron as well as ion conduction.\[^{[158]}\] The \( \text{CO}_2 \) capture capacity of MOFs in particular presents considerable opportunities for pMFC technologies. An increase in the available \( \text{CO}_2 \) concentration causes larger growth rates of photosynthetic organisms to a certain extent.\[^{[159]}\] Utilising MOFs to enrich pMFC cultures with \( \text{CO}_2 \) from the air would therefore correspond to higher carbon capture efficiency.

The broad scope of MOF application is based on the flexibility in selective functionalisation on either the organic linkers or the unsaturated metal sites. MOFs are therefore also promising for the surface modification of electrodes to yield hierarchical structures with large surface area as well as more efficient exchange of substrates and electrons at the interface to biofilms. Organic linkers can be modified before or post-synthesis, and metal sites can act as Lewis acids for grafting routes following the MOF synthesis. The organic component of MOFs results in a low thermal stability and usually restricts the applications to a maximum of 400 °C.

MFCs have to operate at much lower temperatures to sustain the life of the microbial organisms, so implementation of a MOF is more favourable than using zeolites. A greater hindrance for the application of MOFs in pMFCs is their inherent sensitivity to hydrolysis from water, which collapses the MOF into its amorphous phase.\[^{[160]}\] Furthermore, a life cycle analysis has found that the solvent intensive solvothermal synthesis of MOFs is environmentally questionable and uneconomical.\[^{[161]}\] MOFs usually have to be heated to desorb the captured \( \text{CO}_2 \) molecules and this energy input is equivalent to the release of additional \( \text{CO}_2 \) into the environment. Thus, the implementation of MOFs potentially reduces the sustainability of pMFCs, considering that \( \text{CO}_2 \) capture and reduction is naturally performed by photosynthetic organisms.

However, the combination of MOFs with heat sensitive microorganisms in pMFCs means that the captured \( \text{CO}_2 \) has to be made available to the culture without the
requirement to heat the MOF. Photosynthetic cultures can fix CO\textsubscript{2} from various sources, including dissolved carbonates, so it is possible that these organisms could act as catalysts for the regeneration of MOFs.\textsuperscript{[162]} This would result in an improved life cycle impact of both MOF and pMFC technologies. MOF research is a young and emerging field with countless unexplored MOF modifications. Life cycle analysis at this stage is useful to highlight problem areas for future research, but synthesis and operation protocols have not been optimised for economic feasibility so far.

### 2.5. Supporting analysis techniques

#### 2.5.1. Atomic force microscopy

Atomic force microscopy (AFM) is a scanning probe technique for the investigation of topography and electrostatic charge on the surface. It has mainly been used for the examination of electrode surface roughness within this work, which is crucial to the biofilm formation in turbulent flow, as described in 2.3.8.

The sample is scanned with a tip that is mounted on a cantilever spring and moved across the surface with a piezo actuator, which is capable of tip displacements in the range of 1 Å to 100 μm.\textsuperscript{[163]} Tips come in conical, pyramidal and tetragonal shape and are usually fabricated from silicon or silicon-nitride with optional metal coatings for investigations on conductivity and magnetism. Crucial tip characteristics are the radius of tip curvature and the aspect ratio, which is the ratio of tip height to tip width.\textsuperscript{[164]} The radius of curvature is usually smaller than 10 nm and can be further enhanced by functionalising the tip with a small molecule to measure single atoms.\textsuperscript{[165,166]}

A topographic image is created from the tip position on the sample in relation to the cantilever deflection, which results from the Born repulsion between the electron orbitals of the tip and the sample. Electrostatic charges and additional material dependent forces between the tip and sample also contribute to the cantilever deflection.\textsuperscript{[167]} The cantilever deflection is usually measured with the optical lever method, where a laser is focused on the cantilever side opposite the tip. A photodiode array measures the position of the reflected laser beam with increasing sensitivity to displacement as the distance to the cantilever becomes longer. Coatings on the backside of the cantilever are sometimes used to enhance its reflectivity, if the measuring medium impairs the sensitivity by diminishing the laser light intensity.
Image quality is critical for the data evaluation, since most calculations of surface characteristics are performed on the topography map. The minimisation of sources for imaging artefacts is therefore even more important for the reliability of the results than the tip quality. Most artefacts can be avoided with regular calibration and adjustment of scan speed, setpoint and signal amplification according to the sample properties.\textsuperscript{168}

### 2.5.2. Scanning electron microscopy

Electrode material analysis within this work was supplemented with high resolution imaging and elemental analysis using scanning electron microscopy (SEM). The probe in this technique is a focused electron beam, usually with a diameter of 10 to 20 nm that results in higher resolution images of surface features than achievable with light microscopy. Contrary to AFM, the probe penetrates into the sample and therefore allows information to be acquired on its elemental and phase composition, which can also be used to enhance image contrast.

The main experimental factors crucial to the image quality are sample preparation, electron acceleration voltage, spot size and detector position. The acceleration voltage is proportional to the penetration depth of the electrons into the sample, where these are scattered due to elastic and inelastic collisions.\textsuperscript{169} The fraction of elastic to inelastic collisions depends on the atomic number $Z$ of the sample material and determines the shape as well as the size of the electron beam interaction volume. Collisions with atoms that have low $Z$ values result in greater penetration depths and are mainly inelastic. The loss in energy causes the electrons to form a pear shaped interaction volume, in the size region of micrometers. Increasing the acceleration voltage would only increase the penetration depth for a given $Z$ and not the interaction volume. The elasticity of collisions increases with $Z$, resulting in larger scattering angles and smaller, more spherical interaction volumes. Elemental and phase composition of a sample therefore have a strong influence on the energy and scattering angle of the reflected electrons and compositional analysis becomes more reliable the larger the differences in $Z$ are.

Two types of reflected electrons are distinguished and require separate detectors, the backscattered electrons with a kinetic energy higher than 50 eV and the secondary electrons with lower energy.\textsuperscript{170} The high energy backscattered electrons result from elastic collisions with deflection angles larger than 90° and are measured for the compositional analysis, while secondary electrons have lost kinetic energy as a result of inelastic collision and are detected at lower deflection angles for imaging. Consequently, the fraction and energy of backscattered electrons increases with the atomic number of a sample, which aids the
compositional analysis. In practice, high acceleration voltage and large spot size are employed to increase the count of backscattered electrons and reduce error in the compositional analysis. However, these settings are detrimental for the image resolution, since they increase the probe size. Backscattered electrons also originate from a larger interaction volume than the secondary electrons measured with the same acceleration voltage and spot size, which further diminishes usefulness of backscattered electrons for imaging. High resolution image acquisition is therefore performed separately from the compositional analysis at low acceleration voltage and small spot size to minimise the probe size and the interaction volume. In certain cases, where phase contrast is preferred to resolution, backscattered electrons are used for imaging as well. Phase contrast is also called Z-contrast, since the atomic number determines the energy difference between electrons reflected from different phases. The phase contrast is improved with the same methods as the compositional analysis. Thus, a compromise between resolution and phase visualisation is necessary when such measurements are performed.

Interference from charging and radiation damage is an additional problem for the imaging of less conductive samples, especially when biological specimen are investigated. The sputter coating of several nanometres of gold, palladium, chromium or platinum becomes necessary, if the sample is not sufficiently conductive to discharge into the sample stub. Biological samples also have to be chemically fixed with cross-linking agents and dehydrated before the metal coating, since the water would otherwise evaporate under the high vacuum conditions of the measurement and scatter the electron beam. These requirements reduce the data representativeness of the sample in its natural form and make the in situ observation of biological processes impossible. However, environmental scanning electron microscopy (ESEM) has been developed as an SEM adaption without the need for most sample preparation steps, in order to overcome these problems. The high vacuum is only maintained around the electron beam for most of the beam path through a series of pressure limiting apertures and differential pumping, while the sample is kept in low vacuum. Specimens in ESEM are therefore imaged closer to the electron source to reduce the scattering from gas molecules. Such a technology presents a promising combination of the benefits from electron microscopy and in situ biofilm imaging and can help in future investigations of exoelectrogenic activity of biofilms on electrodes.
2.6. Equations in theory chapter

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58. Scabarozi, T., et al., *Electronic and thermal properties of Ti$_3$Al(C$_{0.5}$N$_{0.5}$)$_2$, Ti$_3$Al(C$_{0.5}$N$_{0.5}$) and Ti$_2$AlN.* Journal of Applied Physics, 2008. 104(7).


2 Theory


2 Theory

Chapter 3

Experimental
3. Experimental

3.1. Water

All water used within this work was ultrapure water of 18.2 MΩcm specific electrical resistance and with a temperature of 25 °C at the moment of leaving the water purifier after successive steps of filtration and deionisation. Therefore, within this work the terms “water”, or “Milli-Q water” are used to summarise exactly these qualities.

3.2. Cleaning procedures

3.2.1. Glass

All glassware was cleaned using a sonicator to break up contaminating particles or films, dissolve them in combination with heat and a sequence of varying solvents and ultimately remove them during the rinsing steps. The glassware was protected from impurities in the ultrasonic bath solution by keeping it separate in a receptacle filled with the cleaning solution of the respective cleaning step. Decon 90 from Fisher Scientific was employed as an alkaline, bactericidal and phosphate-, enzyme-, chlorine dioxide-free surfactant. For this purpose, the Decon 90 concentrate was diluted with water to a 5 vol% solution before use. In each rinsing step the glassware receptacle was completely filled with Milli-Q water for the stated amount of iterations. The following cleaning protocol was applied:

1) 15 minutes of sonification in 5 vol% Decon 90 at 80 °C,
2) 15 times rinsing in Milli-Q water,
3) 15 minutes of sonification in 5 vol% Decon 90 at 80 °C,
4) 15 times rinsing in Milli-Q water,
5) 15 minutes of sonification in Milli-Q water at 80 °C,
6) 15 minutes of sonification in isopropanol at 80 °C, and
7) 15 minutes of sonification in Ethanol at 80 °C.
3 Experimental

3.2.2. Sterilisation

Biological contamination of experiments and cultures was minimised by several means of sterilisation prior to the use of the respective equipment. The general measures taken to minimise biological contamination during the procedures are described here, and the specific decontamination methods are described in the respective experimental sections.

All equipment that could sustain the conditions was sterilised in an autoclave by exposing it to high pressure, saturated water vapour at 121 °C for 15 minutes.

Culture vessels were opened inside a class II microbiological safety cabinet, MSC Advantage, from VWR Jencons. Contamination of the environment was prevented by drawing 505 m$^3$ h$^{-1}$ air underneath the work surface to prevent any air from inside the cabinet to leak outside the cabinet. The assimilated air was filtered through HEPA H 14 EN 1822 filters with a guaranteed exclusion of 99.99 % of particles down to a size of 0.3 μm. Cross contamination between samples was minimised through the constant top down stream of filtered air above the work surface. All surfaces inside the cabinet, including gloves, were cleaned before and after use as well as between work steps with 70 vol% ethanol in water.

Metal sheet electrodes and other metallic equipment were sterilised by iterative heating to incandescence in the oxidising and in the reducing part of the Bunsen burner flame.

3.2.3. Electrodes

Electrodes were rinsed with water, polished and rinsed with water again before and after use as well as in between experiments to remove deposits and oxide scales. Alumina particles of 0.1 μm diameter on a wetted microcloth were used to polish the electrode by moving its surface in a figure of eight motion through the alumina slurry.

Platinum electrodes were additionally flame cleaned by repeated heating to incandescence in the oxidising and the reducing part of the Bunsen burner flame in order to remove biological deposits and burn off contaminations.
3 Experimental

3.3. Microbiological culture

3.3.1. Model organisms – algae and cyanobacteria

The organisms used in this work were unicellular, photosynthetic cultures of either green algae or cyanobacteria. All eukaryotic green algae cultures, the respective taxonomical information and the culture collection, which provided the stem cultures, are summarised in table 1. The specified cultures were separated into fresh water cultures and marine cultures, which determined the respective growth medium used as a nutrient source. In this work, the fresh water cultures were all of the division chlorophyta and marine cultures of the division heterokontophyta. Fresh water green algae cultures were grown in Bold Basal Medium with 3-fold Nitrogen and Vitamins – modified and marine green algae cultures in sea-salt media. Further details on composition and preparation of these media are given in 3.3.2. However, it should be noted that Nannochloropsis could also thrive in fresh water.

Table 1: Green algae cultures used within this work, their respective taxonomy and provider. The providers are abbreviated as: Cantab Plantsci for University of Cambridge Department of Plant Sciences; SAG for University of Göttingen Culture Collection of Algae.

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
<th>Division</th>
<th>Class</th>
<th>Provider</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorella</td>
<td>vulgaris</td>
<td>Chlorophyta</td>
<td>Trebouxiophyceae</td>
<td>Cantab Plantsci</td>
</tr>
<tr>
<td>Chlorella</td>
<td>sorokiniana</td>
<td>Chlorophyta</td>
<td>Trebouxiophyceae</td>
<td>SAG</td>
</tr>
<tr>
<td>Chlorella</td>
<td>emersonii</td>
<td>Chlorophyta</td>
<td>Trebouxiophyceae</td>
<td>SAG</td>
</tr>
<tr>
<td>Muriella</td>
<td>zofingiensis</td>
<td>Chlorophyta</td>
<td>Chlorophyceae</td>
<td>SAG</td>
</tr>
<tr>
<td>Nannochloropsis</td>
<td></td>
<td>Heterokontophyta</td>
<td>Eustigmatophyceae</td>
<td>Ex-Alga</td>
</tr>
<tr>
<td>Thalassiosira</td>
<td>pseudonana</td>
<td>Heterokontophyta</td>
<td>Coscinodiscophyceae</td>
<td>Cantab Plantsci</td>
</tr>
<tr>
<td>Phaeodactylum</td>
<td>tricornutum</td>
<td>Heterokontophyta</td>
<td>Bacillariophyceae</td>
<td>Cantab Plantsci</td>
</tr>
</tbody>
</table>
The prokaryotic cyanobacteria cultures and the respective information on taxonomy and stem culture provider are summarised in table 2. *Synechococcus WH5701* (SCo) and *Spirulina maxima* (SMa) were used as representatives of marine cyanobacteria, while *Synechocystis PCC6803* (SCy) was chosen to investigate fresh water cyanobacteria.

Table 2: Cyanobacteria cultures used within this work, their respective taxonomy and provider. The providers were abbreviated as: **CCAP** for Culture Collection of Algae and Protozoa; **Cantab Biochem** for University of Cambridge Department of Biochemistry.

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
<th>Division</th>
<th>Class</th>
<th>Provider</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Synechococcus</em></td>
<td>WH 5701</td>
<td>Cyanobacteria</td>
<td>Cyanophyta</td>
<td>CCAP</td>
</tr>
<tr>
<td><em>Synechocystis</em></td>
<td>PCC 6803</td>
<td>Cyanobacteria</td>
<td>Cyanophyta</td>
<td>Cantab Biochem</td>
</tr>
<tr>
<td><em>Spirulina</em></td>
<td>Maxima</td>
<td>Cyanobacteria</td>
<td>Cyanophyceae</td>
<td>CCAP</td>
</tr>
</tbody>
</table>

### 3.3.2. Growth media preparation

The preparation of stock solutions, dilutions and mixing of growth media was carried out in a biological safety cabinet as elaborated in 3.2.2. All media ingredients were dissolved in ultra pure Milli-Q water. Every container, piece of equipment and water used to prepare the growth media was autoclaved according to 3.2.2 prior to use. Except for vitamin solutions, the stock solutions were also autoclaved directly before mixing them into fresh media solutions. Vitamin stock solutions were kept refrigerated at 2 °C, while media solutions were kept and applied to cultures at room temperature. Table 3 gives an overview of all stock solutions which were used to prepare the following growth media and the mass as well as molar concentration of each chemical in the solutions. Sodium metasilicate nonahydrate was purchased from Fisher Scientific, all remaining chemicals in this table were received from Sigma Aldrich.
Table 3: Stock solutions for growth media preparation, with the mass concentration $\beta$ of the respective chemical and the corresponding molar concentration $c$.

<table>
<thead>
<tr>
<th>No.</th>
<th>Solute</th>
<th>$M$ (g mol$^{-1}$)</th>
<th>$\beta$ (g L$^{-1}$)</th>
<th>$c$ (mol L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$K_2HPO_4$ (≥ 99.95 %)</td>
<td>174.20</td>
<td>75.00000</td>
<td>4.305$\times$10$^{-1}$</td>
</tr>
<tr>
<td>2</td>
<td>$KH_2PO_4$ (≥ 99.0 %)</td>
<td>136.09</td>
<td>175.00000</td>
<td>1.286</td>
</tr>
<tr>
<td>3</td>
<td>$MgSO_4\cdot H_2O$ (≥ 97 %)</td>
<td>138.38</td>
<td>42.10857</td>
<td>3.043$\times$10$^{-1}$</td>
</tr>
<tr>
<td>4</td>
<td>$NaNO_3$ (≥ 99.0 %)</td>
<td>84.99</td>
<td>250.00000</td>
<td>2.941</td>
</tr>
<tr>
<td>5</td>
<td>$CaCl_2\cdot 2H_2O$ (≥ 99.0 %)</td>
<td>147.01</td>
<td>25.00000</td>
<td>1.701$\times$10$^{-1}$</td>
</tr>
<tr>
<td>6</td>
<td>$NaCl$ (≥ 99.5 %)</td>
<td>58.44</td>
<td>25.00000</td>
<td>4.278$\times$10$^{-1}$</td>
</tr>
<tr>
<td>7</td>
<td>EDTA-Na$_4$ (≥ 99.0 %)</td>
<td>380.17</td>
<td>50.00000</td>
<td>1.315$\times$10$^{-1}$</td>
</tr>
<tr>
<td></td>
<td>KOH (≥ 85 %)</td>
<td>56.11</td>
<td>31.00000</td>
<td>5.525$\times$10$^{-1}$</td>
</tr>
<tr>
<td>8</td>
<td>$FeSO_4\cdot 7H_2O$ (≥ 99.0 %)</td>
<td>278.05</td>
<td>4.98000</td>
<td>1.791$\times$10$^{-2}$</td>
</tr>
<tr>
<td></td>
<td>$H_2SO_4$ (≥ 95.0-98.0 %)</td>
<td>98.08</td>
<td>1.84000</td>
<td>1.876$\times$10$^{-2}$</td>
</tr>
<tr>
<td>9</td>
<td>$H_3BO_3$ (≥ 99.5 %)</td>
<td>61.83</td>
<td>11.42000</td>
<td>1.847$\times$10$^{-1}$</td>
</tr>
<tr>
<td>10</td>
<td>$ZnSO_4\cdot 7H_2O$ (≥ 99.0 %)</td>
<td>287.53</td>
<td>14.12000</td>
<td>4.911$\times$10$^{-2}$</td>
</tr>
<tr>
<td></td>
<td>MnCl$_2$·4H$_2$O (≥ 99 %)</td>
<td>197.91</td>
<td>2.32000</td>
<td>1.172$\times$10$^{-2}$</td>
</tr>
<tr>
<td></td>
<td>CuSO$_4$·5H$_2$O (≥ 98.0 %)</td>
<td>249.70</td>
<td>2.52000</td>
<td>1.009$\times$10$^{-2}$</td>
</tr>
<tr>
<td></td>
<td>Co(NO$_3$)$_2$·6H$_2$O (≥ 98.0 %)</td>
<td>291.03</td>
<td>0.80000</td>
<td>2.749$\times$10$^{-3}$</td>
</tr>
<tr>
<td>11</td>
<td>$Na_2MoO_4\cdot 2H_2O$ (≥ 99.5 %)</td>
<td>241.95</td>
<td>1.92000</td>
<td>7.936$\times$10$^{-3}$</td>
</tr>
<tr>
<td>12</td>
<td>Citric acid (≥ 99.5 %)</td>
<td>192.12</td>
<td>6.00000</td>
<td>3.123$\times$10$^{-2}$</td>
</tr>
<tr>
<td>13</td>
<td>Ferric ammonium citrate</td>
<td>265.00</td>
<td>6.00000</td>
<td>2.264$\times$10$^{-2}$</td>
</tr>
<tr>
<td>14</td>
<td>$Na_2CO_3$ (≥ 99 %)</td>
<td>105.99</td>
<td>20.00000</td>
<td>1.887$\times$10$^{-1}$</td>
</tr>
<tr>
<td>15</td>
<td>$H_3BO_3$ (≥ 99.5 %)</td>
<td>61.83</td>
<td>2.86000</td>
<td>4.626$\times$10$^{-2}$</td>
</tr>
<tr>
<td></td>
<td>MgSO$_4$·H$_2$O (≥ 97 %)</td>
<td>138.38</td>
<td>1.40363</td>
<td>1.014$\times$10$^{-2}$</td>
</tr>
<tr>
<td></td>
<td>$ZnSO_4$·7H$_2$O (≥ 99.0 %)</td>
<td>287.56</td>
<td>0.22000</td>
<td>7.651$\times$10$^{-4}$</td>
</tr>
<tr>
<td></td>
<td>CuSO$_4$·5H$_2$O (≥ 98.0 %)</td>
<td>249.69</td>
<td>0.07900</td>
<td>3.164$\times$10$^{-4}$</td>
</tr>
<tr>
<td></td>
<td>$Na_2MoO_4$·2H$_2$O (≥ 99.5 %)</td>
<td>241.95</td>
<td>0.02100</td>
<td>8.679$\times$10$^{-5}$</td>
</tr>
<tr>
<td></td>
<td>Co(NO$_3$)$_2$·6H$_2$O (≥ 98.0 %)</td>
<td>291.03</td>
<td>0.04940</td>
<td>1.697$\times$10$^{-4}$</td>
</tr>
</tbody>
</table>
3 Experimental

<table>
<thead>
<tr>
<th>No.</th>
<th>Solute</th>
<th>σ (mL L⁻¹)</th>
<th>c (mol L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>K₂HPO₄</td>
<td>1</td>
<td>4.31·10⁻⁴</td>
</tr>
<tr>
<td>2</td>
<td>KH₂PO₄</td>
<td>1</td>
<td>1.29·10⁻³</td>
</tr>
<tr>
<td>3</td>
<td>MgSO₄·H₂O</td>
<td>1</td>
<td>3.04·10⁻⁴</td>
</tr>
<tr>
<td>4</td>
<td>NaNO₃</td>
<td>3</td>
<td>8.82·10⁻³</td>
</tr>
<tr>
<td>5</td>
<td>CaCl₂·2H₂O</td>
<td>1</td>
<td>1.70·10⁻⁴</td>
</tr>
<tr>
<td>6</td>
<td>NaCl</td>
<td>1</td>
<td>4.28·10⁻⁴</td>
</tr>
<tr>
<td>7</td>
<td>EDTA-Na₄</td>
<td>1</td>
<td>1.32·10⁻⁴</td>
</tr>
<tr>
<td></td>
<td>KOH</td>
<td></td>
<td>5.53·10⁻⁴</td>
</tr>
<tr>
<td>8</td>
<td>FeSO₄·7H₂O</td>
<td>1</td>
<td>1.79·10⁻⁵</td>
</tr>
<tr>
<td></td>
<td>H₂SO₄</td>
<td></td>
<td>1.88·10⁻⁵</td>
</tr>
<tr>
<td>9</td>
<td>H₃BO₃</td>
<td>1</td>
<td>1.85·10⁻⁴</td>
</tr>
</tbody>
</table>

3.3.2.1. Bold Basal Medium with 3-fold Nitrogen and Vitamins - modified

This vitamin containing growth medium, developed by Bischoff et al. and Andersen et al., was used for all fresh water cultures of green algae and was abbreviated as 3N-BBM+V in this work.[1,2] 3N-BBM+V could not be autoclaved following its preparation, since the vitamins cyanocobalamin and biotin were not stable at 121 °C. Therefore, it was only prepared directly before utilisation. The components and required volumes of stock solution as well as the resulting concentrations in the medium are summarised in Table 4.

Table 4: Bold Basal medium with 3-fold Nitrogen and Vitamins – modified (3N-BBM+V) composition, required stock solution volume in one litre of medium and resulting volume concentration σ (mL L⁻¹) and molar concentration c (mol L⁻¹).
3.3.2.2. BG-11

The vitamin-free BG-11 growth medium was originally designed for cyanobacteria growth by Allen, Hughes and Watanabe et al., and prepared as recommended by the National Center for Marine Algae and Microbiota.[3-5] BG-11 growth medium was autoclaved and allowed to reach room temperature before usage, since it did not contain any vitamins. The required volumes of stock solutions and the resulting concentration of each ingredient in the finished medium are presented in table 5.

Table 5: BG-11 growth medium composition, required stock solution volume in one litre of medium and resulting volume concentration $\sigma$ (mL L$^{-1}$) as well as molar concentration c (mol L$^{-1}$) in the finished medium.
Sea salt medium was used as the nutrient source for all marine green algae cultures, and was prepared by dissolving a sea salts mixture as received from Sigma Aldrich (Cat. No.: S9883-500G) in autoclaved water under sterile conditions, as described in 3.2.2. The mass concentration of sea salts in water was adjusted for the respective target culture according to the advice from collaborators at the University of Cambridge Department for Plant Sciences, specified in table 6. The sea salt medium was autoclaved before each use and kept at room temperature.

Table 6: Sea salt mass concentration in Milli-Q water for the respective culture.

<table>
<thead>
<tr>
<th>Sea salt media</th>
<th>Target culture</th>
<th>Mass concentration $\beta$ of sea salts (g L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phaeoactylum tricornutum</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Thalassiosira pseudonana</td>
<td>31</td>
</tr>
</tbody>
</table>

### 3.3.3. Growth conditions

#### 3.3.3.1. Agar plates

Petri dishes filled with agar gels, called agar plates, were used to inspect cultures for purity, keep pure stock culture reserves, propagate cultures and perform toxicity testing (3.3.5.1). The agar solution was created in borosilicate screw cap bottles (VWR Jencons) from a 2 wt% dispersion of agar powder, supplied from Sigma Aldrich, in the appropriate growth media for the target culture. Specific information on the media used for each organism in this work is given in 3.3.2. The agar dissolved into the aqueous growth medium during the following autoclave run.
and was henceforth only handled under the sterile conditions of a microbiological safety cabinet as described in 3.2.2. Required vitamin supplements were added after the agar solution temperature had fallen below 60 °C. Each Petri dish was filled with approximately 20 mL of agar solution and left to set for at least one hour, before the closed agar plate was sealed with acrylic Parafilm from Fisher Scientific and stored at 2 °C until use.

Cultures were inoculated with large concentration gradients across the agar plates, in order to isolate and inspect single colonies. This was achieved by streaking 10 μL of liquid culture with an inoculation loop from Sigma Aldrich five times into one direction of the agar plate, followed by streaking across the previous five lines with a fresh inoculation loop. Repeating this procedure multiple times on the respective previous streaks resulted in a dilution pattern as shown in figure 24.

![Figure 24: Streaking of 10 μL liquid culture samples onto agar plates resulted in a dilution down to the isolation of single cells, which then formed distinct and separated colonies. In each of the steps a) to e) the cell density of the previous streaks was diluted by dragging a certain culture volume across the agar plate four times.](image)

The plated cultures were sealed with acrylic Parafilm again and incubated at room temperature on fluorescent light boxes from Lightbox UK with diurnal cycles of 12 hour light/dark periods. The luminous intensity at the surface of the light boxes was 1500-1800 cd m$^{-2}$ with a light colour temperature of 7000-8000 K, in order to mimic daylight properties.

### 3.3.3.2. Liquid culture

All liquid cultures were incubated in 250 mL borosilicate glass Erlenmeyer flasks from VWR Jencons on a Stuart SSL 1 orbital shaker set at 120 rotations per minute (rpm) at room temperature. Sterility with concurrent vital gas exchange was ensured by using polyurethane stopper foams from Fisher Scientific, which were additionally covered with aluminium foil to minimise media evaporation. The
Erlenmeyer flasks, including stopper foams and aluminium foil were autoclaved and handled in a microbiological safety cabinet according to 3.2.2.

Diffuse light was provided 12 hours a day using a fluorescent light box beneath the culturing flasks as illustrated in figure 25.

![Image of Erlenmeyer flasks](image)

**Figure 25**: Liquid culture incubation on a Stuart SSL 1 orbital shaker with 120 rpm at room temperature and fluorescent lighting from underneath the cultures.

Previously conducted work within our group on the growth phases of our cultures indicated that cells enter a stationary growth phase at 20-30 days. Therefore, re-inoculation was carried out every one to two months with approximately $5 \cdot 10^8$ cells from a stem culture in 100 mL sterilised fresh media, in order to keep the organisms in the exponential phase of growth. Section 3.3.2 elaborates on the types of media used for specific cultures.

### 3.3.4. Cell counting

Two methods were employed to determine cell densities of cultures: manual counting using a microscope in combination with a Neubauer improved haemocytometer for sporadic culture inspection, and automated flow cytometry for rapid screening of multiple cultures.

#### 3.3.4.1. Microscopic cell counting using a haemocytometer

Improved Neubauer haemocytometers made out of glass from Millscience Counting Chambers and disposable plastic haemocytometers from PEQLAB limited were used to determine cell densities. While the plastic haemocytometers did not require cleaning, the glass haemocytometers and corresponding cover slips were first cleaned with DECON 90 surfactant, then rinsed with ethanol and finally dried with nitrogen before and after every cell count. Verification of the glass haemocytometer cleanliness and subsequent cell counting was performed on either
an Ortholux optical microscope from Ernst Leitz GmbH Wetzlar, or a L3001 fluorescence microscope from GX Microscopes.

Culture samples were diluted with the appropriate growth medium to give cell counts of 50 to 500 per counting grid. In order to determine the dilution factor, an initial cell count with a 100 times diluted sample was performed. For every cell count a 7 µL sample of the diluted culture was pipetted onto each of the two grids on the haemocytometer. The cell density in one millilitre of the original culture was calculated by multiplying the average amount of cells on one counting grid with the volume ratio of media to culture used for the dilution and with the haemocytometer specific factor 1·10^6.

3.3.4.2. Flow cytometry

Flow cytometry was conducted on a Guava Easy Cyte Flow Cytometer from Millipore, with Guavasoft 2.2.2 software. A measurement protocol with optimised yellow and red laser light intensity as well as forward and side scatter range for the most sensitive detection of changes in cell shape and chlorophyll content was first established for each culture, and consistently used throughout the experiments.

The capillary was cleaned with Guava Instrument Cleaning Fluid from Millipore before and after each experiment, and with Milli-Q water in between measurements.

Samples were diluted with Milli-Q water to keep the cell density between 5·10^4 and 5·10^5 cells mL^{-1}, in order to remain in the optimal confidence interval of the flow cytometer. The cell density of every sample was homogenised with a vortex mixer immediately before each measurement and at least 3000 cells were counted to calculate the cell density. Furthermore, falsification of the results due to the formation of a cell density gradient inside the test vessel was prevented by setting the count threshold appropriate to a measuring time between 1-2 minutes.

3.3.5. Cytotoxicity of metal-organic framework MIL-101

The cytotoxicity of the chromium carboxylate based metal-organic framework MIL-101 and its amine-modification MIL-101(Cr)-NH₂ on green algae and cyanobacteria was tested using samples produced by our collaborator at the University of Bath, Dr. Dongmei Jiang, and received as powder. Cytotoxicity aspects relating to an implementation of metal-organic framework (MOF) materials in microbial fuel cells (MFCs) were investigated with exclusion zone microscopy on agar plate cultures and cell counting on liquid cultures.
3.3.5.1. Exclusion zone tests

All exclusion zone tests were conducted on cultures grown on agar gels according to 3.3.3.1. The MOF MIL-101 powder was pressed into tablet form for easier handling on the agar plates and for controllable borders to the tested organisms using a 30 Tonne Press H30-I MK.2 from Research and Industrial Instruments Company England. The pressing was carried out with 8 lb inch\(^{-2}\) pressure for one minute.

Exclusion zone testing was performed on the green algae cultures *Chlorella vulgaris* (CVu), *Chlorella sorokiniana* (CSo), *Muriella zofingiensis* (MZo), a mixed culture of green algae and cyanobacteria (Mix) as well as the cyanobacteria cultures SMa and SCo.

Each culture was inoculated on the agar gel immediately before a fragment of the MOF MIL-101 tablet was applied on top of the gel. The mixed culture was disseminated on an agar plate made from 3N-BBM+V medium. Subsequently, the Petri dishes were sealed with Parafilm from Fisher Scientific and stored on a lightbox with the same light properties and irradiation times as elucidated in 3.3.3.1. After the organisms had been cultured for 41 days the periphery between the MOF fragment and the biofilms was examined for exclusion zones with a L3001 Series fluorescence microscope from GX Microscopes to evaluate the respective organism tolerance for the MOF MIL-101 material.

3.3.5.2. Growth curves

The toxicity effect of the two MOFs MIL-101 and its amine functionalised modification, MIL-101(Cr)-NH\(_2\), on green algae and cyanobacteria was investigated on the basis of cell growth rate and chlorophyll content per cell.

Initial investigations were only focused on the growth rates of the green algae cultures CVu, CSo and MZo as well as on the cyanobacteria cultures SCo and SCy. In each case, two biological replicates of one parent culture were taken and one culture of each pair was exposed to 48 mg of MOF MIL-101 per 100 mL of culture, while the other one served as control. Haemocytometry was employed for the cell quantification every seven days on average for a period of 42 days.

The study was then extended by comparing the growth rates of control cultures to cultures that were either exposed to MOF MIL-101, or that were grown under conditions known as toxic. For this second part of the investigation, 25 mg MOF MIL-101 was used per 150 mL of culture. This MOF MIL-101 concentration already corresponded to a concentration of chromium (III) that was 40 times higher than
the EC<sub>50</sub> for freshwater algae and six times higher than the EC<sub>50</sub> for marine diatoms.<sup>[6,7]</sup> Therefore, a toxic effect from the possible leaching of chromium (III) was expected to be detected with this MOF concentration as well. A known toxic environment was created using ferricyanide (K₃[Fe(CN)₆]) and ferrocyanide (K₂[Fe(CN)₆]), which has commonly been employed as artificial redox mediator couple in MFCs. Each was added at a concentration of 2.5 mol L⁻¹. The analysis was restricted to the green algae culture <i>Chlorella emersonii</i> (CEm) and the cyanobacteria culture SCy. Six biological replicates of each culture were divided into pairs, of which one pair was exposed to MOF MIL-101, the other pair to the toxic redox mediator mixture and the last pair served as controls. Cell quantification with concurrent inspection of cell size, shape and chlorophyll content were performed on a Guava Easy Cyte Flow Cytometer from Millipore for 27 days; on average a count was performed every four days, with a final count two months later.

In addition, the toxicity of the amine functionalised MOF MIL-101(Cr)-NH₂, and its efficiency in increasing the biologically available CO₂ concentration in liquid culture were examined with respect to the green algae cultures CSo and MZo as well as to the cyanobacteria culture SMa. This MOF-MIL101 modification in particular was tested as a result of the recommendation of our collaborator, Dr. Dongmei Jiang from the University of Bath, as the most promising for carbon capture applications. Following its synthesis, the MOF MIL-101(Cr)-NH₂ was washed three times in ethanol and subsequently one time in water before it was dried at 50 °C over night. 25 mg of the MOF was autoclaved directly in the flasks according to 3.2.2. The MOF in half of these flasks was preloaded with CO₂ for 5.5 hours. Afterwards, all test cultures were incubated with 150 mL per flask. Each culture was tested for five conditions:

1) no exposure, serving as control culture,
2) exposed to MOF MIL-101(Cr)-NH₂ without CO₂ preloading,
3) exposed to MOF MIL-101(Cr)-NH₂ with CO₂ preloading,
4) exposed to MOF MIL-101(Cr)-NH₂ without CO₂ preloading in constant CO₂ stream, and
5) exposed to CO₂ stream without MOF MIL-101(Cr)-NH₂ presence.

And each of these conditions was confirmed with three replicates per culture as summarised in table 7 and illustrated in figure 26.
3 Experimental

Table 7: The sample setup for MOF MIL-101(Cr)-NH₂ toxicity investigations on a single culture comprised 15 samples, which were divided into sets of three samples under equivalent growth conditions. The application of this examination procedure on the green algae cultures CSo and MZo as well as on the cyanobacteria culture SMA resulted in a collection of 45 samples.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>MIL-101(Cr)-NH₂</th>
<th>CO₂ Preloading</th>
<th>CO₂ stream</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-6</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-9</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>10-12</td>
<td>Yes</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>13-15</td>
<td></td>
<td></td>
<td>Yes</td>
</tr>
</tbody>
</table>

Figure 26: Incubation array of CSo, MZo and SMA to test the effects of MOF MIL-101(Cr)-NH₂ presence, MOF MIL-101(Cr)-NH₂ preloading with CO₂, MOF MIL-101(Cr)-NH₂ performance in constant CO₂ stream.

The cultures were incubated under the conditions described in 3.3.3.2 in all toxicity experiments. In order to assure comparability, a volume of 50 mL from a single parent culture in its exponential growth phase was taken for each of the test cultures and filled up with the appropriate media to the stated volumes. Low volume samples of less than 100 µL were extracted in a microbiological safety cabinet, as described in 3.2.2, for each analysis. All measurements were conducted on the day of the sample extraction.

3.4. Electrode manufacture

3.4.1. Sputter coating

Hot pressed electrodes, based on Zorflex Activated Carbon Cloth FM10 (ZACC FM10) from Chemviron Carbon and Nafion® perfluorinated membrane 115 (Nafion® 115) from Sigma Aldrich, were coated in platinum employing an Agar
Sputter Coater 6001. Each substrate was coated for 60 seconds at a current of 30 mA in a 10 Pa argon atmosphere, which caused the formation of several nm thick Pt islands in previous experiments on FTO glass within the group.

### 3.4.2. Membrane materials

Nafion® membranes in liquid and solid form as well as two solid types of cation exchange membranes were applied to the tested cathode materials:

1) Nafion® perfluorinated resin solution 5 wt. % in a mixture of lower aliphatic alcohols and water, containing 45% water from Sigma Aldrich,

2) Nafion® perfluorinated membrane 115, thickness 0.005 in. from Sigma Aldrich,

3) cation exchange membrane 551652U from VWR, and

4) CMI-7000S Cation Exchange Membrane Lot 277 from Membranes International Inc.

The Nafion® resin solution was painted onto the carbon paper and dried overnight in the fume cupboard. Solid membranes were hot-pressed to the respective cathode corresponding to 3.4.4.

### 3.4.3. Comparison of Nafion® resin and Nafion® membranes on carbon paper

Carbon paper was chosen as a template to compare the applicability of Nafion® resin to solid Nafion® membranes, since it exhibited the smoothest and most uniform surface out of the tested carbon materials. The Nafion® perfluorinated resin solution (5 wt% in a mixture of lower aliphatic alcohols and water, containing 45 % water) was painted onto the carbon paper and dried in the fume cupboard over night. The solid Nafion® 115 membrane was hot-pressed onto the carbon paper according to 3.4.4.

Falcon tubes with a volume of 15 mL from Fisher Scientific were sealed onto the top of the membrane side of each sample with silicone. This exposed an area of approximately 1.3 cm² of the tested material to a volume of 15 mL solution above it. A flow of water inside the falcon tubes was mediated by a peristaltic pump at high flow rates overnight as shown in figure 27.
3.4.4. Hot pressing of cathode-membrane assemblies

Two iron blocks with polished surfaces were heated at an approximate rate of 4 °C min⁻¹ on a hot plate from Stuart Scientific until the metal probe thermometer indicated a temperature of 100 °C on the top side of the blocks.

Cathode and membrane material were both placed simultaneously between the blocks and hot-pressed using a 30 tonne press (H30-I MK.2 from Research and Industrial Instruments Company England) for 30 seconds with a pressure of 0.85 MPa, delivered from a ram diameter of 8.6 cm. The hot-pressed electrodes were gently removed from the iron blocks using the swelling of the ion exchange membrane in the presence of water.

Figure 28 depicts the examined combinations of carbon based cathode materials with ion exchange membranes. The presented combinations were a result of preceding material durability tests on several more materials and represent the samples that were examined further. The carbon based cathode materials included: ZACC FM10, carbon paper (C-paper) from the University of Cambridge Department of Biochemistry and carbon felt (C-felt) from Le Carbone. The tested ion exchange membranes were Nafion® 115 with a thickness of 0.127 mm from Sigma Aldrich and the CMI-7000S Cation Exchange Membrane (CMI-7000S) with a thickness of 0.45 mm from Membranes International Incorporated.
3 Experimental

3.4.5. Polyaniline coating of conductive substrates

The electrochemical coating of fluorine doped tin oxide coated glass (FTO glass) with an IR reflectance of 87% and a surface resistance of 6-9 $\Omega$ sq$^{-1}$ (Tec 8) from Pilkington glass with polyaniline (PANI) was performed within the scope of a collaboration with Cambridge University on the comparison of anode materials in photo-microbial fuel cells (pMFCs). The electrochemical polymerisation reaction was conducted in three electrode mode as described in 2.2.8 and shown in figure 29. FTO glass substrates were cleaned according to 3.2.1 and provided with an improved electrical contact in the form of a copper strip (AT528 50 Micron Copper Foil Shielding Tape from Advance Tapes International), in order to maximise the uniformity of the current density throughout the sample. It was assured that the copper tape was not immersed in the coating solution. A high surface platinum net counter electrode and a Ag/AgCl reference electrode were employed and cleaned in between experiments as described in 3.2.3. The conductive side of the FTO glass was pointed towards the reference electrode and the distance between these two electrodes was kept minimal. The counter electrode and the FTO glass working electrode were positioned reasonably far away from each other. This was done to
avoid irregular coating of the substrate as a result of short electrode separation, whilst preventing the electrolyte resistance from interfering with the PANI deposition in case that the distance between the electrodes became too large.

Figure 29: Arrangement of platinum counter electrode (CE), fluorine doped tin oxide (FTO) coated glass substrate working electrode (WE) and Ag/AgCl reference electrode (RE) for the electrochemical coating of FTO glass with PANI.

The coating solution was prepared fresh before each experiment by dissolving 0.1 mol L\(^{-1}\) Aniline from BDH Laboratory Supplies in 0.1 mol L\(^{-1}\) aqueous sulphuric acid with sonication at room temperature for one hour.

PANI deposition, process monitoring and subsequent characterisation were conducted via cyclic voltammetry in staircase mode, using an Autolab PGSTAT12 from Windsor Scientific Limited.

The polymerisation of PANI was performed in a voltage range of 0.0 V to 9.5 V with a scan rate of 10 mV according to Kalaji \textit{et al.} \cite{9}. Five scans were carried out with these parameters on each substrate. Following the deposition, excess PANI was washed off by rinsing the samples with Milli-Q water.

The characterisation of the dried, polymerised PANI film on the FTO glass was conducted in a fresh, aqueous solution of 1 mol L\(^{-1}\) H\(_2\)SO\(_4\). A potential range of -0.3 V to 0.4 V with a scan rate of 50 mV was applied in order to avoid causing irreversible oxidation of the polymerised PANI at further positive potentials.
3.4.6. Ceramic electrode manufacturing

All ceramics were produced with a replication process, using polyurethane foams from Sydney Heath & Son Ltd as a template. These foams were dip coated in a slip containing the metal precursor powder and subsequently evaporated through thermolysis, while the ceramic slip was sintered. The fabricated ceramics were either based on TiO\textsubscript{2}, or on Ti\textsubscript{2}AlC and were compared to the alternative bio-anode material Duocel Reticulated Vitreous Carbon foam from ERG Aerospace Corporation.

3.4.6.1. TiO\textsubscript{2} ceramics

Reticulated polyurethane foams with porosity between 10 and 30 pores per inch (ppi) were tested. The ceramic slip composition and sintering procedure according to the work of Thorne et al. was used to fabricate the TiO\textsubscript{2} ceramics.\cite{10,11} First, the liquid components of the slip, consisting of the solvent water, the dispersant poly(ethylenglycol) and the surfactant Dispex GA40 were mixed. Subsequently, the solids composed of the thickener methyl cellulose, the binder polyvinylpyrrolidone K30, the plasticizer poly(vinyl alcohol) and the TiO\textsubscript{2} powder were mixed into the liquids. The amounts of all slip ingredients per coating and respective providers were summarised in table 8.

Table 8: TiO\textsubscript{2} ceramic slip composition according to the work of Thorne et al.\cite{10,11}

<table>
<thead>
<tr>
<th>Liquid components</th>
<th>Solid components</th>
</tr>
</thead>
<tbody>
<tr>
<td>3g poly(ethylenglycol) from Sigma Aldrich</td>
<td>1.5g methyl cellulose from Sigma Aldrich</td>
</tr>
<tr>
<td>125 mL Milli-Q water</td>
<td>1.5g polyvinylpyrrolidone K30 from Sigma Aldrich</td>
</tr>
<tr>
<td>2.5 mL Dispex GA40 from Ciba AG</td>
<td>3g poly(vinyl alcohol) 99+% hydrolysed from Sigma Aldrich</td>
</tr>
<tr>
<td></td>
<td>300g TiO\textsubscript{2} powder 0.3 µm mean particle size, 99.5% pure from Pi-kem Ltd</td>
</tr>
</tbody>
</table>

64 g of zirconia grinding media were added to the mixture in table 8 and used in combination with a ball mill model 2VS, from Capco Test Equipment Ltd., to avoid agglomeration and to provide the desired rheology of the TiO\textsubscript{2} slurry by milling for 24 hours. After impregnation of the reticulated polyurethane foam template with
the slip, excess material was removed with high velocity air from a MM4 Airtrack compressor, manufactured by TQ Education & Training Limited. The coating was left to dry for 24 hours at room temperature, followed by the application of a 1 cm wide continuous TiO\textsubscript{2} slip strip, which was also left to dry for 24 hours.

Evaporation of the template and sintering of the ceramic slip was performed on TiO\textsubscript{2} powdered Al\textsubscript{2}O\textsubscript{3} ceramic tiles according to the heating procedure presented in figure 30 in air, using a muffle furnace from Elite Thermal Systems Limited. A one hour dwelling period at 600 °C served to guarantee the complete thermolysis of the polyurethane foam template.

![Figure 30](image.png)

**Figure 30:** TiO\textsubscript{2} ceramic sintering procedure in air, including an one hour dwelling period at 600 °C for complete thermolysis of the polyurethane foam template.

All TiO\textsubscript{2} ceramics applied in MFCs were coated in fluorine doped tin oxide (FTO) with chemical vapour deposition (CVD) by Solaronix SA.

An electrical contact was added by the attachment of a stainless steel wire to the non-porous ceramic fundament strip using silver loaded epoxy adhesive from RS Components Limited. The cured contact was insulated with quick set epoxy adhesive from RS Components Limited and subsequently sealed with the biocompatible, high modulus polyether sealant, Wet Water Sticky Stuff, from Warehouse Aquatics, as shown in figure 31.
3.4.6.2. Single coated Ti$_2$AlC ceramic for electrochemical investigations

Ti$_2$AlC ceramics were produced in collaboration with Professor Christopher Rhys Bowen and Tony Thomas from the Department of Mechanical Engineering at the University of Bath. A replication process based on polyurethane foam with a pore size of 10 ppi as a template for the electrode geometry was implemented. These foams were dip coated in a slip containing the Ti$_2$AlC precursor powder “Maxthal 211 ceramic engineering material” obtained from Kanthal. Optimal material retention with the most uniform coating was assured with a slip composition of 2 mL polyethylene glycol, 55.5 mL water, 1 mL Dispex GA40, 3 g methyl cellulose and 125 g Ti$_2$AlC precursor powder. Liquids were mixed before adding the solids in order to guarantee slip homogeneity. 64 g of 5 mm ceramic beads were added to the suspension to aid the mixing in the ball mill for 36 hours. Following the dip coating the sample was treated with high velocity compressed air to ensure uniform coating as well as the removal of excess slip from the pores. The general preparatory steps of the replication process are shown in figure 32.

Figure 31: FTO coated TiO$_2$ ceramic electrode with non-porous ceramic layer underneath the electrical contact and biocompatible silicone as insulation.

Figure 32: Preparatory steps of the replication process: a) dip coating of the polyurethane foam template into the slip and assuring a uniform coating of the interior through repeated compression; b) removal of excess slip with high velocity compressed air; c) prevention of pore clogging while drying.
In order to provide the foundation for an electrical contact, the pores of one narrow side of each sample were filled with slip (see figure 34a). The slip was allowed to set for at least 24 hours before sintering.

Two methods to facilitate the slow evaporation of all solvents were explored, since the comparatively large heating rate during the sintering process would have caused crack formation due to rapid solvent evaporation and subsequent slip hardening. Both methods were optimised towards maximum solvent evaporation, while avoiding oxidation of the slip components in air. In method 1 the samples were dried at room temperature in air for at least 24 hours and were then heated to 400 °C in an argon atmosphere before the slip was sintered. A 24 hour setting period at 50 °C was implemented in method 2 in order to reduce the formation of the competing phase TiC at the high setting temperatures of method 1. In this chapter no differentiation was made between samples from either setting methods, since the same electrochemical results were obtained in all tests.

The samples were sintered in an argon atmosphere by heating from 20 °C to 1400 °C with a continuous ramp of 150 °C h⁻¹, dwelling for three hours and cooling at 150 °C h⁻¹. Figure 33 summarises the differences in heat treatment and atmospheric conditions between the two setting methods with subsequent sintering conditions.

![Figure 33](image)

**Figure 33:** Setting and sintering conditions of Ti₂AlC ceramics used for electrochemical material analysis with the white background representing the sample being in air and blue background in argon atmosphere: a) Setting method 1 leading to higher TiC content; b) setting method 2 resulting in lower TiC content.

Following the sintering, electrical contacts were soldered onto the non-porous foundation and insulated with Wet Water Sticky Stuff (figure 34b and c). Good conductivity between the end of the wire and several points on the Ti₂AlC ceramic was confirmed with a digital Volt-Ohm meter before the application of the samples.
Furthermore, a non-porous Ti$_2$AlC ceramic with an even, uniform surface was produced for surface analysis investigations, since the Ti$_2$AlC ceramics with a porosity of 10 ppi were unsuitable for the creation of an adequate measuring interface. These surface measurements included four terminal sensing, described in 3.5.1.6, and atomic force microscopy, elaborated in 3.5.2.

For this purpose, 3.7629 g Ti$_2$AlC precursor powder was pressed into a cylindrical mould with a hydraulic press. The compacted powder pellet was then sintered according to the procedure shown in figure 33b.

Two additional 10 ppi porous samples were produced from the same amount of precursor powder and under the same conditions as the non-porous sample respectively. These samples were used in electrochemical and pMFC investigations (described in 3.5 and 3.6.7.1 respectively), in order to obtain a comprehensive Ti$_2$AlC material analysis through the combination with the surface analysis of the non-porous Ti$_2$AlC ceramic. It was necessary to mix the precursor powder with the appropriate amounts of solvents into a slip, since the porous samples had to be realised with the replication process, as described in 3.1. Material differences were not expected from this variation of the non-porous sample fabrication process, since the additional chemicals as well as the polyurethane foam template were completely vaporised and removed by the argon stream at lower temperatures before the sintering.

3.4.6.3. Double coated Ti$_2$AlC ceramic

Double coating was explored as an improvement of the structural properties of Ti$_2$AlC ceramics in collaboration with Professor Christopher Bowen and Tony Thomas from the Department of Mechanical Engineering at the University of Bath.
3 Experimental

In the course of this investigation, the Ti$_2$AlC ceramic fabrication procedure, described in 3.4.6.2, was extended with a second coating step after the first coating had been dried at room temperature for 24 hours. Impregnation of the hardened sample structure was achieved with a second slip mixture, which only differed from the first one by a higher water content of 75 mL. The second coating was also allowed to set for 24 hours at room temperature in air, before the samples were sintered in argon atmosphere, as shown in figure 35.

![Figure 35: Setting and sintering procedure of double coated Ti$_2$AlC ceramics with 24 hour setting periods after each coating step and an extended dwelling period at 1400 °C.](image)

An extended dwelling period of five hours at 1400 °C allowed for the thicker Ti$_2$AlC slip coating. All equipment, materials, argon flow rate during the sintering and the protocol for the application of the electrical contact after the sintering used for the fabrication of single coated Ti$_2$AlC ceramics in 3.4.6.2 were also applied for the manufacture of double coated Ti$_2$AlC ceramics.

3.4.7. Prussian blue coated electrodes

Prussian blue coating of electrodes as replacement for the toxic redox mediator couple ferricyanide and ferrocyanide was investigated on TiO$_2$ ceramics and fluorine doped tin oxide coated glass (FTO glass). Two differing protocols for a pyrrole based, current free deposition of Prussian blue, as described by Koncki et al. and Borisova et al. respectively, were compared for coating the non-conductive TiO$_2$ ceramics.$^{[12,13]}$ Furthermore, a third method of electrodepositioning Prussian blue according to Puganova et al. was conducted on FTO glass as part of a preliminary feasibility study for FTO coated TiO$_2$ ceramics.$^{[14]}$

All experiments regarding Prussian blue deposition had to be conducted in the fume cupboard, since the use of potassium hexacyanoferrate(III) and hydrochloric
Experimental acid solutions released toxic HCN gas. The required glass wear was cleaned according to 3.2.1.

TiO₂ ceramics were prepared according to 3.4.6.1, but were not coated in electrically conductive fluorine doped tin oxide. An aqueous growing solution of 0.1 mol L⁻¹ HCl, 0.1 mol L⁻¹ KCl, 2 mmol L⁻¹ K₃[Fe(CN)₆] and 2 mmol L⁻¹ FeCl₃ was prepared. The TiO₂ substrates were immersed into the growing solution and then 98 % pyrrole from Sigma Aldrich was added to initiate the formation and deposition of Prussian blue onto the TiO₂ surface. At this point, the difference between the two Prussian blue deposition protocols of Koncki and Borisova was investigated by either leaving the samples to dry in the dark, or irradiating them with a 15W UV light during the drying process. All samples were washed in Milli-Q water after the drying phase.

The electrochemical deposition was conducted on TEC 15 FTO glass from Nippon Sheet Glass Co., Ltd. with a sheet resistivity of 12 to 14 Ω sq⁻¹. Cyclic voltammetry in staircase mode was conducted using a Ag/AgCl reference electrode (RE) and a platinum net counter electrode (CE) connected to an Autolab PGSTAT12. The platinum CE was flame cleaned corresponding to 3.2.3 before and after use. An aqueous electrolyte containing 0.1 mol L⁻¹ HCl, 0.1 mol L⁻¹ KCl, 4 mmol L⁻¹ K₃[Fe(CN)₆] and 4 mmol L⁻¹ FeCl₃ was prepared as growing solution according to Puganova et al. The Prussian blue films were first grown through continuous cyclic voltammetry in the potential range between 0.3 V to 0.8 V with a scan rate of 20 mV s⁻¹ until reproducible cyclic voltammogram (CV) shapes indicated no further Prussian blue deposition. This was achieved after 50 CVs per sample. The subsequent activation of the Prussian blue films was executed with 100 scans in a voltage range of -0.05 V to 0.35 V at a scan rate of 20 mV s⁻¹.

3.5. Anode characterisation

An Autolab PGSTAT12 from Windsor Scientific Limited was used as potentiostat/galvanostat for all electrochemical investigations in this section. Glassware and electrodes were cleaned before and after experiments according to 3.2.1 and 3.2.3 respectively. The experiments were performed in a Faraday cage with blackened walls to minimise light pollution during investigations on light sensitivity and in order to protect light sensitive chemicals. Metallic parts of the setup stage were earthed per electrical connection to the same location on the inside of the Faraday cage. The Faraday cage was earthed to the appropriate connection in the potentiostat. All experiments were conducted at room temperature of 298 ± 5 K and at a pressure of approximately 100 kPa (RTP).
3 Experimental

3.5.1. Electrochemical characterisation of Ti$_2$AlC ceramic as working electrode

3.5.1.1. Electrochemical stability

The electrochemical stability study was conducted on two Ti$_2$AlC working electrodes (WE) in two common background electrolytes; aqueous solutions of KCl and NaNO$_3$ each at a concentration of 0.1 mol L$^{-1}$. In all experiments the reference electrode (RE) was Ag/AgCl and the counter electrode (CE) was a platinum net electrode (Pt net CE) connected to an Autolab PGSTAT12 (figure 36).

![Figure 36: Ti$_2$AlC ceramic long-term oxidation setup with Ag/AgCl RE (blue) and Pt net CE (green) connected to Ti$_2$AlC WE (red) over an Autolab PGSTAT12 potentiostat.](image)

All electrodes were rinsed with Milli-Q water between experiments and the Pt net CE was flame cleaned during the cleaning cycles.

The Ti$_2$AlC electrodes were analysed for both photosensitivity and electrochemical window. Photosensitivity was tested by recording CVs in dark and under illumination using a 1 W, 625 nm diode held close to the electrochemical cell. This type of illumination source was chosen as it was deemed appropriate for use in biological studies in later experiments.

3.5.1.2. Exhaustion of the Ti$_2$AlC oxidation with continuous cyclic voltammetry

The Ti$_2$AlC ceramic was subjected to 166 CVs at a scan rate of 1 mV s$^{-1}$ in the potential range from -0.3 V to 0.7 V vs. Ag/AgCl reference electrode. Data obtained during initial studies on Ti$_2$AlC in 6.3.3.3 showed a more defined current response to the oxidation process the slower the scan rate was. Furthermore, it was found
that this potential range was sufficient to trigger the process. The experiment was stopped when the oxidation current was no longer visible in the on-line CV graph.

After the experiment at 1 mV s\(^{-1}\) was completed the same procedure was repeated on the same electrode with 202 CVs at a reduced scan rate of 0.5 mV s\(^{-1}\) to analyse the current response with lower charging currents.

### 3.5.1.3. Scan rate dependence of electrochemical Ti\(_2\)AlC oxidation

A range of scan rates was applied (500, 400, 300, 200, 100, 50, 20, 10, 5, 1 and 0.5 mV s\(^{-1}\)) within a consistent potential range of -0.3 to 0.7 V vs. Ag/AgCl reference, using the same Ti\(_2\)AlC ceramic working electrode as described in 3.5.1.1 and 3.5.1.2.

The initial investigation of Ti\(_2\)AlC ceramics as working electrodes in 3.5.1.1 indicated that the electrochemical oxidation of Ti\(_2\)AlC could only be measured at sufficiently slow scan rates. Results analysed in 6.3.3.4 suggested that more material would be oxidised at slower scan rates, since a greater flow of oxidation charge was measured at 0.5 mV s\(^{-1}\) than was predicted at 1 mV s\(^{-1}\). A more structured investigation of the scan rate effect on Ti\(_2\)AlC oxidation was therefore essential due to two limitations within this work. The measurements described in 3.5.1.1 not only varied in scan rate, but also in the range of the applied potential windows. Furthermore, the results in 6.3.3.4 were inconclusive, since only two different scan rates were analysed.

### 3.5.1.4. Potential window of Ti\(_2\)AlC ceramic electrodes

The Ti\(_2\)AlC ceramic was subjected to CVs with a constant scan rate of 1 mV s\(^{-1}\) at varying potential ranges. Firstly, the largest potential window from -0.3 V to 0.7 V vs. Ag/AgCl reference was applied to confirm Ti\(_2\)AlC oxidation. Afterwards, the voltage range was sequentially narrowed down by 0.1 V on each end of the range until the minimum potential window of 0.0 V to 0.4 V was recorded.

It should be noted that this study was deliberately conducted after it was ensured that the CV shapes changed negligibly with scan number. Consequently, the irreversible Ti\(_2\)AlC oxidation had been exhausted to a certain extent and was not as distinct as presented in CVs which were recorded before this investigation. However, the manual manufacturing process led to a certain variance in electrode surface area between Ti\(_2\)AlC ceramic samples, and therefore differing current amplitudes. As a result of this it was preferred to conduct this study on the same electrode as previously utilised to ensure comparability.
3.5.1.5. Analysis of Ti$_2$AlC ceramic oxidation based on charge flow

To avoid misinterpretation by accidently picking outliers, the data had to be translated into a format that enabled presentation of the 166 CVs at 1 mV s$^{-1}$ and 202 CVs at 0.5 mV s$^{-1}$ in a continuous, comprehensive manner. Therefore, the measured currents were converted into the amount of charge that flowed.

Using the trapezoidal rule approximation as explained in 7.1.1, the CVs were integrated to give three types of charge that had flown throughout the experiments. The primary data output of Faradaic and non-Faradaic current was translated into absolute charge, $Q_{\text{abs}}$. To analyse the contributions to $Q_{\text{abs}}$ separately, the charge due to non-Faradaic currents was subtracted as background. This enabled the calculation of the amount of charge that had flown to oxidise, $Q_{\text{ox}}$, and the charge to reduce, $Q_{\text{red}}$, the material. Each type of charge was first calculated for each CV separately and then added to the sum of previously built up charge of that type, giving an overview of cumulative charge transferred. These data were then extrapolated to analyse the non-reversible oxidation of Ti$_2$AlC at CV scan numbers beyond the scope of this experiment. The residual sum of squares $R^2$ between each data set and its fitting function was calculated to evaluate the quality of the proposed process dynamics. The mathematical background and work steps involved in the conversion of current to the different types of charge flow as well as a detailed justification for the fitting functions can be found in 7.1.

3.5.1.6. Four terminal sensing

A flat and continuous surface is required for four terminal sensing investigations. The porous Ti$_2$AlC ceramics based on 10 ppi porous polyurethane foams were therefore unsuitable for the measurement. Instead, 3.7629 g Ti$_2$AlC precursor powder were pressed into a cylindrical mould with a hydraulic press. The compacted powder pellet was then sintered under the same conditions as the porous Ti$_2$AlC samples, as given in 3.4.6.2.

All samples were analysed at 20 °C using a Multiheight Probe setup from Jandel Engineering Limited with tungsten carbide electrodes spacing of 1.00 mm ±10 µm and a tip radius of 300 µm. The measurement accuracy was verified on a Jandel Engineering reference sample of 150 nm of indium tin oxide (ITO) on glass with a nominal sheet resistivity of 12.52 ohm sq$^{-1}$ giving 12.5 ±0.159 ohm sq$^{-1}$ when measured in triplicate. Full system specifications and sample preparation for this method can be found in 7.3. Polishing was carried out with silicon carbide abrasive sheets and followed by thorough washing with Milli-Q water.
The bulk resistivity was analysed instead of sheet resistivity, since the probe spacing of 1 mm would have required a maximum sample thickness of 625 μm to determine the latter within an acceptable error range. Both non-porous Ti$_2$AlC ceramic samples, one from our laboratories and the other from Kanthal, were more than 5000 μm thick.

### 3.5.2. Atomic force microscopy

Surface topographies were measured with a Nanosurf easyScan 2 FlexAFM system from Windsor Scientific. The system augmentations comprised a Nanosurf Environmental Control Chamber, rested on the vibration isolation system Nanosurf Isostage and encased in a Nanosurf Acoustic Enclosure 300, which also contained metallic foam lining for electromagnetic shielding. Recordings were either taken in tapping mode with TAP190-G cantilevers, or in continuous mode with Cont Al-G cantilevers, both from Budget Sensors. The cantilever holders Cantaclip SA, or Cantilver holder AO were mounted on 10 x 10 μm or 100 x 100 μm scan heads appropriate to the measuring environment.

The presented data and evaluation were based on images with 512 x 512 lines and at least one second measuring time per line into one scan direction in order to avoid edge artifacts. Each line of the image was scanned forward and backward to monitor the tracking quality of the AFM probe, allowing a total scan time of at least two seconds per line. Both laser direction and focus, were adjusted before each experiment. For measurements performed in tapping mode, the vibration frequency was optimised before each sample approach. All measurements were taken without cooling, at RTP.

### 3.5.3. Contact angle goniometry

Surface hydrophobicity was evaluated using the static sessile drop method using Milli-Q water. The measurements were taken on a FTA1000 B Class contact angle goniometer from First Ten Ångstoms at RTP, shown in figure 37.
Prior to every measurement, the sample surfaces were cleaned from surface contaminations by rinsing with Milli-Q water, followed by rinsing with ethanol and finally dried in a stream of nitrogen. The presented values are averages of multiple measurements in several locations of the samples, in order to account for inhomogeneities of the materials.

### 3.5.4. Confocal microscopy

The biofilm support quality of single and double coated Ti$_2$AlC ceramics was assessed by utilising the natural chlorophyll fluorescence of photosynthetic organisms with a LSM 510 META confocal microscope from Carl Zeiss, as shown in figure 38.
This measurement did not require any additional cross linking agents or dyes, so that biofilms could be analysed in natural form. Each image consisted of a white light optical microscopy image and a confocal image of the same area using an argon laser with the wavelengths 458, 477, 488 and 514 nm. A filter was applied, which only permitted the detection of light with wavelengths above 505 nm, in order to assure that the chlorophyll fluorescence was measured exclusively.

Porous, single and double coated Ti$_2$AlC ceramics were produced as described in 3.4.6.2 and in 3.4.6.3 respectively and were incubated in the same CVu culture according to 3.3.3.2 for more than three weeks. Fragments of the biofilm coated ceramics were extracted under sterile conditions and were examined under the microscope within two hours. The maximum fluorescence intensity was determined through a lambda measurement prior to the analysis, which consisted of imaging the same area of the sample at certain wavelengths between 502 nm to 748 nm in 11 nm intervals. Magnifications greater than 40 times were avoided, as the working distance would have become too short to focus on the uneven samples. The chlorophyll a fluorescence at 684 nm was coloured red and overlaid with the simultaneously taken bright-field images in order to gain a better understanding of biofilm thickness and coverage of the lightproof Ti$_2$AlC ceramic.

### 3.5.5. Scanning electron microscopy and energy dispersive X-ray spectroscopy

Scanning electron microscopy (SEM) was performed on Ti$_2$AlC ceramic samples using a JEOL 6480 LV scanning electron microscope equipped with an INCA X-act X-ray detector from Oxford Instruments. This microscope was chosen for SEM characterisation, since it was equipped for simultaneous energy dispersive spectroscopy, which was used for elemental mapping of regions of interest (ROI).

The inherent electrical and thermal conductivity of Ti$_2$AlC ceramic rendered coating of the samples unnecessary, which was beneficial for the elemental analysis of the sample. Samples for surfaces analysis were mounted on carbon tape and earthed with silver paint.

Surfaces or cross sections of samples were studied in secondary electron imaging (SEI) mode with an electron acceleration voltage of 10 V and a spot size of 10-20 nm in order to achieve high resolution, but avoid excessive charging of the samples and to prevent the electrons from penetrating too far into the possibly inhomogeneous material. The elemental analysis of a ROI was then carried out by switching into backscattered electron composition (BEC) imaging mode to improve the phase contrast. The acceleration voltage in this mode was set to 20 V and the spot size to 60 nm, which diminished the image resolution, but improved electron
count rate at the detector and confidence in the energy dispersive spectroscopy data.

The geometry of strut cross sections of single coated porous Ti$_2$AlC ceramics was evaluated graphically on samples, which were sintered according to setting method 1 in 3.4.6.2. Each strut was approximated as cylindrical and the average strut diameter determined from four measurements per strut in different angles. The cross section of the hollow strut interior was approximated as triangle, so that its area could be calculated from the measurement of its three sides according to Heron’s formula as described in 7.4. These calculations were performed on five strut cross sections to give an idea of coating thickness as well as space and electrode surface available to exoelectrogenic cells in Ti$_2$AlC ceramic MFCs.

### 3.5.6. Compression testing

The mechanical strength of cubical Ti$_2$AlC ceramics with an edge length of 20 mm and a porosity of 10 ppi was assessed with compression testing with a uniform, uniaxial compressive load in collaboration with the mechanical engineering department of the University of Bath, as shown in figure 39.

![Uniform and uniaxial compression testing](image)

*Figure 39: Uniform and uniaxial compression testing was performed on Ti$_2$AlC ceramics to assess their mechanical strength on the basis of compressive strength.*

The compressive stress was recorded while the compressive load was continuously raised until the ultimate stress point caused the material to fracture. Ultimate stress points could be determined with greater precision than yielding points, since the fracture resulted in a clear drop in compressive stress. Furthermore, the low ductility of ceramics led to little differences between ultimate stress and yielding points. For these reasons, the ultimate stress point was chosen as the sole indicator of mechanical strength.
3.6. Electrochemical study of microbial exoelectrogenic activity

Additional to the culturing conditions and regular re-inoculation of cultures, given in 3.3.3.2, several sterility measures were taken during microbiological experiments to prevent culture contamination. Glassware was first cleaned according to 3.2.1, then either closed with aluminium foil, or enclosed into another container, before it was autoclaved together with all other autoclavable materials. Electrodes were cleaned as elucidated in 3.2.3 and additionally sterilised with 70 vol% ethanol in water. Experiments on cultures in non-airtight containers were performed in a microbiological safety cabinet, MSC Advantage, from VWR Jencons, as described in 3.2.2. Further laboratory work was conducted in compliance with biosafety level 1 regulations as defined by the Centers for Disease Control and Prevention.[15]

3.6.1. Electron mediator

Potassium hexacyanoferrate(III), also called ferricyanide, and potassium hexacyanoferrate(II) trihydrate, known as ferrocyanide, from Sigma Aldrich were employed as electron mediators between organisms and electrodes. Experiments with these chemicals in potentially acidic environments were carried out in the fume cupboard to account for the generation of toxic and flammable HCN gas. Solutions of the light sensitive ferri- and ferrocyanide were protected from light to avoid concentration changes and formation of impurities.

3.6.2. Cathode material characterisation in a batch pMFC

All tested cathode materials were hot-pressed to the respective types of ion-exchange membrane as described in 3.4.4. In addition to creating the required concentration gradient, the membrane also assured a watertight seal between the liquid culture filled anodic chamber and the air-cathode. The applied characterisation methods did not require long time scales, thus a batch pMFC with a stagnant solution was employed, since it guaranteed shorter system equilibration periods. All components of this pMFC, shown in figure 40, were engineered and fabricated between our group and our collaborators at the University of Cambridge Department of Biochemistry. Transparent Perspex layers, separated by silicone layers, facilitated an adjustable bio-reactor volume and allowed light penetration into the anodic chamber, so that the photo-response of the photosynthetic cultures could be investigated. The silicone layers were produced with a Sylgard 184 Silicone Elastomer Kit from Dow Corning.
Figure 40: Batch pMFC cell for cathode testing showing the sequence of transparent Perspex and silicone layers with polyelectrolyte membrane (PEM) hot-pressed to the cathode material at the bottom in the side view and the position of the cylindrical 1.6 cm in diameter and 1.9 cm high anodic chamber in the top view.

All investigations presented in this work utilised the layer configuration shown in figure 40, which resulted in an anodic chamber height of 1.9 cm and a corresponding anodic chamber volume of 3.8 mL. A fluorine doped tin oxide (FTO) coated TiO$_2$ ceramic anode with 20 ppi was produced as described in 3.4.6.1 and was consistently applied in all experiments. Taking the volume of the anode into account, a comparatively small solution volume of 3.1 mL was used in these experiments to further minimise the equilibration time of the system. In this setup the tested cathode material was clamped beneath the anodic chamber with the polyelectrolyte membrane (PEM) facing towards the organism solution above. Consequently, the oxygen was supplied from beneath the cathode through an array of tubes in the Perspex layer. The anodic chamber was filled with solution through the injection orifice after the pMFC had been assembled to keep the time period between culture addition and measurement at a minimum.

The pMFC was operated in a lightproof container, only exposed to monochromatic diffuse light from an array of nine LEDs at a wavelength of 652 nm and at a distance of 24.0 cm to the middle of the LED array. Figure 41 shows the position of the cathode characterisation pMFC with regard to the light source.
Figure 41: Illumination geometry of the cathode testing batch pMFC from an array of nine LEDs with a wavelength of 625 nm.

The incident light energy onto the photosynthetic culture behind the surface of the Perspex lid was measured as 39.03 W m\(^{-2}\) with a calibrated photodiode. This amounted to 7.847 mW onto a solution surface of 2.0 cm\(^2\). The solution in the fuel cell chamber contained CVu as photosynthetic organism as well as 2.5 mmol L\(^{-1}\) potassium ferricyanide and 2.5 mmol L\(^{-1}\) potassium ferrocyanide trihydrate as redox mediators in equal amounts to reach the steady state conditions of the system faster. Before each experiment a fresh solution was prepared by centrifuging 1 mL aliquots of algae parent solution for three minutes at 13200 rpm, discarding the old supernatant and replacing it with the same volume of fresh media.

An overview of the carbon based cathode materials and combinations with ion-exchange membranes which were tested in this system, is given in 3.4.4.

The FTO coated TiO\(_2\) ceramic anode was connected to the air-cathode over a variable resistor (RM6 Decade Box, from RS Components Ltd) and the voltage generation from the pMFC over this external resistance was monitored using a High Resolution Data Logger (ADC-24, from Pico Technology) in combination with a PC, as shown in figure 40.

Polarisation curves, described in 3.6.3, as well as the photo-response to diurnal 12 hour light and dark cycles were used to evaluate the suitability of a cathode. After the resistance at the maximum power point (optimal external resistance/load) for a certain bio-electrochemical system (BES) was identified with polarisation curves, the light and dark response was measured with this resistance to gain insight into its feedback performance. At first, the light and dark response was measured under open circuit conditions and subsequently at the optimal external load.
During the experiments the injection orifice of the anodic chamber was sealed after the chamber had been filled, so that solution evaporation was minimised. The photo-response of ZACC FM10, from Chemviron Carbon, plus Nafion® 115, from Sigma Aldrich, and of carbon paper, from the University of Cambridge Department of Biochemistry, plus Nafion® 115 was used as an instrument to verify whether the reduced gas exchange had an impact on the system. Both cathodes were tested at optimal external resistance with an open and a sealed injection orifice respectively, in order to exclude misinterpretations due to cathode attributes.

The contribution of the photosynthetic microorganism CVu to the signal was examined by photo-response measurements on two BES, which differed in cathode material and respective optimal external resistance. Both systems were tested with and without CVu at optimal resistance, using the cathode assemblies ZACC FM10 plus CMI-7000S membrane and carbon felt plus Nafion® 115.

The assembly of platinum coated ZACC FM10 cathode plus Nafion® 115 was characterised through photo-response measurements at open circuit and at the optimal external resistance of the BES with the non-platinated ZACC FM10, 10 kΩ. Three samples of platinum coated ZACC FM10 assemblies were examined to validate the results and to account for possible inequalities during the sputter coating.

3.6.3. Polarisation and power curves

Polarisation curves were acquired by measuring the voltage over a sequence of different external resistances between the anode and the cathode of a pMFC. The voltage was thereby monitored with a High Resolution Data Logger (ADC-24, from Pico Technology) in combination with a PC, while the external resistances were changed on the variable resistor (RM6 Decade Box, from RS Components Ltd). A broad overview of the system response to a wide range of resistances was generated initially and the measurement repeated at a resistance range, which was concentrated around the maximum current/power generation, if necessary. The resistances were applied in sequence from large to low magnitudes, since this presented the least initial deviation from the normal operating conditions and therefore, the least disturbance and smallest equilibration periods of the BES. The initial sequence comprised the resistances: open circuit (infinite resistance); 10 MΩ, 1 MΩ, 100 kΩ, 50 kΩ, 10 kΩ, 7.5 kΩ, 2.5 kΩ and 100 Ω; with a greater data resolution for the resistances between the resistances 50 kΩ and 2.5 kΩ, since previous investigations within our group frequently revealed optimal resistances within this region. The external resistance was changed to the next value in the sequence if the mean voltage changed less than 2% of its value within 10 minutes, in order to obtain equilibrium values of the system. Current and power values were
calculated concurrent to the voltage measurement and lower resistances of the sequence were omitted, if the maximum power point was clearly crossed.

3.6.4. CV and SWV of differently fabricated Ti$_2$AlC ceramics in culture

Cyclic voltammetry (CV) on single coated, double coated and compacted Ti$_2$AlC ceramics in CVu culture was employed to investigate the interactions of exoelectrogenic organisms with this novel electrode material. Additionally, square wave voltammetry (SWV) was performed to examine the varying cyclic voltammetry responses from these bio-anodes in more detail.

The Ti$_2$AlC ceramics were produced as described in 3.4.6.2 and 3.4.6.3 and subsequently oxidised with 345 cyclic voltammograms (CVs) in the potential range 0.0 V to 0.7 V with a scan rate of 5 mV s$^{-1}$. These CVs were performed in an aqueous solution of 0.1 mol L$^{-1}$ KCl using a Ag/AgCl reference electrode and a platinum net counter electrode connected to an Autolab PGSTAT12. White precipitate from the ceramic was filtered out of solution and analysed by XRD following the CVs. All Ti$_2$AlC ceramics were washed in Milli-Q water, autoclaved according to 3.2.2 and incubated in the same CVu growth flask under the conditions described in 3.3.3.2.

CVs and SWV in a potential range of -0.3 V to 0.4 V were sporadically recorded in culture between 13 to 36 days of incubation using a platinum foil counter electrode and a Ag/AgCl reference electrode in combination with the Autolab PGSTAT12. A range of frequencies and amplitudes were tested to optimise the signal to noise ratio during the SWV. As a result, anodic currents were recorded in a frequency range of 80 to 100 Hz and an amplitude of 40 mV, while cathodic currents were measured from 8 to 12 Hz and at 5 mV.

3.6.5. Solution pH to photo-response relation from a biofilm on Ti$_2$AlC ceramic

A membraneless pMFC was created in a flask to evaluate the feasibility of a more cost-effective realisation of pMFCs, without a Nafion® membrane, and in order to simultaneously monitor the pH shifts as well as viability of a biofilm that was incubated under constant electron drain on the anode.

A single coated Ti$_2$AlC ceramic was fabricated and equipped with an electrical contact according to 3.4.6.2 before it was sterilised in an autoclave as described in 3.2.2. The sterile electrode was incubated in a CVu culture without electron starvation from an external circuit for 115 days under the conditions described in 3.3.3.2. All measuring equipment was sterilised, assembled and operated in a microbiological safety cabinet, described in 3.2.2. The biofilm coated Ti$_2$AlC anode
was then reinoculated in fresh 3N-BBM+V and connected to a platinum foil cathode over an external resistance of 10 MΩ as shown in figure 42.

Figure 42: Membraneless pMFC with a precultured CVu biofilm on a 10 ppi, single coated Ti$_2$AlC ceramic anode, which was connected to a Pt foil cathode over an external resistance of 10 MΩ. Voltage generation over the external resistance and solution pH were monitored in stirred and unstirred culture.

The voltage generation across the external resistance was measured with a High Resolution Data Logger (ADC-24, from Pico Technology), while the solution pH was monitored with a Jenway 3505 pH/mV/Temperature Meter. Fluorescent light was provided for 12 hours every day from either side of the pMFC by two 21.0 cm x 29.7 cm light boxes from Lightbox UK with a luminous intensity of 1500-1800 cd m$^{-2}$ at the surface of the light boxes and a colour temperature of 7000-8000 K. The photoresponse measurements were recorded in an unstirred solution for seven days and subsequently in stirred solution for 57 days. Initial experiments in stirred solution were conducted prior to the 57 day period to verify that the magnetic stirring plate did not cause interference with the measurements.

3.6.6. Flat FTO glass and ITO plastic anodes in air-cathode flow pMFCs

Four air-cathode pMFC, facilitating long-term investigations through a continuous flow of solution into and out of the cell, were produced in collaboration with the University of Cambridge Department of Biochemistry.

The anodes consisted of 2.5 cm x 5.5 cm pieces of either fluorine doped tin oxide coated glass (FTO glass) with an IR reflectance of 87% and a surface resistance of 6-9 Ω sq$^{-1}$ (Tec 8) from Pilkington glass, or indium tin oxide coated polyethylene terephthalate (PET) sheets (ITO plastic) from Sigma Aldrich with a surface resistance of 40 Ω sq$^{-1}$ in the 3 cm x 6 cm anodic flow cell chamber. Two flow cells were equipped with FTO glass anodes and the other two flow cells with ITO plastic anodes. Stainless steel wires were attached onto the conductive sides of the anodes.
and insulated after the contact had cured, using a sequence of silver loaded epoxy adhesive and Quick Set Epoxy Adhesive from RS Components Limited respectively. The hardened epoxy resin was coated in the biocompatible, high modulus polyether sealant, Wet Water Sticky Stuff, from Warehouse Aquatics.

Each flow cell was equipped with two ZACC FM10 cathodes, which were hot-pressed to Nafion® 115 membranes according to 3.4.4. The two cathodes were interconnected with gold wire and then sealed to the Perspex casing with Wet Water Sticky Stuff. The electric circuit was closed over a 10 kΩ external resistance and the voltage generation over this load monitored with a High Resolution Data Logger (ADC-24, from Pico Technology). Oxygen for the recombination reaction with protons and electrons from the anodic chamber was provided through 10 air holes in the Perspex casing per cathode, as shown in figure 43, in order to protect the flexible cathodes against deformation.

![Diagram of flow cell](image)

**Figure 43:** Air cathode flow cells were developed for studies on FTO glass and ITO plastic anodes (green) in pMFCs, which were connected to two ZACC FM10 cathodes (black) over an external resistance of 10 kΩ respectively. The voltage over this resistance was measured with High Resolution Data Logger ADC-24 (V).

The 1 cm high anodic chamber contained a volume of 18 mL, which was reduced by the particular anode and anode contact in each flow pMFC. Culture solution volumes varied slightly between the flow pMFCs, depending on the type of anode used and thickness of the anode contact insulation. Table 9 gives an overview of the nomenclature and the respective culture solution volumes of each pMFC.
Table 9: Culture solution volumes and identification of each of the air-cathode pMFCs with FTO glass and ITO plastic anodes.

<table>
<thead>
<tr>
<th>Flow Cell</th>
<th>ITO plastic 1</th>
<th>ITO plastic 2</th>
<th>FTO glass 1</th>
<th>FTO glass 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (mL)</td>
<td>12.0</td>
<td>13.0</td>
<td>11.0</td>
<td>10.75</td>
</tr>
</tbody>
</table>

Transparent silicone tubing of 1 mm inner diameter from Fisher Scientific was used to transport the culture solution between the four pMFCs and up to two external stock cultures, so that regular checks for clogging of the tubing could be carried out. The continuous stirring of the media with Mini Basic Hytrel magnetic stirrers from Fisher Scientific and the constant flow of solution, mediated by an ECOLINE VC-MS/CA8-6 peristaltic pump from Ismatec in combination with three-stop Pharmed BPT pump head tubing from Cole-Parmer Instrument Co. Ltd, replenished the nutrients in the pMFCs and prevented clogging of the tubing. Solution was pumped in and out of the flow pMFC, in order to minimise leakage resulting from pressure fluctuations.

The stock cultures were kept in the dark, while the pMFCs were exposed to a controlled light environment using an array of nine LEDs at a wavelength of 652 nm. The distance between the middle of the LED array and the respective centres of the pMFCs was 26.7 cm, as shown in figure 44.

Before the light irradiated the photosynthetic organisms inside the flow cell chamber, it passed through a 4.5 mm Perspex lid and a 2.5 mm silicone layer, which was prepared with the Sylgard 184 Silicone Elastomer Kit from Dow Corning. The light intensity after absorption by the Perspex layer and the subjacent silicone layer was measured as 6.774 W m\(^{-2}\) with a calibrated photodiode. Consequently, a culture surface of 3 cm x 6 cm was irradiated with 12.19 mW. The cultures were exposed to light under these conditions for 12 hours every 24 hours and were kept
in the dark for the remaining day. This was done in order to analyse the photo-
response from the flow pMFCs and allow for the respiratory metabolism of the
organisms.

The extraction of samples and modifications to the solutions in the flow cells were
performed in a microbiological safety cabinet, as described in 3.2.2, using
borosilicate glass stock solution bottles from Fisher Scientific. Nine holes of 5 mm
diameter were drilled into each of the polypropylene bottle caps, in order to enable
vital gas exchange, run tubing into the bottle, and allow sampling. The air flow
between the cultures and the laboratory environment was filtered with 38 mm
Stopper Foams from Fisher Scientific underneath the bottle caps to protect the
cultures from contamination. All tubing that was run through the stopper foams
was sealed to the bottle cap with silicone and could be disconnected from the rest
of the system through straight, barbed fittings from Cole-Parmer Instrument Co. Ltd.

Two preliminary system tests were conducted using the green algae culture CVu in
all flow pMFCs, followed by a long-term investigation, which additionally
investigated the marine cyanobacteria culture SCo. The first of the preliminary tests
investigated the system sustainability and comparability between the individual
flow cells. As depicted in figure 45a, the same stock solution of CVu plus the
artificial redox mediator mix of 2.5 mmol L\(^{-1}\) potassium hexacyanoferrate(III) and
2.5 mmol L\(^{-1}\) potassium hexacyanoferrate(II) trihydrate was employed for all flow
cells, so that respectively two flow cells worked at equivalent conditions. Following
the confirmation of the comparability between the flow cells, the second
preliminary test examined the impact of the artificial redox mediator mix by
utilising two stock solutions, one equivalent to the stock solution from the first
preliminary test and the other stock solution without the mediator mix. Each stock
solution supplied a pair of FTO glass and ITO plastic anode flow cells, as shown in
figure 45b.
Figure 45: Work plans on system sustainability and effect of the artificial redox mediator mixture of 2.5 mmol L\(^{-1}\) ferrocyanide (Fe\(^{2+}\)) with 2.5 mmol L\(^{-1}\) ferricyanide (Fe\(^{3+}\)) on air-cathode flow pMFCs with FTO glass anodes and ITO plastic anodes employed the green algae culture CVu in its growth medium Bold Basal Medium with 3-fold Nitrogen and Vitamins – modified (3N-BBM+V).

The subsequent long-term investigations explored the growth of a mixed culture of green algae and cyanobacteria in the two media, Bold Basal Medium with 3-fold Nitrogen plus Vitamins – modified (3N-BBM+V) and BG-11. Each medium supplied a pair of FTO glass and ITO plastic anode containing pMFCs. Growth media, redox mediator and culture were added in sequence and characterised with photo-response measurements respectively before the next solution component was added. In this way, each preceding solution served as control for the following one. Following the characterisation of the artificial redox mediator couple, ferricyanide and ferrocyanide each at a concentration of 2.5 mmol L\(^{-1}\), the respective culture solutions were added and the final long-term photo-response investigations performed. The whole work plan and characterised factors of the system are summarised in figure 46.
3.6.7. Porous Ti$_2$O and Ti$_2$AlC ceramics as bio-anodes in continuous flow pMFCs

3.6.7.1. Ti$_2$AlC ceramic as bio-anode in an immersed Pt cathode pMFC

The quality of Ti$_2$AlC ceramic as an electron acceptor in pMFCs was investigated with a mixed culture of photosynthetic green algae and cyanobacteria. Anodic and cathodic compartments were separated with a proton selective Nafion® 115 membrane. Each compartment was supplied with a constant flow of the BG-11 growth medium (3.3.2.2) from separate stock bottles. An ECOLINE VC-MS/CA8-6 peristaltic pump from Ismatec with 3-stop Pharmed BPT pump head tubing from
Cole-Parmer Instrument Co. Ltd, in combination with transparent silicone tubing with 1 mm inner diameter from Fisher Scientific, mediated the solution flow at a rate of approximately 1 mL min\(^{-1}\). The anodic stock solution was incubated with respective 20 mL volumes of the green algae cultures CSo, CEm and MZo as well as with the cyanobacteria culture SMa. Both 1 litre stock solutions were constantly mixed using Mini Basic Hytrel magnetic stirrers from Fisher Scientific. Gas exchange between the stock solutions and the ambient air was facilitated through Stopper Foams from Fisher Scientific underneath the perforated bottle caps. The renewal of growth medium was performed through Millex-GS Syringe Filter Units consisting of mixed cellulose esters membranes with 0.22 \(\mu\)m pore size. Anode and cathode were connected over a variable resistor (RM6 Decade Box, from RS Components Ltd) and the voltage generation was monitored with a High Resolution Data Logger (ADC-24, from Pico Technology), as depicted in figure 47.

![Nafion 115 membrane](image)

**Figure 47:** The dual liquid chamber glass pMFC utilised a 10 ppi porous Ti\(_2\)AlC ceramic anode as electron acceptor for a mixed culture of photosynthetic green algae and cyanobacteria, both grown in BG-11 media. The electric connection to the BG-11 media immersed platinum foil cathode was closed over a variable resistance and the voltage generation over this resistance was measured. A proton selective Nafion\(^{\circledR}\) 115 membrane separated anodic from cathodic chamber.

For the proof of concept, the pMFC was operated in ambient light for 80 days. The following in depth studies were conducted through the continuation of the already running pMFC under controlled light conditions for an additional 222 days. For this purpose, the ambient light was blocked with multiple layers of black canvas and the pMFC was only exposed to monochromatic light of 625 nm from a 1 W LED light source with variable light intensity (from Schott) for 12 hours every day.
The long-term response of the mixed culture to changes in external resistance, light intensity and starvation were examined on the basis of voltage generation and photo-response. In addition, polarisation curves were measured regularly according to 3.6.3, in order to evaluate the short and long term effects of these changes in operating conditions on the pMFC power generation efficiency.

The pMFC was operated with an external resistance of 10 kΩ for the first 33 days, followed by a reduced resistance of 1 kΩ for 51 days and a subsequently elevated load of 80 kΩ for 218 days. Starvation of the culture from fresh media for 162 days was studied during the last period at 80 kΩ.

Furthermore, an investigation on optimal lighting conditions was conducted by measuring polarisation curves while the pMFC was exposed to either monochromatic red light at a wavelength of 625 nm, fluorescent light, or kept in dark. The first and last condition were realised with the standard setup and fluorescent light was provided from two flat 21.0 cm x 29.7 cm light boxes (Lightbox UK), above and below the pMFC. Each lightbox exhibited a luminous intensity of 1500-1800 cd m\(^{-2}\) at its surface and was positioned at a distance of 15 to 30 cm from the glass walls of the pMFC. Investigations on the power generation from either light source were conducted during the daily routine 12 hour illumination period and polarisation curves in the dark were accordingly performed during the respective dark incubation period. A set of polarisation curves was measured with especially long equilibration times to explore the time scale for representative measurements. As a consequence of this investigation, the pMFC was operated in the dark for nine days after it had been run under controlled diurnal illumination cycles for 61 days. Finally, the LED was pointed away from the pMFC after 129 days and instead shone onto the anodic stock bottle for the remaining 94 days. All system operation investigations are summarised in one timeline, shown in figure 48.
Figure 48: Timeline overview of the experimental conditions during the long-term investigations on the two-liquid-chamber pMFC consisting of a 10 ppi porous Ti$_2$AlC ceramic as bio-anode in a mixed culture of green algae and cyanobacteria, which was separated from the Pt foil cathode by a Nafion® membrane.

The experiment was completed by disassembling the pMFC under sterile conditions, so that samples of the Ti$_2$AlC ceramic bio-anode could be extracted for cross section SEM as well as EDX analysis as described in 3.5.5. Biofilm samples were reinoculated in fresh BG-11 media for further use in pMFCs.

3.6.7.2. Fabrication of air-cathode flow MFCs for the screening of anodes

Flow pMFCs were manufactured to contain the TiO$_2$ and single coated Ti$_2$AlC ceramic anodes that were too large for application in the flow pMFCs described in 3.6.6. The air-cathode pMFC design was continued, so that the previously characterised cathode assembly of ZACC FM10 hot-pressed to a Nafion® 115, described in 3.4.4, could be utilised again. The airtight locking mechanism from a Lock & Lock Rectangular Container (HPL805 from Hitch & Hike), with an inner volume of 180 mL and outer measurements of 108 mm x 88 mm x 48 mm, was adapted for quicker exchange of electrode materials and easier machinability than previously possible with the flow pMFCs described in 3.6.6. The cathode was clamped between two plates on the sides of the casing, as shown in figure 49, in order to prevent biofilm formation on the cathode and simultaneously reduce the shading of the anode by the cathode.
Aluminium plates of 3.0 mm thickness on the outside of the cell served as electrical cathode contacts, while 5.0 mm Perspex counterplates assured that the watertight proton exchange membrane was pressed sufficiently tight against the casing. The size of the elliptical cathode window and required measurements for the production of the anode contact are illustrated in figure 50. Transparent silicone tubing with 1 mm inner diameter from Fisher Scientific was employed to transport the anodic electrolyte between the flow pMFCs and the stock solution, as described in 3.6.7.1. The Lock & Lock containers were operated upside down with the anodes fixed to the container lid. Hence, the opaque plastic bottom of the original container was replaced with clear Perspex, in order to improve the light intensity inside the pMFC, and anodes were swapped by exchanging the whole container lid.

3.6.7.3. FTO coated TiO$_2$ ceramics in a continuous flow air-cathode pMFC

Fluorine doped tin oxide (FTO) coated TiO$_2$ ceramics were produced at a size of 6.0 x 7.9 x 0.5 cm$^3$ according to 3.4.6.1 and used for primary investigations on the
continuous flow air-cathode pMFCs described in 3.6.7.2. The internal surface area of these electrodes was approximated to be 50 to 60 cm$^2$ on the basis of previous characterisations of FTO coated TiO$_2$ ceramics with the same porosity through cyclic voltammetry in ferricyanide by Rebecca Thorne.$^{[11]}$ The lighting conditions and external resistance of 10 k$\Omega$ during the experiments in 3.6.6 were resumed to allow comparisons between pMFC systems.

Four pMFCs were divided into pairs and connected to their respective light screened stock bottle with transparent silicone tubing of 1 mm inner diameter from Fisher Scientific. The impact of each solution component on the photo-response from the pMFCs was investigated by stepwise addition of the components to the stock bottles under sterile conditions. Straight, barbed fittings from Cole-Parmer Instrument Co. Ltd were used as decouplers and kept in sterile containers, when the solution modifications were carried out. The sequence of analysed solution components comprised growth media, photosynthetic culture, artificial redox mediator at low concentration and then at 10 times higher concentration. Consequently, one stock bottle was inoculated with the green algae culture CEm in 3N-BBM+V growth medium and the other bottle was filled with the cyanobacteria culture SCy in BG-11 growth medium. Both cultures and growth media are described in 3.3.1 and 3.3.2 respectively. A mixture of equal amounts of ferricyanide and ferrocyanide was employed as an artificial redox mediator. The initial lower concentration contained 0.25 mmol L$^{-1}$ of each mediator ingredient and the following higher concentration contained 2.5 mmol L$^{-1}$. Vital gas exchange between culture and air was facilitated through Stopper Foams from Fisher Scientific underneath the perforated bottle caps. The constant solution exchange between pMFCs and stock bottles was realised by an ECOLINE VC-MS/CA8-6 peristaltic pump from Ismatec with 3-stop Pharmed BPT pump head tubing from Cole-Parmer Instrument Co. Ltd with the same flow rate for each pMFC. Both stock cultures were constantly stirred with Mini Basic Hytrel magnetic stirrers from Fisher Scientific and reinoculated in the respective fresh media every month.

### 3.6.7.4. Ti$_2$AlC ceramic and vitreous carbon foam anodes in air-cathode pMFCs

The photo-response and polarisation curves of four double coated Ti$_2$AlC ceramics were compared to two samples of the likewise inherently conductive Duocel Reticulated Vitreous Carbon (RVC) foam from ERG Aerospace Corporation. All Ti$_2$AlC ceramics were manufactured on the basis of templates with 10 ppi at a size of 2.5 x 8.0 x 5.5 cm$^3$ and provided with an electrical contact according to 3.4.6.3. The RVC foams were received in the size 8.8 x 3.5 x 0.6 cm$^3$ with 10 ppi and 20 ppi. The specific surface area of the 10 ppi RVC foam was determined as 27.72 cm$^2$ and of the 20 ppi samples as 64.68 cm$^2$ corresponding to the company’s own multipoint
BET adsorption measurements with krypton gas at cryogenic temperatures.\textsuperscript{[16]} Cyclic voltammetry was conducted at different scan rates on an additional Ti\textsubscript{2}AlC ceramic sample in 3 mmol L\textsuperscript{-1} ferricyanide plus 100 mmol L\textsuperscript{-1} KCl background electrolyte and the results on specific surface area were used to determine the surface area of the four Ti\textsubscript{2}AlC ceramic bio-anodes. This was done in order to exclude any possible material changes in the Ti\textsubscript{2}AlC ceramic during the surface area characterisation affecting the pMFC measurements. The specific surface area of each Ti\textsubscript{2}AlC ceramic was determined as 356.0 cm\textsuperscript{2} according to the evaluation of the background corrected peak currents at 0.31 V in combination with ferricyanide diffusion coefficients found by Konopka et al.\textsuperscript{[17]} All measured quantities were normalised per square meter of specific electrode area.

The electrodes were all applied in the air-cathode pMFCs elucidated in 3.6.7.2. Monochromatic light with a wavelength of 625 nm was provided from an array of LEDs for 12 hours every day in a controlled light environment, as described in 3.6.6. All flow cells were supplied from the same light screened stock bottle, which contained the mixed culture of green algae and cyanobacteria that was harvested from the electrode biofilm at the end of experiment 3.6.7.1. As opposed to previous systems, the culture solution was not fed back into the stock bottles after passing through the pMFCs. The buildup of toxic substances during culture growth and the contamination of the stock culture was slowed down by guiding the stock solution through dripping points before it entered the pMFCs, which did not permit any backflow of solution into the stock culture. The excess culture solution from the pMFCs was collected in an overflow bottle positioned at a lower level, as shown in figure 51.
Figure 51: Air-cathode flow pMFC setup for investigations on Ti$_2$AlC ceramic anodes in mixed culture with dripping points and collection of pMFC effluent in an overflow bottle instead of recirculation to minimise contamination and premature culture death resulting from buildup of toxins.

The flow of stock culture into the pMFCs was mediated by an ECOLINE VC-MS/CA8-6 peristaltic pump from Ismatec for 10 minutes every 12 hours with a flow rate 0.5 mL min$^{-1}$ per flow cell. Air exchange was facilitated through Stopper Foams from Fisher Scientific underneath the perforated stock culture bottle caps and fresh BG-11 media was added through Millex-GS Syringe Filter Units. The stock culture was constantly stirred with Mini Basic Hytrel magnetic stirrers from Fisher Scientific.

3.6.8. PMFC power generation in relation to bio-anode surface features

The influence of surface morphology and surface energy of anode materials in pMFC power generation was investigated in collaboration with the University of Cambridge’s Departments of Biochemistry, Materials Science and Metallurgy, Chemical Engineering, and Biotechnology.

Fluorine doped tin oxide coated glass (FTO glass) with an IR reflectance of 87 % and a surface resistance of 6-9 $\Omega$ sq$^{-1}$ (Tec 8), from Pilkington glass, was cut into 10.0 cm x 2.0 cm pieces and electrochemically coated in PANI according to 3.4.5 at the University of Bath. These PANI FTO glass electrodes were compared to equally sized pieces of indium tin oxide coated polyethylene terephthalate (PET) sheets (ITO plastic) from Visiontek Systems Ltd with a surface resistance of 60 $\Omega$ sq$^{-1}$, carbon paper (C-paper) from the University of Cambridge Department of Biochemistry, and 304 stainless steel sheets of 1 mm thickness from MetalOffCuts Sheet Metal Store.
UK. Atomic force microscopy, as described in 3.5.2, was employed in continuous mode with Cont Al-G cantilevers from Budget Sensors to determine the average surface roughness of each anode on the basis of 10 μm x 10 μm scans in different locations on the respective sample. Regarding the PANI FTO glass electrodes, this was done before and after the PANI coating. The contact angles of Milli-Q water on the sample surfaces were measured as described in 3.5.3.

All anode materials were characterised at the University of Cambridge in an air-cathode pMFC with five anode chambers made from Perspex, each 12.0 cm x 16.0 cm x 1.0 cm, as shown in figure 52.

![Figure 52](image)

**Figure 52:** Multichannel pMFC for the comparison of stainless steel, PANI coated FTO glass, carbon paper and ITO plastic anodes under equal conditions. The technical drawing (a) and the image of the actual device with a steel electrode in each anodic chamber for demonstration (b) were adapted from the joint publication on our investigations.\(^8\)

Carbon paper from Alfa Aesar was coated in 3 mg platinum per m\(^2\) and served as air-cathode at a size of 4.5 cm x 1.6 cm in each of the five chambers. The cathodes were positioned below the anodic solution level, at a distance of 0.5 cm from the anodes and connected with them over an external resistance of 1 MΩ. The voltage over this external resistance was monitored in one minute intervals with a High Resolution Data Logger (ADC-24, from Pico Technology). The cyanobacteria culture *Oscillatoria limnetica*, from the Culture Collection of Algae and Protozoa, was used as an electron source in BG 11 growth medium in the anodic chambers. For this purpose, the samples of one parent culture were centrifuged at 4000 g for 10 minutes, resuspended in fresh BG 11 medium and inoculated in every anodic chamber 12 hours prior to closing the electric circuit. Photo-response measurements were performed with two hour cycles alternating between light and dark. The chlorophyll content of biofilm samples from each chamber was determined 12 hours, four days and eight days after inoculation, in order to account for differences in growth rate between the chambers by normalising the
power generation per cell. After four days polarisation curves were conducted in light with approximately 8 W m$^2$ as well as in dark with external resistances ranging from 10 MΩ to 5 kΩ and readings were taken, if the voltage changed less than 0.1 mV min$^{-1}$. The final polarisation curves and the power curves resulting from these were the result of at least seven repeats.
3.7. References in experimental chapter

Chapter 4

Metal-organic framework MIL101 toxicity in green algae and cyanobacteria
4. MOF MIL101 toxicity in green algae and cyanobacteria

4.1. MOF MIL101 toxicity abstract

The growth of green algae and cyanobacteria cultures in the presence of the chromium carboxylate based metal-organic framework (MOF) MIL101 and its amine-modified version MIL-101(Cr)-NH$_2$ was assessed to evaluate toxic and growth enhancing effects prior to applications in photo-microbial fuel cells (pMFCs). Flow cytometry confirmed that MOF MIL101 is harmless to the tested cultures in concentrations up to 480 mg L$^{-1}$ and MIL-101(Cr)-NH$_2$ did not exhibit toxic effects at a concentration of 167 mg L$^{-1}$. Healthy biofilms were grown without exclusion zones to MOF pellets on agar plates. However, dispersions of any of the two MOFs did not reveal evidence that indicated an improved bio-availability of CO$_2$ during culturing in ambient air or in a CO$_2$ saturated atmosphere.

Metal poisoning from the electron mediator mixture of ferricyanide and ferrocyanide, commonly utilised in MFC research, was shown to diminish chlorophyll contents per cell of *Chlorella emersonii* and *Synechocystis PCC6803* within six days.$^{[1-3]}$ This caused growth rates to stagnate irrecoverably for 66 days and demonstrated that these cultures are not suited for bioremediation of heavy metals in wastewater.

None of the metal poisoning effects were seen in the presence of MOF MIL101 for 72 days, or with MIL-101(Cr)-NH$_2$ for 62 days. The potential wastewater contamination risk from the disposal of MOF MIL101 containing waste is therefore considered small.
4.2. The potential of MOF MIL101 for pMFCs

MOFs have shown great potential for applications in gas storage, gas separation and catalysis due to their high selectivity and flexibility in functionalisation of both organic linkers and metal sites.\[^{[4]}\] The use of organic linkers between metal clusters allows larger pores sizes and expands the range of target molecules compared to zeolites.\[^{[5]}\] In particular the capacity for CO\(_2\) capture is promising for the application of MOFs in pMFCs. Improved accessibility to CO\(_2\) results in larger growth rates of photosynthetic cultures and this translates into higher carbon capture rates of pMFCs. Transfer of CO\(_2\) from the MOF to the organism can also improve the regeneration time of the MOF for further CO\(_2\) capture.\[^{[6]}\] So far, the sensitivity to hydrolysis was a major difficulty for the application of MOFs in pMFCs.\[^{[7]}\]

However, the MOF MIL101 (MIL standing for Materials of Institute Lavoisier) has distinguished itself from other MOFs by remaining stable in boiling water for a week and exhibited record capacities for carbon dioxide uptake of 40 mmol g\(^{-1}\) at room temperature.\[^{[8]}\] BET fitting of N\(_2\) adsorption isotherms estimated its surface as 4230 m\(^2\) g\(^{-1}\) and the pore volume as 2.15 cm\(^3\) g\(^{-1}\). The captured CO\(_2\) is usually desorbed from MOFs at increased temperature. This energy input effectively also releases CO\(_2\) into the environment and reduces the carbon efficiency of MOFs.\[^{[9]}\] An implementation of MOFs in pMFCs could therefore be beneficial for the efficiency of both technologies, if the captured CO\(_2\) could be made accessible to the organisms without heating the MOF. Furthermore, the work of collaborators showed that the MOF selectivity for CO\(_2\) could be improved through the modification of the organic linker with an amine group, resulting in the MOF MIL-101(Cr)-NH\(_2\).\[^{[10]}\]

MOF MIL101 is synthesised from chromium(III) salts and terephthalic acid in the presence of HF during a hydrothermal process at 220-240 °C.\[^{[7]}\] CO\(_2\) molecules can coordinate to the chromium sites in the resulting chromium(III) terephthalate. Furthermore, these chromium sites have been found to act as a catalyst for oxidation reactions.\[^{[11]}\]

Thus, toxicological studies of MOF MIL101 and MIL-101(Cr)-NH\(_2\) are required prior to an application in pMFCs, in order to assess possibly harmful oxidative effects and the chance for chromium leaching into the environment. Investigations were performed on the basis of green algae and cyanobacteria growth rates, since these cultures are commonly employed in pMFCs.
4.3. Results and discussion of MOF MIL101 toxicity

4.3.1. MOF MIL101 effect on cell density and chlorophyll content

The growth of the green algae culture *Chlorella emersonii* (CEm) and of the cyanobacteria culture *Synechocystis PCC6803* (SCy) was examined with flow cytometry in pure culture, in the presence of an artificial electron mediator and in a dispersion of MOF MIL101. A mixture of 2.5 mmol L\(^{-1}\) ferricyanide and 2.5 mmol L\(^{-1}\) ferrocyanide served as a mediator additive, as it was used in several pMFC experiments in this work and represents the effects of a toxic environment.\(^{[1]}\) MOF MIL101 was used at a concentration of 167 mg L\(^{-1}\), which was equivalent to a six times higher chromium concentration than the EC\(_{50}\)s for marine diatoms and a 40 times higher concentration than the EC\(_{50}\)s for fresh water algae. As a result of this, a toxic effect would have been identified, even if the chromium had only partially leached into the solution.\(^{[12,13]}\)

The CEm controls without additives as well as the CEm cultures with MOF resulted in cell density variations of more than a magnitude between the cultures, while the presence of mediator caused consistently lower cell densities. This variation was caused by initially diminishing cell densities in one control and one MOF culture, which continued into an approximately 14 day long lag phase before positive growth took place. The lag phase in the other control and MOF cultures was equally long, but was not preceded by such a strong decline in cell density. Consequently, the control and MOF culture without initial death phase were grouped together in figure 53a and the ones with initial cell density decline are shown in figure 53b. Both sets are compared to the averaged CEm plus mediator cultures.

**Figure 53:** CEm cell densities of controls without additives (black), in presence of either 2.5 mmol L\(^{-1}\) ferricyanide and 2.5 mmol L\(^{-1}\) ferrocyanide (red), or 167 mg L\(^{-1}\) MOF MIL101 (blue): a) Largest growth rates of CEm resulting in a multiplication of 73 times the initial cell density in presence of MOF MIL101, while the cell...
density only multiplied 20 times without additives; b) The lower CEm growth rates resulted in a multiplication of the initial cell densities by 3.9 times in presence of MOF and 0.7 times in the control. The addition of mediator reduced the cell density to 18 % of the initial count by the end of a and b respectively.

The CEm culture in the presence of MOF (blue) increased its initial cell density 73 times within 27 days in figure 53a, but only by 3.9 times within 72 days in figure 53b, while the initial number of cells in the control cultures (black) increased 20 times in figure 53a and decreased to 74 % in figure 53b. The results therefore showed that maximum CEm growth can be achieved in the presence of MOF MIL101 and did not suggest a toxic effect of MOF MIL101 on CEm; however, indications for enhanced growth were also not seen. A clear toxic effect was seen in the presence of the ferricyanide and ferrocyanide mixture, which decreased the cell density to 12 ±1 % within six days and kept the culture at consistently low cell densities of 18 ±4 % at day 27 and 18 ±10 % at day 72.

All SCy cultures entered the exponential growth phase within the first two days of incubation, but continuation into the stationary phase occurred at varying times corresponding to the additives in the culture, as shown in figure 54.

![Figure 54: SCy cell densities of controls without additives (black) multiplied by 127 times, in the presence of 167 mg L\(^{-1}\) MOF MIL101 (blue) by 98 times, while the number of cells only doubled in a mediator mixture of 2.5 mmol L\(^{-1}\) ferricyanide and 2.5 mmol L\(^{-1}\) ferrocyanide (red).](image)

The exponential growth of SCy in the presence of the mediator (red) abated after two days and entered the stationary phase following day 6, with a maximum of 7.8 ±0.2 times of the initial cell density at day 16, which was followed by a continuously decreasing cell count down to 2.0 ±0.1 times by day 72. MOF cultures (blue) exhibited exponential growth for 13 days and then entered the stationary phase, while the growth rate of the control cultures (black) continued to gradually...
decrease until the stationary phase was entered after 27 days of growth. The initial 13 days of incubation therefore did not suggest a toxic effect of MOF MIL101 on SCy, but the earlier start of the stationary phase indicated a growth limitation due to the MOF. At day 72 the number of cells in the control culture had multiplied by 127 ±6.9 times, while the SCy cells in presence of MOF MIL101 confirmed inhibited growth with a multiplication of 97 ±9.7 times.

The chlorophyll content per cell was monitored simultaneously to the cell density and showed declining levels over time in the presence of the toxic mediator mixture in CEm and SCy cultures, while the addition of MOF MIL101 did not cause such an effect. The reliability of these results was assured with two filters during the flow cytometry measurements. One filter selected according to the cell morphology through measurement of the forward and side scattering of the lasers; which corresponded to the cell size and shape respectively. The plot of cell number against red fluorescence intensity resulted in a peak corresponding to the typical chlorophyll a content of a culture and the minimum fluorescence intensity of this peak was used a second filter. Both filters were defined during measurements of control cultures and applied to distinguish the data on the target culture from chlorophyll containing cell debris and possible contaminations. Data on green algae cells was shown in graphs of yellow against red fluorescence intensity, in order to account for as many photosynthetic pigments as possible. [14,15] Cyanobacteria lack chlorophyll b, so the corresponding data is presented in graphs of forward laser scatter against red fluorescence intensity. [16]

The chlorophyll content of CEm at different stages of growth is shown in figure 55 by an array of graphs, which are separated into three columns for control, mediator and MOF cultures and vertically ordered according to the time of measurement. Healthy photosynthetic cells have been denoted by the red circles, which varied in size according to the refinement of the filter conditions.
Figure 55: Chlorophyll content per cell of CEm cultures in a plot of yellow against red fluorescence. The array of graphs compares the chlorophyll content of the control (left column) to the effect of 2.5 mmol L⁻¹ ferricyanide and 2.5 mmol L⁻¹ ferrocyanide (middle column) as well as to the impact of 167 mg L⁻¹ MOF MIL101 (right column) over a vertical time axis. The red circles resulted from a forward scatter filter for cell size and side scatter filter for cell shape.

The chlorophyll content per cell started to diminish in the presence of mediator after the initial decline in cell density had stopped, as seen by the decreasing red fluorescence intensity at day 6. The amount of cells with reduced chlorophyll content gradually increased during the lag phase until day 27 and a recovery towards larger contents was not observed until day 72. Smaller amounts of chlorophyll per cell were also detected more frequently in control and MOF cultures as the cultures aged. However, these low chlorophyll particles did not
match the size requirements for CEm cells and were probably agglomerating cell debris. The chlorophyll content per cell did therefore not suggest a toxic effect of MOF MIL101 on CEm.

SCy cultures exhibited the same response of decreasing chlorophyll a content to mediator addition, as illustrated in figure 56. As opposed to the CEm cultures, the incipient diminishment in chlorophyll a content in SCy cells was preceded by an exponential growth phase instead of a decline in cell density. However, the amount of chlorophyll per cell did not recover in mediator cultures until day 72 of the experiment, indicating metal poisoning due to ferricyanide and ferrocyanide. This effect was not observed in the chlorophyll content of MOF cultures, which suggested that their earlier transition to the stationary phase and the lower cell densities compared to the control cultures were not caused by a toxic effect of the MOF MIL101.
Figure 56: Chlorophyll content per cell of SCy cultures in plots of forward laser scatter (cell size) against red fluorescence intensity (chlorophyll content). The red circles distinguish the SCy culture from cell debris and resulted from a side versus forward scatter filter and a cell count versus red fluorescence filter. Compared to the cultures without additives (left column), the addition of 2.5 mmol L$^{-1}$ ferricyanide and 2.5 mmol L$^{-1}$ ferrocyanide (middle column) caused diminishing chlorophyll content over time, while the absence of this effect in presence of 167 mg L$^{-1}$ MOF MIL101 (right column) suggested that it is not toxic to SCy.

It should be noted that the density of data points in flow cytometry graphs is dependent on the dilution of the sample and not an indication for the actual amount of cells with certain characteristics. The measurement required varying dilutions to adjust the sample cell density to the flow cytometer confidence range as the cell density increased over time, which caused an unequal amount of data points between graphs.
Preliminary cell counts were conducted with haemocytometry on further green algae and cyanobacteria cultures. This examination was performed on one pair of control and MOF MIL101 culture for each species, so a large error can be assumed. These experiments were therefore only carried out as a qualitative control to identify cultures which were not suited for work with MOF MIL101. For this reason, a nearly threefold larger dose of 480 mg L⁻¹ MOF MIL101 was added to the cultures.

The green algae cultures *Chlorella sorokiniana* (CSo), *Chlorella vulgaris* (CVu) and *Muriella zofingiensis* (MZo) did not exhibit an explicit toxic response to MOF MIL101, as shown in figure 57.

![Figure 57: Preliminary cell counts of green algae cultures did not show a toxic effect of MOF MIL101 in a concentration of 480 mg L⁻¹ (red) compared to controls (black) in the case of: a) Chlorella sorokiniana; b) Chlorella vulgaris; and c) Muriella zofingiensis.](image)

Variation in cell density was observed between the control and MOF cultures, but the cell counts remained within the same magnitudes and all the MOF cultures of green algae remained in the exponential growth phases for over six days.

The tests on cyanobacteria involved *Synechococcus WH5701* (SCO) and confirmed the tolerance of SCy to MOF MIL101 in three times larger concentration, as illustrated in figure 58.
Figure 58: Preliminary cell counts of cyanobacteria cultures in the presence of 480 mg L\(^{-1}\) MOF MIL101 (red) compared to controls (black) of: a) Synechocystis PCC6803, which did not exhibit a toxic response; and b) Synechococcus WH5701, which indicated a toxic response with diminishing cell counts.

An initial decrease in SCy cell density in both the control and MOF culture in response to the inoculation in fresh media suggested that the parent culture had already reached too high cell densities. The exponential growth phase commenced from day 12 and caused the SCy population in the presence of MOF to exceed the control in all further cell counts. This higher growth rate in the presence of the three times larger dose of MOF MIL101 suggested that the lower SCy cell densities with MOF during the flow cytometry experiment did not result from MOF MIL101 toxicity. SCo cultures showed diminishing cell counts in the presence of MOF, while the control culture cell density increased throughout the initial 21 days, which would imply a toxic effect. However, the SCo control culture also exhibited an early death phase without indications for a stationary phase between day 21 to 30 and culture death due to unsuitable culturing conditions is therefore more likely.

4.3.2. Exclusion zones around MOF MIL101 pellets on agar colonies

Plans for the implementation of MOFs in pMFCs required pronounced culture growth in the vicinity of the MOF, so that captured CO\(_2\) could be utilised to improve growth rates and consequently power generation in the pMFC. The MOF MIL101 powder was therefore pressed to pellets of approximately 1-2 cm size, which were placed on agar plates. The plates were inoculated with freshly prepared green algae and cyanobacteria cultures on agar plates immediately before the pellet was added, in order to evaluate the MOF effect on biofilm density. Hence, this method distinguished itself from cytometry by revealing localised information on long-term growth, rather than giving averaged results on the whole culture.

An exclusion zone between the biofilm and the MOF pellet was not observed with any culture, which confirmed the cytometry results on the toxicological harmlessness of MOF MIL101. Promoted growth in the proximity of the pellet was
observed in most cultures before a continuous biofilm covered the agar plate, as shown in figure 59, on the basis of the green algae cultures CSo (a), CVu (b), MZo (c) and with a mixed culture of green algae and cyanobacteria (d). The respective period of incubation was indicated above the images and a second row of microscopy images with 40 times magnification underneath the camera pictures confirmed that an exclusion zone was also not formed on a micrometer scale.

Figure 59: Exclusion zones to MOF MIL101 pellets on agar plates were not observed with a camera after five and 41 days, or with 40 times magnification under a microscope following 10 and 40 days of incubation with: a) Chlorella sorokiniana; b) Chlorella vulgaris; c) Muriella zofingiensis; d) Mixed culture of green algae and cyanobacteria. All cultures exhibited promoted growth in the vicinity of the MOF MIL101 pellet.

The camera images of CSo and mixed culture after five days as well as MZo after 41 days demonstrate that the densest biofilms formed in some distance from the pellet, which varied in the range of micro- to millimetres along the edges of each pellet. Microscopy with 40 times magnification confirmed that biofilms formed without exclusion zones between the pellets and each culture. It should be noted that colour differences between the microscopy images were only due to changes in the image recording settings and are not representative of changes in chlorophyll content.
The cyanobacteria cultures SCo and *Spirulina maxima* (SMa) also did not show exclusion zones between biofilms and MOF pellet, which gave further evidence that MOF MIL101 was not toxic and that the declining SCo cell densities in the 480 mg L\(^{-1}\) MOF MIL101 dispersion in 4.3.1 resulted from unsuitable culturing conditions. The SMa biofilm density was affected by the MOF pellet in a more complex manner, as demonstrated in figure 60a.

![Figure 60: Camera pictures after five and 41 days as well as microscopy images after 10 and 40 days of incubation with a MOF MIL101 pellet on an agar plate did not show any separation between the pellet and the cyanobacteria biofilms of a) *Spirulina maxima*; and b) *Synechococcus WH5701*. Both cultures formed denser biofilms in the vicinity of the pellet.](image)

Contrary to the green algae biofilms, the SMa culture grew densest on the circumference of several circles along the MOF pellet edges and displayed reduced biofilm density within the circles. Figure 60b shows the sparse growth of SCo biofilm on the agar plate during 41 days of incubation and continuous SCo biofilms were only recognised several months later. The SCo agar plate was prepared from the same BG-11 media as the SCo test solutions in 4.3.1, which suggests that the low growth rates in both experiments could be improved with a change in growth medium.

### 4.3.3. CO\(_2\) capture capability and toxicity of MOF MIL-101(Cr)-NH\(_2\)

Our collaborators functionalised MOF MIL101 with an amine group on the organic linker, in order to increase the flexibility for further modifications and enhance its affinity for CO\(_2\), which are promising qualities for applications in pMFCs.\(^{[10]}\) The resulting MOF MIL-101(Cr)-NH\(_2\) was therefore assessed for its effects on growth and chlorophyll content of the green algae cultures CSo and MZo as well as of the cyanobacteria culture SMa. The study was focused on investigating whether dispersions of MOF MIL-101(Cr)-NH\(_2\) can support growth by increasing the concentration of available CO\(_2\).\(^{[17]}\) Thus, cultures were either incubated with 167 mg L\(^{-1}\) doses of CO\(_2\) preloaded MOF, or MOF without previous CO\(_2\) treatment and
the latter was divided into cultures with and without constant CO₂ bubbling. MOF-free control cultures were also grown with and without CO₂ bubbling, which resulted in five different culturing conditions for the growth of each organism. The respective culturing conditions were produced in triplicate and the flow cytometry data is shown as the resulting average.

Neither growth rates, nor chlorophyll contents per cell of green algae and cyanobacteria were affected by MOF MIL-101(Cr)-NH₂, but the CO₂ bubbling caused long lag phases, which resulted from excessive cell destruction according to the data on chlorophyll content.

Figure 61 shows the cell densities for all growth conditions of CSo (a) and MZo (b).

*Figure 61:* Green algae growth curves without additives (black), in presence of 167 mg L⁻¹ MOF MIL-101(Cr)-NH₂ (red), with CO₂ pre-treated MOF MIL-101(Cr)-NH₂ in the same concentration (blue), under constant CO₂ enrichment without further additives (green) and with 167 mg L⁻¹ MOF MIL-101(Cr)-NH₂ (orange). a) Chlorella sorokiniana exhibited a second lag phase in both cultures with CO₂ enrichment between 10-25 days, while MOF MIL-101(Cr)-NH₂ did not inhibit growth; b) Muriella zofingiensis cultures with CO₂ enrichment remained in the lag phase for at least one month and indicated inhibited growth in the presence of CO₂ pretreated MOF MIL-101(Cr)-NH₂ while untreated MOF did not affect growth.

All CSo cultures exhibited an initial lag phase of approximately four days for the adaptation to fresh media before the exponential growth phase began. The growth of CSo with CO₂ bubbling (green and orange) was inhibited from day 10, but continued from day 24 until similar cell densities as in the other CSo cultures were observed. CSo control (black), non-treated MOF culture (red) and preloaded MOF culture (blue) showed all stages of a standard growth curve and only differed in the cell density decline rate during the death phase from day 32, when the cell counts of the non-treated MOF culture decreased less than the other two cultures. The CSo control, non-treated and preloaded MOF cultures achieved maximum multiplication of the initial cell densities at day 32, which were 54 ±12 times, 79 ±17 times and 40 ±2 times, respectively. In comparison, the CO₂ enriched control culture (green) had multiplied 27 ±6 times at day 48 and the CO₂ enriched MOF culture (orange) achieved 39 ±3 times the initial cell density at day 60.
The constant CO$_2$ enrichment caused MZo cultures to remain in the lag phase for at least 32 days before the population started to increase and therefore showed a greater sensitivity of MZo to the adverse effects than seen in CSo cultures. Initial cell densities were only multiplied by 4 ±3 with MOF and 7 ±2 times without MOF. The remaining MZo cultures displayed continuously increasing cell densities and the presence of untreated MOF did not influence the growth rates. A lower growth rate was observed in the MZo cultures in the presence of preloaded MOF, which could have also resulted from the 12 times larger starting cell density compared to the control and non-treated MOF culture. The growth relative to the initial cell density was correspondingly low in the preloaded MOF culture and only increased by threefold, while the MZo control increased 96 ±4 times and the untreated MOF culture 109 ±10 times.

The solution pH of all cultures was tested on day 54, in order to assess if the increased CO$_2$ levels caused an acidification of the medium, which could have inhibited the growth. However, the pH of all CSo cultures and CO$_2$ enriched MZo cultures was in the range of 6-8 and only the remaining MZo cultures showed a slightly alkalised pH of 8-10.

Chlorophyll a and b content per cell is depicted in graphs of yellow against red fluorescence and arranged into an array of columns according to the culturing condition and rows corresponding to the measuring date. The graphs on CSo chlorophyll content are shown in figure 62 with the same signal amplification and graph scales to allow a direct comparison between all graphs.
Figure 62: Chlorophyll content of *Chlorella sorokiniana* (C) is presented in graphs of yellow against red fluorescence over a vertical time axis. The presence of 167 mg L\(^{-1}\) MOF MIL-101(Cr)-NH\(_2\) in the culture was denoted with (M), CO\(_2\) pretreatment with (L) and constant CO\(_2\) enrichment with (B) in the letter code above the regarding column. The red circles identify the culture according to size exclusion and cell count versus fluorescence filters.

A general accumulation of particles with gradually decreasing chlorophyll a content was observed in all samples as the cultures aged. These particles are depicted outside the red circles, since their diameter was smaller than expected of green algae cells, which indicated that these were naturally agglomerating cell debris. Samples of CO\(_2\) enriched CSo cultures (CB and CMB) exhibited a disproportionally large amount of cell debris in the period from day 10 to day 24. This suggested that the stagnating cell densities during this time were not caused by nutrient limitation or growth inhibition, but by excessive destruction of cells. The second growth phase of CO\(_2\) enriched CSo cultures resulted in a smaller ratio of cell debris to healthy cells in the graphs of day 48, while the remaining cultures showed increasing amounts of cell debris as the death phase advanced.

MZo cell density differences between CO\(_2\) enriched and the remaining cultures were greater and this was reflected in larger ratios of cell debris to healthy cells, as illustrated in figure 63.
Figure 63: Chlorophyll content of *Muriella zofingiensis* (M) in plots of yellow against red fluorescence, which were arranged in columns according to sample composition and ordered along a vertical time axis. The addition of 167 mg L\(^{-1}\) MOF MIL-101(Cr)-NH\(_2\) was denoted with an additional (M), CO\(_2\) pretreatment with (L) and constant CO\(_2\) enrichment with (B) in the letter code above the regarding column. Filters corresponding to cell shape against cell size and cell count against chlorophyll content were used to identify the culture with the red circles.

The MZo culture with preloaded MOF (MML) only showed minor chlorophyll fluorescence from particles smaller than MZo cells, which verified that the reduced growth rate under this culturing condition was rather due to the high initial cell density than caused by a cell destructive effect.

SMa growth was also not affected by the addition of MOF, but the constant CO\(_2\) enrichment caused the cell densities of these corresponding cultures to remain a magnitude lower than the control. As shown in figure 64, the SMa control, untreated MOF culture and preloaded MOF culture did not exhibit a lag phase for longer than three days and grew with the same rate until the stationary phase was reached by day 20.
Figure 64: *Spirulina maxima* cell densities showed a lag phase of less than four days. The following growth phase ended in cultures under constant CO$_2$ enrichment without additives (green) and with 167 mg L$^{-1}$ MOF MIL-101(Cr)-NH$_2$ (orange) following approximately four days of incubation and these cultures remained in the stationary phase until the end of the experiment. MOF MIL-101(Cr)-NH$_2$ containing cultures without CO$_2$ enrichment showed similar cell densities as the control (black), regardless whether the MOF was CO$_2$ pre-treated (blue), or not (red).

The maximum cell density of the SMa control was achieved by day 34 and corresponded to a multiplication of the initial cell density by 42 ±2, while the untreated and preloaded MOF cultures continued to grow to 55 ±6 and 54 ±10 times respectively until the end of the experiment. In comparison, the CO$_2$ enriched control and MOF culture only exhibited growth until day 4 and multiplied by 7 ±2 and 6 ±2 times. The pH of all SMa cultures was between 8-10 at day 54, so an acidification due to CO$_2$ enrichment could be excluded.

The chlorophyll a measurements indicated the same cell destructive process in cultures with CO$_2$ bubbling as it was seen with green algae cultures. However, the differentiation between healthy cells and cell debris was more complicated, since the particles with lower chlorophyll content did not differ in size, as shown by the plots of forward scatter against red fluorescence on day 45 in figure 65.
Figure 65: *Spirulina maxima* (S) chlorophyll content is shown in plots of forward scatter against red fluorescence to distinguish the relatively smaller cyanobacteria cells (green) from cell debris. The graphs were arranged in columns according to samples composition, which was denoted along the top with: M) 167 mg L\(^{-1}\) MOF MIL-101(Cr)-NH\(_2\); L) CO\(_2\) pre-treatment of the MOF; B) Constant CO\(_2\) enrichment of the culture flask.

The side scatter resulting from the cell shape was similar between high and low chlorophyll content particles and a filter on yellow fluorescence could not be applied, since cyanobacteria lack chlorophyll b.\(^{[18]}\) Differentiation was only possible using the filter of cell count against red fluorescence intensity, which usually gives a peak with the width corresponding to the variation in chlorophyll a content. The number of particles with decreased chlorophyll a content was a magnitude lower than the main culture count and was evenly distributed across the red fluorescence intensities, so that no further peaks arose in the filter. The horizontal limits of the red circles in figure 65 therefore indicate the estimated minimum red fluorescence of the peak.

A further difference to the chlorophyll content analysis of green algae was that increased fractions of particles with diminished chlorophyll a content were not detected simultaneously with the retardation of growth. This indicated that the initial growth stagnation could have been caused by limiting factors and only later resulted from cell destruction.
4.4. Conclusion on MOF MIL101 toxicity

4.4.1. Cytotoxicity of MOF MIL101 and its modified analogue MIL-101(Cr)-NH$_2$

The results from four green algae cultures (CEm, CSo, CVu, MZo) and three cyanobacteria cultures (SCy, SCo, SMa) suggest that MOF MIL101 is harmless to these organisms in concentrations up to 480 mg L$^{-1}$ and also showed no toxic responses to its amine modification, MIL-101(Cr)-NH$_2$, in concentrations up to 167 mg L$^{-1}$. Any sample that implied a potential toxic response did not show all of the required characteristics to draw a definitive conclusion. Therefore, it is more probable that such anomalous results were caused by unsuitable culturing conditions.

The SCy cell densities in presence of MOF MIL101 only reached 71% of the control cultures in 4.3.1. However, the growth of control and of MOF culture was similar during the initial 13 days of incubation and a toxic effect should have influenced the cell densities from the first day, as it was seen in the SCy culture, which contained the mixture of ferricyanide and ferrocyanide. Furthermore, the chlorophyll content per cell was not affected by the presence of MOF MIL101, although metal poisoning generally results in an inhibition of growth and photosynthesis.$^{[19]}$ Over a period of 72 days, the metal poisoning from ferricyanide and ferrocyanide affected the chlorophyll content per cell more strongly with increasing duration of exposure, but growth rates were significantly lower within several days already. It is therefore unlikely that a toxic effect of MOF MIL101 was not detected due to insufficiently long testing periods.

The incubation of MZo cultures with preloaded MIL-101(Cr)-NH$_2$ also resulted in reduced cell densities, as shown in 4.3.3. Considering that MZo growth was not impaired by the untreated MOF, a toxic effect of the MOF material could be excluded. Exposing the MOF to CO$_2$ at room temperature before the experiment does not change the material and the chlorophyll contents per cell did not indicate any cell destructive effect of the preloaded MOF similar to the CO$_2$ enriched cultures. The logical conclusion is that the 12 times larger inoculation cell density in the preloaded MOF cultures caused the lower growth rate, since the concentration of metabolites was accordingly higher, which is known to affect growth rate.$^{[20]}$

Further interference with the toxicity analysis could have resulted from diminished light intensity within the cultures due to the addition of MOF powder. However, solutions did not visibly darken after the addition of MOF and the cultures were grown under constant agitation, which allowed cells to absorb light from various angles. Identical growth rates between MOF containing and MOF-free cultures
could be achieved in 4.3.3, so that the error regarding light availability was considered insignificant.

### 4.4.2. Growth in dispersions of MOF MIL101 and MIL-101(Cr)-NH$_2$

Cytometry did not reveal any evidence for increased growth rates in presence of MOF, so that a greater CO$_2$ availability due to MOF dispersion in the cultures could not be confirmed. The results in 4.3.2 showed promoted growth of green algae and cyanobacteria in the vicinity of MOF MIL101 pellets, which formed the highest biofilm densities in several micrometres to millimetres distance from the edge of the pellets. However, a beneficial effect on growth rate was not seen in the flow cytometry analysis of MOF dispersions in liquid culture. Furthermore, the separation between highest biofilm density and MOF pellet suggested that there was no material specific advantage, such as leaching of useful ions into the agar, which would have supported growth of denser biofilms. An alternative reason for the denser growth around the pellets could be the condensation of water in the sealed Petri dishes, which dragged cells across the surface whenever the Petri dish was moved. More of the water droplets and cells would have been retained by the edges of the MOF pellet than by the agar surface. Petri dishes were incubated on energy saving LED lightboxes, but the necessary temperature gradient for condensation in the humid atmosphere of a sealed Petri dish is not high. It is therefore possible that condensation happened following the switch from 12 hour light to a 12 hour dark cycle. Preferential growth of green algae or cyanobacteria in the vicinity of MOFs can therefore not be confirmed, but the dense biofilms without exclusion zones to the edges of the pellets demonstrated the long-term biocompatibility of MOF MIL101.

### 4.4.3. Benefit of metal-organic frameworks for microbial fuel cells

MOF MIL101 did not show an elevation of bio-available CO$_2$ in dispersions, which represented the least complicated implementation in MFCs. Immobilised pellets of MOF in the vicinity of the MFC electrodes would have allowed to utilise continuous flow pMFCs with large volumes, but the results indicate that the MOF pores are filled with water in liquid culture and do not serve the purpose to concentrate CO$_2$.

Successful enhancement of algae and cyanobacteria biomass production as well as elevated MFC power output has already been achieved using mixed cultures with heterotrophic bacteria.\cite{17,21,22} These improvements were attributed to the elevated CO$_2$ concentration in presence of heterotrophic bacteria, which was identified as the growth limiting factor in most autotrophic cultures. CO$_2$-enrichment with
bacterial cultures has the advantage that the suitable amount of released CO$_2$ is continuously self-regulated through the symbiotic relation with the autotrophic culture, which serves as nutrient source for the heterotrophs.

Preliminary life cycle analysis by our collaborator Owen Glyn Griffiths has estimated that the solvothermal synthesis of MOF MIL101 consumes 236 MJ per gram, which would negatively affect the revenue and life cycle impact of pMFCs compared to the alternative CO$_2$ enrichment with mixed cultures.

**4.4.4. Culture choice for toxicity tests**

Cyanobacteria provided more significant results than green algae in toxicity investigations with growth curves due to higher growth rates and correspondingly larger differences in cell density. The maximum growth rates of cyanobacteria are usually lower than those of green algae; however, under nutrient limiting conditions and comparatively low light conditions in pMFCs larger growth rates can be expected of cyanobacteria.$^{[23]}$

Cytotoxicity of MOF MIL101 had to be tested on green algae as well as cyanobacteria, since both types of photosynthetic cultures are employed in pMFCs.$^{[24]}$ However, it should be taken into consideration that the growth media cannot be replaced during the measurement of a growth curve, which naturally limits the testing period before the death phase of the respective culture starts. Inoculation of low cell densities in the magnitude of $10^3$ cells mL$^{-1}$ allows to extend this time frame slightly, but also involves the risk of premature culture death if the culturing conditions are not ideal. The naturally high growth rates of cyanobacteria therefore allowed identification of the extent of toxicity with greater differences in cell densities in shorter periods than green algae, which reduced the statistical errors and supported the evaluation.

**4.4.5. CO$_2$ enrichment of green algae and cyanobacteria cultures**

The continuous enrichment of cultures with CO$_2$ did not result in acidification within 54 days, but cell densities were decreased due to excessive cell destruction, as shown on the basis of chlorophyll content. A possible reason could have been the strong flow rates and high shear stress in the vicinity of the nozzle, which ruptured the cell walls. The expulsion of oxygen with large amounts of CO$_2$ could have also limited growth rates, since oxygen is required for the respiratory metabolism of photosynthetic cultures. However, gas exchange with the ambient air was possible through stopper foams and oxygen starvation would have not
resulted in high ratios of cell debris in the cultures. Lower CO\textsubscript{2} flow rates through wider nozzles should therefore be applied in future experiments.
4.5. Prospects of MOF research

The presented work has conclusively proven that MOF MIL101 cannot be used in direct contact with the liquid culture to enhance bio-available CO$_2$ concentration. Trapping of water within the pores could be avoided with hydrophobic coatings on MOF membranes, but the issue of environmental impact and low cost efficiency of MOF implementation in pMFCs remains, as described in 4.4.3. Additionally, the application of such a membrane at the interface between liquid culture and ambient air would result in further risks for leakage and reduce the available surface area for air-cathodes and exposure to light.

However, advances in MOF research have shown encouraging results on novel MOFs for carbon capture in the presence of water. Fracaroli et al. captured CO$_2$ from air with 65 % relative humidity using a magnesium oxide based MOF with amine functionalised terphenylene organic linkers (IRMOF-74-III-CH$_2$NH$_2$).[25] The adsorption efficiency did not decline as a result of the competition with water, since the CO$_2$ uptake was facilitated by the amine groups and not by the magnesium sites. Magnesium ions are environmentally benign and would therefore also not pose a risk for high growth rates of photosynthetic cultures. The IRMOF-74-III-CH$_2$NH$_2$ illustrates the rapid progress in MOF research. Although it is not suitable for carbon capture in water, MOFs in general are likely to become a valuable target for future work on pMFCs.

Enhancement of microbial growth via CO$_2$ enrichment is an important aspect of pMFC research. Further investigations on this issue should therefore also include the optimisation of mixed culture systems of photo-autotrophic and heterotrophic microbial communities.
4.6. References in chapter on MOF MIL101 toxicity in green algae and cyanobacteria


Chapter 5

Carbon Cathodes in Microbial Fuel Cells
5. Carbon Cathodes in Microbial Fuel Cells

5.1. Cathode materials abstract

Various carbon materials were hot-pressed to ion-exchange membranes and then analysed with polarisation curves as well as photo-response measurements in a batch photo-microbial fuel cell (pMFCs) to identify the most suitable air-cathode for further pMFC investigations. A combination of Zorflex Activated Carbon Cloth FM10 with the proton-selective Nafion® 115 membrane provided over 30 times larger maximum power outputs compared to the second most efficient assembly of carbon paper and Nafion® 115. The combination of the same carbon cloth with an alternative cation-exchange membrane, CMI-7000S, only achieved 0.05 % of the maximum power generation. Hot-pressing according to previously reported optimal conditions resulted in unnecessary material disintegration and caused irreversible material changes in the Nafion® membranes.\[1\] A pressure of 8.66 kg cm\(^{-2}\) at 100 °C for 30 seconds provided membrane-cathode assemblies with sufficient adhesion for months of continuous utilisation as air-cathodes in pMFCs.
5.2. Cathodes in MFC research

The minimisation of activation, ohmic and mass transport losses due to the cathodic compartment is an extensively investigated topic in MFC research and is of equal importance for the power generation as optimisation of the anodic compartment. While this work mainly focused on materials and processes in the anodic compartment, studies have also been conducted on a small range of carbon materials for the application as air-cathodes in pMFCs.

The aim of the work on cathodes was to minimise the cathodic current limitation, so that the research on anodic materials could be conducted without interference. A cathode’s purpose in a MFC is to facilitate the recombination of protons from the anodic compartment with oxygen to water, which assures a proton gradient from the cathodic to the anodic compartment. The metabolic activity of organisms in the anodic chamber provides the electrons for this process and continuously lowers the pH, which drives the proton flux into the cathodic chamber and therefore also the current through the external circuit. Slow oxygen reduction at the cathode is a known limiting factor for the rate of this process and it can be accelerated with inorganic catalysts, such as platinum, or with biological catalysts and artificial electron mediators, if the cathode is immersed in a liquid compartment. Alternatively, air-cathodes can be employed in single chamber MFCs to increase the cathodic oxygen concentration and mass transport as well as to reduce the operation costs.

The air-cathode approach to the oxygen limitation was also chosen in this work, since it additionally reduced the disturbance of anode investigations by keeping the cathodic compartment as simple as possible. However, this pMFC design requires a watertight seal between the electrode compartments, which simultaneously allows protons to pass through. Nafion® is the commonly used separator membrane in air-cathode MFCs due to its high proton selectivity, but air-cathode assemblies with the cation exchange membrane CMI-7000S were also examined in this work. The cathode materials were limited to carbon based electrodes, since these represent the most promising combination of conductivity and flexibility in design, in addition to being suitable for heat bonding of cathode to membrane. Furthermore, the large surface area of activated carbon has recently attracted the attention of MFC research, especially in air-cathode MFC designs.
5 Carbon Cathodes in Microbial Fuel Cells

5.3. Results on carbon air-cathodes

5.3.1. Painted versus solid Nafion® ion exchange membranes

Slow diffusion through ion-exchange membranes can limit the current in a pMFC, which decreases the potential power output as well as sensitivity to biological signals.\textsuperscript{[9,10]} Nafion® perfluorinated resin was therefore investigated as an alternative to solid Nafion® membranes, since it would allow thinner layers of Nafion® with shorter diffusion lengths. The resin was painted directly onto the carbon cathode, allowed to dry completely before use and the sample was not bent throughout the investigation. The cathode research was aimed at the identification of suitable materials for air-cathodes, so initial examinations were conducted on watertightness.

The hardened Nafion® perfluorinated resin remained watertight for hours with a standing solution of 15 mL above it; however, it exhibited leakage within seconds after a solution flow was mediated by the peristaltic pump. In contrast, the solid Nafion® perfluorinated membrane 115 stayed watertight even at high solution flow rates until the experiment was stopped after 12 hours.

5.3.2. Hot-pressing of air cathodes

As a result of the insufficient watertightness of Nafion® resin, research was conducted on combining solid perfluorinated Nafion® 115 membranes (Nafion® 115) and CMI-7000S cation exchange membranes (CMI-7000S) with various carbon materials by hot-pressing.\textsuperscript{[1]} Temperature and pressure were adjusted to achieve optimal adhesion between the membranes and the Zorflex Activated Carbon Cloths FM30K and FM10 (ZACC FM30K and ZACC FM10), carbon paper (C-paper) as well as carbon felt (C-felt).

The Nafion® 115 membrane became less flexible and exhibited shrinkage during the hot-pressing procedure between 100 to 150 °C. This made each Nafion® 115 sample unusable for a second cathode material combination attempt. This material change was not reversible by soaking the Nafion® 115 in water or in 0.5 mmol L\textsuperscript{-1} sulphuric acid overnight. Additionally, conducting the hot-pressing procedure at temperatures around 150 °C caused a colour change from transparent to yellow, which was also irreversible. As a result of this, all cathode-membrane assemblies, which were tested in further experiments, were hot-pressed at 100 °C. Materials such as C-felt and ZACC FM30K mostly disintegrated at all pressures and thus, lost most of the large surface area advantage compared to other carbon based...
materials. C-paper often exhibited cracks after the procedure, which caused material waste, especially considering that the Nafion® 115 membrane could only be used once in the hot-pressing procedure. ZACC FM10 possessed sufficient flexibility and durability to sustain the hot-pressing procedure in most cases.

The adhesion between CMI-7000S and ZACC FM10 was equally as good as was observed with Nafion® 115. Additionally, the CMI-7000S exhibited greater heat stability than Nafion® 115, since it did not undergo any colour changes or deformations during the hot-pressing at 150 °C.

5.3.3. Polarisation and power curves

Polarisation curves of the various hot-pressed air-cathodes were measured under equal conditions in a batch pMFC to evaluate the maximum power generation with each material and identify the respective resistance at the maximum power point (optimal external resistance) for subsequent photo-response investigations. An FTO coated TiO₂ ceramic anode and the green algae culture Chlorella vulgaris (C.vulgaris) were used in each experiment, since these were the most extensively characterised anode material and exoelectrogenic organism in this batch cell.[11] Furthermore, the application of newly developed ceramic anodes was the ultimate aim for future pMFCs within this work.[12] The examined air-cathodes consisted of perfluorinated Nafion® 115 membranes with Zorflex Activated Carbon Cloth FM10 (ZACC FM10+Nafion® 115), with carbon paper (C-paper+Nafion® 115) and with carbon felt (C-felt+Nafion® 115). Samples of ZACC FM10 were also hot-pressed to CMI-7000S cation exchange membranes (ZACC FM10+CMI-7000S) and excelled during the investigations in the achievable length of testing periods. Presented currents and power values were normalised per anode area to allow comparisons with results in other chapters.

Initial experiments were performed to assess the effect of sealing the injection orifice to prevent concentration changes resulting from solution evaporation, compared to leaving it open and thereby allowing gas exchange between microbial culture and environment. The data was not affected by this setup change within the analysed time frame and therefore a distinction between the corresponding data was not made.

ZACC FM10+Nafion® 115 provided higher voltages at all applied resistances than the other cathode materials, as shown in figure 66a; more detail is given on ZACC FM10+CMI-7000S as well as on C-felt+Nafion® 115 in figure 66b. The potentials for maximum power generation are indicated with dashed lines in the colour corresponding to the material dataset.
The current density of ZACC FM10+Nafion® 115 at 100 Ω was 176 times higher than the current density of ZACC FM10+CMI-7000S and the open circuit potential (OCP) was six times larger. This suggested that the thicker CMI-7000S membrane increased the internal resistance of the pMFC with either longer diffusion paths, or with greater resistance to proton diffusion. C-paper+Nafion® 115 exhibited a 14 times smaller current density at 100 Ω than ZACC FM10+Nafion® 115 and half of the OCP, which was attributed to the reduced surface area of carbon paper compared to activated carbon cloth. Carbon felt inherently possess greater surface area; however, its fragility caused the majority of each sample to crumble away during both the hot-pressing and when it was clamped between the batch pMFC layers, which reduced surface area and electrical connection between the fibres.

Power curves of each cathode material are illustrated in figure 67a with dashes indicating the respective maximum power output. The power curves of ZACC FM10+CMI-7000S as well as C-felt+Nafion® 115 are shown in figure 67b in more detail.
Figure 67: a) Power curves calculated from the polarisation curves in figure 66 revealed that ZACC+Nafion® 115 provided a 32 times larger maximum power generation per anode area than achieved with C-paper+Nafion® 115; b) ZACC+CMI-7000S and C-felt only generated 0.5 % and 0.2 % of the maximum ZACC+Nafion® 115 power output respectively.

The maximum power outputs of C-paper+Nafion® 115, ZACC FM10+CMI-7000S and C-felt+Nafion® 115 reached 3 %, 0.05 % and 0.02 %, respectively, of the maximum power generation with a ZACC FM10+Nafion® 115 cathode.

The resistance of a pMFC circuit should be kept as low as possible, in order to present the anode as a beneficial electron acceptor for the exoelectrogenic organisms. It was therefore important that maximum power generation could be achieved with minimal external resistances. ZACC FM10+Nafion® 115 outcompeted the other tested materials in both regards, since its optimal external resistance of 10 kΩ was five times smaller than the external resistance for C-paper+Nafion® 115 and 100 times less than the optimal external resistances of the remaining two cathodes. Figure 68 shows all examined cathode materials and respective optimal external resistances in order of decreasing OCP.
Figure 68: Summarising the open circuit potentials (purple) and optimal external resistances (blue) of the tested cathode materials illustrated that ZACC+Nafion®115 promised higher power generation and simultaneously allowed operation of the pMFCs at lower external resistances, which supported the exoelectrogenic interaction between organisms and anode.

5.3.4. Photo-response amplitude and power generation at optimal resistance

The investigations on cathodes were performed to identify the most suitable combination of ion-selective membrane and cathode material for pMFC research. This commonly involved biological examinations on the basis of power output variation in response to changes in lighting conditions, widely known as photo-response. The greatest sensitivity to photo-responses can be expected at the optimal external resistance of a pMFC. Hence, the photo-response to alternating 12 hour cycles of light and dark was examined with each cathode material at its optimal external resistance in the same batch cell with C.vulgaris, which had been used in 5.3.3.

Additionally, the most promising air-cathode, ZACC+Nafion®115, was sputter-coated in platinum to investigate if oxygen reduction catalysis could reduce the internal resistance of the pMFC and thereby improve power output as well as photo-response sensitivity.

Photo-responses could be measured with all cathode materials and consistently comprised larger power generation in the light than in the dark. A summary of the average difference between light and dark power density (green) over the whole
testing period as well as the power densities during illumination (red) and in the dark (blue) on the second examination day is presented in figure 69. Absolute power outputs were only stated for the second day of each experiment, since the static solution and small volume in the batch cell did not allow attainment of steady state conditions in long-term experiments. These values were therefore only stated as comparison to the photo-response amplitude.

The platinum coating (Pt ZACC+Nafion® 115) reduced the photo-response amplitude to 39 % of the values that were achieved without coating (ZACC+Nafion® 115). This indicated that the sputter coating reduced the cathode surface area by clogging the low volume pores of the activated carbon surface. Furthermore, the rate of oxygen reduction was either not limiting, or it was not sufficiently improved to increase the overall power output.

The average photo-response with ZACC+Nafion® 115 represented 51 % of the light power density on the second day, which was a higher fraction than exhibited by the other materials. Additionally, the trends between the power curves of each material in 5.3.3 were confirmed with the average power densities during several days of operation at the respective optimal external resistance. ZACC+Nafion® 115 therefore showed the highest sensitivity to photo-responses and promised the largest signal to noise ratios out of the tested carbon cathodes.
5.3.5. Batch pMFC performance

The 2 cm$^2$ top face of the anodic chamber was irradiated with a light energy of 7.847 mW. Table 10 demonstrates the percentage of electrical energy that could be generated from the incident light energy with the examined cathode materials at their respective optimal external resistances.

*Table 10:* The maximum electrical power achieved with the respective cathode at its optimal external resistance was divided by the incident light energy per area, which was equal to 7.847 mW onto the 2 cm$^2$ top face of the anodic chamber. Carbon material and ion-exchange membrane of each cathode are stated separately and the ratio of electrical energy produced from the incident light energy is given as a percentage.

<table>
<thead>
<tr>
<th>Carbon Material</th>
<th>Ion-Exchange Membrane</th>
<th>Optimal Resistance (kΩ)</th>
<th>Max. Power Output (W)</th>
<th>Electrical from Light Energy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZACC FM10</td>
<td>Nafion® 115</td>
<td>10</td>
<td>5.701·10$^{-6}$</td>
<td>7.265·10$^{-2}$</td>
</tr>
<tr>
<td>ZACC FM10</td>
<td>CMI-7000S</td>
<td>1000</td>
<td>2.865·10$^{-9}$</td>
<td>3.651·10$^{-5}$</td>
</tr>
<tr>
<td>C-felt</td>
<td>Nafion® 115</td>
<td>1000</td>
<td>8.718·10$^{-10}$</td>
<td>1.111·10$^{-5}$</td>
</tr>
<tr>
<td>C-paper</td>
<td>Nafion® 115</td>
<td>50</td>
<td>1.738·10$^{-7}$</td>
<td>2.215·10$^{-3}$</td>
</tr>
</tbody>
</table>

It should be noted that the low volume of the batch pMFC did not support long operating times, which precluded the formation of biofilms and made the use of artificial redox mediators necessary. A toxic electron mediator mixture of potassium ferricyanide and potassium ferrocyanide trihydrate, each used at a concentration of 2.5 mmol L\(^{-1}\), was employed for this purpose and its toxic effects are illustrated in chapter 4 on MOF MIL101 toxicity in green algae and cyanobacteria. The batch pMFC was therefore only used as a cathode characterisation device and the results from this study were harnessed in continuous flow pMFCs. These allowed sufficiently long operating times to form anodic biofilms and utilise the larger power generation from the dense consortia in the microbial mat without the necessity for artificial redox mediators.\(^{[17]}\)
5.4. Conclusions on carbon air-cathodes

The aim of the research on air-cathodes was to create a pMFC for the research on the anodic half cell without interference from the cathode. An air-cathode design was therefore preferred to a two compartment pMFC, in order to reduce power output limitations due to oxygen solubility and mass transport loss in the catholyte.\textsuperscript{[2,18]} As a result, it became necessary to create a watertight seal between anodic chamber and cathode with an ion-exchange membrane. This requirement increased the mechanical stress on the cathode during its combination with the membrane and even more so when it was clamped into the pMFC, which limited the selection of cathode materials. The study was focused on carbon based materials, since these presented a good combination of electrical conductivity, large surface area and the required durability as well as heat stability for hot pressing.\textsuperscript{[19]} Furthermore, the engineering of pMFCs benefitted from the application of relatively thin and flexible carbon cloth cathodes.

5.4.1. Choice of membranes

The risk of cathode material damage was lowest if Nafion® perfluorinated resin solution was used to create the ion-selective membrane, acting as a coating over the various cathode shapes and hence preserving the large surface area of more fragile materials. A larger fraction of cathode surface area was in direct contact with the membrane, since the initially liquid resin hardened along the cathode surface. The Nafion® resin could be applied in thin layers, which would have reduced the diffusion length through the membrane and thereby also the internal resistance of the pMFC. However, the occurrence of leakage within seconds after mediating a flow through a 15 mL solution made Nafion® resin impractical in air-cathode pMFCs. Application of a thicker coating to reinforce its structure would have only negated the practical advantage of Nafion® resin. Potential ways of harnessing the benefits of Nafion® resin would be to reduce the volume of the anodic chamber down to the scale of microfluidic devices, or an application in a MFC with liquid compartments on either side of the membrane where pressure fluctuations are better compensated.

The application of Nafion® resin on cathodes with less densely interwoven fibres, such as carbon felt, involved the risk of discontinuous coatings, which would have caused leakage from the anodic pMFC chamber. Solid ion-exchange membranes therefore allowed the exploration of a wider range of materials.

The solid Nafion® membrane was compared to the CMI-7000S cation exchange membrane in assemblies with ZACC FM10. Nafion® 115 exhibited irreversible
shrinkage, hardening and a colour change towards slightly yellow during the hot-
pressing, which was not observed in CMI-7000S. The colour change indicated that
the cation conducting sulfonate groups of the Nafion® 115 membrane were
affected by the heat treatment.\[9]\ Kim et al. concluded that the heat bonding of
Nafion® to cathode materials could negatively affect the ion conduction and
permeability of the membrane.\[20]\ The results therefore suggest that CMI-7000S is
more suited for hot-pressing than Nafion®, considering that ZACC FM10 bonded
equally strong to both membrane materials. However, the batch cell application of
the ZACC FM10+Nafion® 115 cathode resulted in a 1915 times larger maximum
power density than achieved with ZACC FM10+CMI-7000S. The corresponding
optimal external resistance with ZACC FM10+Nafion® 115 was 100 times lower,
which is attributed to the higher ohmic resistance of CMI-7000S membranes.\[10]\ Furthermore, the power density of a membrane pMFC decreases with diminishing
proton selectivity of the membrane, since every charge compensation with cations
other than protons does not result in a recombination with oxygen and electrons to
water at the cathode.\[21,22]\ CMI-7000S membranes are less proton-selective than
Nafion®, which further decreased the power densities.\[23]

Nafion® 115 membranes were observed to swell only on the side that was in
contact with the liquid chamber during operation, which caused deformation of the
air-cathode in pMFCs without a strut section on the cathode side. This could reduce
the contact area between membrane and cathode and especially become a
problem in pMFCs with large cathode areas. Nafion® membranes are usually stored
in deionised water for several hours prior to the application, in order to avoid
swelling during the operation, but air-cathodes will still warp when the air-side of
the membrane dries.\[24]\ Pereira et al. addressed this problem by improving the
structural support of Nafion® membranes with mesoporous silica, which were
functionalised with sulphonic acid groups. The resulting membrane was also
reported to show improved proton conductivity and hydration at elevated
temperatures compared to standard Nafion® membranes, which would be
beneficial for the pMFC power generation in future experiments.\[25]\ An additional factor to consider for the research on bio-electrochemical devices is
the cost efficiency of the implemented materials. It should therefore be noted that
Nafion® 115 was purchased for a 40 times higher cost per area than CMI-7000S,
which will impact the engineering of pMFCs before higher technology readiness
levels can be achieved. Conversely, the focus of this research was on the anodic
compartment of pMFCs and ZACC FM10+Nafion® 115 was identified as the most
promising air-cathode to minimise cathodic limitations and interference during the
investigations.
5.4.2. Hot pressing of air cathodes

The optimal conditions for the heat bonding of ZACC FM10 to Nafion® 115 were similar in temperature, but much lower in pressure than reported by Kim et al. for the assembly of platinum catalysed carbon cloth with a Nafion® membrane.[1] Kim et al. reported the best electrode performance following hot-pressing with 150 kg cm\(^{-2}\) at 120 °C for 30 seconds. Such high pressures resulted in the complete disintegration of both ZACC types and the C-felt and were found to be unnecessary for good adhesion between the cathode and the membrane. Good adhesion between carbon cloth and membrane as well as concurrent preservation of its fibrous structure was achieved with over 17 times lower pressure of 8.66 kg cm\(^{-2}\) at 100 °C. The temperature was reduced, in order to reduce the adverse effects on ion-conductivity and permeability, which were described in 5.4.1. Furthermore, the Nafion® membrane shrunk and hardened before it could be bonded to the cathode at higher temperatures, since the metal blocks were pre-heated and samples placed into the press before pressure was applied. In this state the Nafion® did not adhere to the cathode, so the pressure had to be applied during the transition process. An alternative would be to use a hot-press, which allows heating of the blocks while pressure is applied; however, higher temperatures would still involve the risk of Nafion® material damage.

5.4.3. Choice of air-cathode for following pMFC studies

ZACC FM10+Nafion® 115 excelled compared to the other tested cathodes with much higher maximum power densities. In addition to this, the photo-response amplitude represented the largest fraction of the power output during pMFC operation. The comparatively low optimal external resistance was also beneficial for the utilisation of exoelectrogenic organisms in a pMFC, since large circuit resistances have been observed to evolve cultures with lower exoelectrogenic activity, as discussed in more detail in chapter 6 on Biofilm supporting anode materials. ZACC FM10 exhibited greater durability during the hot-pressing than ZACC FM30K, or C-felt and was successfully combined with Nafion® 115 as well as CMI-7000S membranes. The platinum coating of ZACC FM10+Nafion® 115 resulted in inferior pMFC performance regarding all tested aspects. Additionally, life cycle assessments identified the use of platinum catalysts as a major problem for the cost-efficiency and sustainability of bio-electrochemical systems, considering that the energy intensive production of platinum releases 51.2 tons of CO\(_2\) per kg of refined platinum.[26,27] Thus, the investigation of platinum coated electrodes was discontinued.
5.5. Prospects for air-cathode investigations

Nafion® perfluorinated membranes with 0.125 mm thickness have exhibited good adhesion to carbon electrodes and the greater proton selectivity as well as lower ohmic resistance resulted in larger pMFC power outputs than were achieved with the CMI-7000S membrane. Improving pMFC power densities with the proton selectivity of an ion-exchange membrane includes the disadvantage of limiting the proton mobility to equal the slow diffusion inside the membrane.\[22\] However, the greater coulombic efficiencies and the possibility to overcome the problem of low oxygen solubility in water with air-cathodes have proven to outweigh the mass transport limitation in membranes.\[28\]

The advantages of a membrane could be maintained and the internal resistance due to the mass transport resistance still decreased, if the diffusion length through the membrane was reduced in thinner membranes. Nafion® membranes from Sigma Aldrich with a thickness of 0.125 mm confirmed the feasibility of the hot-pressing procedure and helped to identify the crucial factors during the heat bonding process. A potential further step in the development of this investigation should focus on greater pMFC efficiencies with 0.05 mm thick Nafion® membranes from Alfa Aesar. Considering that this membrane is 2.5 times thinner, hot-pressing might have to be conducted at lower temperatures. In addition to the performance enhancement, the application of a thinner membrane would also improve the sustainability of the pMFC by reducing the material expenditure. A similar sheet size of the thinner membrane costs 10 times less, and a four times larger sheet size becomes up to 60 times cheaper. The high cost of Nafion® has often been reported as a major drawback in pMFC cost-efficiency studies and the future marketability of Nafion® containing pMFCs will benefit from using its advantages with minimal material investment.\[29,30\]
5.6. References in chapter on carbon air-cathodes

11. Thorne, R.J., Bio-photo-voltaic cells (photosynthetic - microbial fuel cells), in Department of Chemistry. 2011, University of Bath: Bath.


Chapter 6

*Biofilm supporting anode materials*
6. Biofilm supporting anode materials

6.1. Bio-anode materials abstract

The characterisation of anode surface properties in relation to sustainable voltage generation during long-term examinations in photo-microbial fuel cells (pMFCs) demonstrated the importance of biofilm formation for high power output and for the required sensitivity in biological sensing applications.

Ti$_2$AlC ceramic has been investigated as novel anode material and biofilm substrate for pMFCs, since more interface area between biofilm and electrode can be provided in a certain electrode chamber volume through the utilisation of ceramic manufacturing procedures. This inherently conductive type of MAX phase ceramic allowed the fabrication of reticulated electrodes with customisable geometries and macroporosity, which enhanced the adhesion of exoelectrogenic cells due to high surface roughness and protection of cells from shear flow, whilst also acting as the final electron acceptor for reduced metabolites. Cyclic voltammetry has shown that after sintering, Ti$_2$AlC is electrochemically stable from -100 mV to +500 mV in both KCl and KNO$_3$ background electrolytes. The electrochemical stability can be extended to larger potential windows by the promotion of surface passivation at positive potentials, whilst maintaining excellent conductivity of the material. In addition to this, the manufacturing procedure has been optimised to achieve 19 times higher compressive strength of the porous systems. In view of application as a bio-electrode in a microbial fuel cell, it has been confirmed that Ti$_2$AlC ceramics are bio-compatible with the unicellular green algae *Chlorella vulgaris* (C.vulgaris/CVu) and that they support the growth of mixed culture biofilms. Confocal and atomic force microscopy studies illustrate the importance of roughness on the macro scale for cell adhesion. Ti$_2$AlC ceramic electrodes provided sustainable power generation and reliable photo-responses in multiple mediator-free pMFCs over several months and outcompeted porous electrode alternatives, such as reticulated vitrified carbon (RVC) foams as well as FTO coated TiO$_2$ ceramics, with up to 36 times larger maximum power densities.

Long-term studies of photo-responses with these materials, as well as with ITO coated plastic and FTO coated glass anodes, were also used to evaluate selective factors for biofilm formation. Changes in photo-response type and amplitude were observed simultaneously with alterations in culture growth phase as well as biofilm formation of green algae and cyanobacteria cultures.
6 Biofilm supporting anode materials

Electrochemical coating of electrode surfaces with Prussian blue and polyaniline increased roughness, beneficial for cell adhesion, but did not improve the electron mediation or power generation in pMFCs.
6.2. Ti$_2$AlC ceramic; a promising electrode material

The electrode geometry needs to be adjustable to the application just as well as the electrode material in order to facilitate the increasingly versatile applications of electrochemistry. Long term biological sensing and energy generation, in particular, present challenges. Biocompatibility of the electrode with the biological species and environmental impact of production, as well as the effect of electrode surface properties and electric circuit resistance on cell adhesion, become factors to consider.$^{[1-3]}$ Metallic electrodes often possess excellent kinetics, but are restricted to simple shapes and the manufacture of them involves wasting a considerable fraction of often precious materials. In addition to this, corrosion presents a constant problem in the long term application of non-noble metal electrodes.$^{[4]}$

A type of conductive ceramic, first discovered in powder form by Nowotny et al. in 1971, and later called MAX phases ($M =$ early transition metal, $A =$ A-group metal, $X =$ C or N, with the general formula $M_{n+1}AX_n$) by Barsoum et al. holds the potential to solve these problems by combining the chemical stability of ceramics with metal-like conductivity.$^{[5,6]}$ Such a material is particularly desirable for biological analytes as the only metallic alternatives; stainless steel and titanium, present poor biofilm supports due to their naturally smooth surfaces.$^{[7,8]}$

Using conductive ceramics, electrode shapes can easily be adapted to the desired application. Thorne et al. have already shown how employing a replication process based on polyurethane foams can be harnessed to create electrodes with complex 3-dimensional macroporous networks of defined pore size.$^{[9,10]}$ Such a technique increases the efficiency of the reactor geometry utilisation and improves mass transport conditions. However, TiO$_2$ ceramics were not electrically conductive and had to be coated with fluorine doped tin oxide by chemical vapour deposition to be usable as electrodes. This necessity represented the main cost factor for these electrodes and complicated the production of electrodes with smaller pore size. For this reason, the advantages of this manufacturing process were combined with the inherent conductivity of MAX phase ceramics for the first time to overcome these problems.$^{[11]}$

Within this work a focus has been made on Ti$_2$AlC, a 211 MAX phase, as a proof of concept for using such ceramic material as electrodes.$^{[12]}$ As described by Barsoum et al., the structure of this material is based on a repetition of one layer of aluminium atoms for every two layers of titanium carbide.$^{[13]}$ The results of soft X-ray emission spectroscopy investigations by Magnuson et al. suggested that the conductivity of this material is not based on the aluminium layers, but rather on the Ti metal bonding. Furthermore, they found that Ti(II) layers contribute more to the
Biofilm supporting anode materials[^14] Ti$_2$AlC ceramic only contains Ti(II) and is therefore expected to exhibit greater conductivity than any alternative 312 MAX phase[^15]. Therefore, based on Magnuson’s findings it was decided to test an electrode application of MAX phases with the Ti$_2$AlC MAX phase first. Within the class of MAX phase ceramics, Ti$_2$AlC in particular is also considered to be among the lightest as well as the most oxidation resistant.[^16,17] Electrode corrosion has been a common problem of long-term sensing or energy generation in the presence of biofilm forming micro-organisms.[^18] The study was therefore also conducted to explore the applicability of Ti$_2$AlC as an alternative to expensive corrosion resistant metals. Furthermore, Ti$_2$AlC permitted the greatest flexibility in its fabrication, since it has been found to be the thermochemically most stable phase in the system of Ti-Al-C compounds.[^19] For these reasons Ti$_2$AlC has already been brought onto the market under the trade name Maxthal 211. However, to our knowledge, a comprehensive study of its qualities as an electrode material is not currently available.

Initial characterisation aimed at assessing the Ti$_2$AlC ceramic behaviour as the working electrode (WE) in environments which were either common in electrochemical methods, or were reported as catalytic for material changes in the working electrode.[^20] This investigation comprised cyclic voltammetry with respectively altered scan rates, potential windows, light conditions, types of background electrolyte and concentration of background electrolyte, while the remaining factors were kept constant.

Through these initial investigations an electrochemically induced slow oxidation in the Ti$_2$AlC was shown. The presented Ti$_2$AlC ceramic study therefore includes additional in-depth analysis to investigate the reversibility of the underlying oxidation process and to identify the oxidation potential.

Since the kinetics of the oxidation were found to be comparatively slow, the Ti$_2$AlC ceramics were subjected to weeks of continuous cyclic voltammetry, in order to promote the oxidation and examine the associated long-term changes. This study was conducted at two different scan rates. Long-term cyclic voltammetry at the first scan rate was analysed on the basis of cumulative charge transfer, which resulted in the identification of an exponential decline in oxidation current with each cycle. This analysis was used to predict the further progression of the oxidation and was subsequently confirmed with the experiments at the second scan rate.

The results of the long-term study suggested an irreversible Ti$_2$AlC oxidation, which did not impair its conductivity to a great extent. Thus, explorations into exhausting the Ti$_2$AlC oxidation prior to its application as an electrode were carried out, in order to assure a reliable response from a Ti$_2$AlC ceramic. Consequently, the oxidation potential was investigated to allow the use of chronoamperometry as an
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electrochemical pre-treatment. This technique represented the most time effective method for exhausting the irreversible oxidation of Ti$_2$AlC. Simultaneously, it would have the advantage of simplified monitoring and extrapolating the magnitude of oxidation current against time.

Finally, the bulk resistivity for semi-infinite volumes, $\rho_{\infty}$, of Ti$_2$AlC ceramics was determined through 4-terminal sensing. Results of Ti$_2$AlC ceramics produced in our laboratories were compared to an industrially fabricated sample from Kanthal in order to analyse how representative our Ti$_2$AlC ceramics were. The bulk resistivity was measured at different stages in the use of Ti$_2$AlC ceramic as an electrode, in order to gain insights into the reasons for the observed Ti$_2$AlC ceramic oxidation.

Porous Ti$_2$AlC ceramics were implemented in pMFCs and compared to alternative electrode materials in long-term tests regarding power generation and sensitivity to biological responses during environmental changes. Amongst the alternative electrode materials were common pMFC anodes, such as FTO coated glass and ITO coated plastic, as well as the promising new materials RVC foam and FTO coated TiO$_2$ ceramic, which can also be fabricated in various shapes with defined porosity.$^{[9,21,22]}$ Anode material research was performed following the identification of the cathode material, Zorflex activated carbon cloth FM10 hot-pressed to a Nafion® 115 membrane, which provided the largest power generation in combination with a FTO coated TiO$_2$ ceramic in a batch pMFC. This cathode material was therefore kept constant in all experiments on pMFC anode materials in our laboratories to ensure that the results could be compared.

A preliminary study using Prussian blue as an electron mediating coating between the biofilm and electrode was conducted with TiO$_2$ ceramic as substrate.$^{[23]}$ The aim was to make the electrode surface more attractive for organisms as an electron acceptor, since it was already known that several metal-reducing cyanobacteria species form biofilms on iron-rich rock to re-oxidise metabolites.$^{[24]}$ In consideration of the high costs involved in the CVD coating of TiO$_2$ ceramics, FTO glass slides and non-CVD treated TiO$_2$ ceramic were coated separately in Prussian blue to evaluate the bonding strength and biocompatibility in an initial investigation. Testing the adhesive force separately on both substrates also addressed the issue that the TiO$_2$ surface was not continuously covered in FTO after the CVD deposition, as shown by Thorne et al.$^{[9]}$
6.3. Results on bio-anodes

It should be noted that results are evaluated neglecting ohmic losses. This simplification became necessary due to the complex nature of the investigated MFCs. Circuit resistance fluctuated sporadically as a consequence of biological processes in culture, and also following redox reactions within the electrode material in the case of Ti$_2$AlC ceramics. Culture changes during an experiment involved factors such as cell viability, organisation and age of biofilms, as well as composition of mixed cultures. A simultaneous on-line measurement of all these changes would have been necessary, together with a sophisticated knowledge about the respective influence on the circuit resistance. The presented work can therefore be considered as a fundamental investigation for such experiments in the future.

6.3.1. Prussian blue coating as mediator between TiO$_2$ ceramics and algae

An additional Prussian blue coating of the by Thorne et al. developed FTO coated TiO$_2$ ceramics aimed at reducing the usage of the toxic redox mediator couple ferricyanide and ferrocyanide by confining the redox mediator to the interface between electrode and algae.$^{[9]}$ Removing this mediator couple from solution had two practical advantages for the experiments discussed within this work. Firstly, the larger solution volumes entailed in the investigation of FTO coated TiO$_2$ ceramics in continuous flow cells would require greater amounts of ferricyanide, effectively decreasing the biocompatibility of such a pMFC technology. Furthermore, the focus of this work was on the growth of biofilms on pMFC anodes, since these were shown to contribute more to the pMFC power output than individual cells in solution.$^{[25]}$ Hence, exposing the cells in solution to redox mediator toxicity would have been likely to result in a reduced power generation due to lower cell density.

The presented study investigated the feasibility of a Prussian blue film on FTO coated TiO$_2$ ceramics by coating a FTO surface and a TiO$_2$ ceramic surface separately. FTO coated glass was used as a substrate for an electrochemical deposition of Prussian blue, while the non-conductive surface of the TiO$_2$ ceramic had to be coated in Prussian blue through pyrrole polymerisation.

6.3.1.1. Electrochemical deposition of Prussian blue onto FTO glass

Prussian blue was successfully deposited on the FTO glass substrate by continuous cycling between 0.3 V and its oxidised form, Prussian green, at 0.7 V, as shown in figure 70a.
Figure 70: a) Electrochemical deposition of Prussian blue on a FTO glass substrate from an acidic solution of 4 mmol L\(^{-1}\) \(K_3[Fe(CN)_6]\) plus 4 mmol L\(^{-1}\) \(FeCl_3\) through repeated cycling to its higher oxidation state Prussian green, followed by b) activation and characterisation of the Prussian blue film by repeated cycling to its lower oxidation state Prussian white.

The solution colour changed rapidly from yellow to dark blue during the first scan, and the CV shape remained approximately unchanged after five scans. However, cycling of the potentials was continued beyond five scans to assure the production of a homogeneous film structure. Subsequent cyclic voltammetry between -0.05 V to 0.35 V, as depicted in figure 70b, activated and confirmed the successful Prussian blue deposition with the characteristic Prussian white peak evident at around 145 mV and the Prussian blue peak around 275 mV wrt. Ag/AgCl.\(^{[26]}\) The Prussian blue film adhered well to the FTO glass during the subsequent washing with Milli-Q water.

6.3.1.2. Current free deposition of Prussian blue by pyrrole polymerisation

Current free deposition of Prussian blue onto the nonconductive TiO\(_2\) ceramics by means of pyrrole polymerisation took place rapidly after the pyrrole was added to the growing solution. An instant colour change from the yellow ferricyanide growing solution to dark blue was observed, paired with excessive HCN gas evolution. The resulting Prussian blue coating, shown in figure 71b in relation to a bare TiO\(_2\) ceramic in figure 71a, did not wash off during the following rinsing in Milli-Q water.
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Figure 71: TiO$_2$ ceramic with 30 ppi a) before Prussian blue coating and b) after the coating with diminished reflectivity and porosity, but with well adhered Prussian blue film.

6.3.1.3. AFM characterisation of electrochemically deposited Prussian blue

AFM imaging of the FTO glass surface before the Prussian blue coating (figure 72a) and after the coating (figure 72b) showed that the FTO surface was uniformly covered in Prussian blue particles of smaller size than the FTO particles after the coating. The average surface roughness decreased from 32.6 nm to 29.4 nm, which indicated that the Prussian blue was filling the cavities between the FTO particles.

Figure 72: AFM study of FTO glass surface morphology a) before and b) after electrochemical Prussian blue coating. Surfaces are represented by a sequence of colour map, 3 dimensional model and line scan tracing respectively. The colour map was used for the surface roughness calculations.
The small difference in average surface roughness between uncoated and coated FTO glass suggested that a comparatively thin layer of Prussian blue was deposited.

6.3.2. PANI coating of FTO glass for enhanced cell adhesion and anode area

Polyaniline (PANI) coating of MFC anodes has already been shown to improve power outputs compared to the equivalent non-coated anodes. However, in a joint study with the University of Cambridge, PANI coated FTO glass anodes were compared to other common MFC anode materials with regards to the influence of surface morphology and surface energy on pMFC power output by applying all tested materials in one multichannel pMFC under identical conditions. Electrochemical coating and surface characterisation of PANI electrodes is presented in this section, while the application in the multichannel pMFC is elucidated in 6.3.5.

The PANI film was meant to improve biofilm adhesion by creating a positive surface charge and thereby improving the interaction between extracellular proteins and electrode surface, in a manner similar to previous adhesion improvements seen after ammonia treatment. Furthermore, the PANI coating was hypothesised to enlarge the available surface area for the exoelectrogenic cells to adhere to, which would improve MFC power output due to the increased number of electron injecting cells.

6.3.2.1. Electrochemical polymerisation of Aniline on an FTO glass electrode

The electrochemical deposition of PANI onto the FTO coated glass substrate, shown in figure 73a, was characterised by larger currents on the scan from positive to negative potentials during the first three cycles, resulting from structural rearrangements within the PANI film. Any additional PANI deposit from further cycling was mostly washed off during the subsequent rinsing process, although these further deposition cycles were found to improve PANI film regularity and long-term adhesion. The characteristic redox peaks Red 1 and Ox 1 for the conversion of leucoemeraldine to emeraldine as well as the polymerisation redox peaks Red 2 and Ox 2 were observed during the aniline polymerisation.
Figure 73: a) Electrochemical polyaniline deposition involved a reversible redox process with oxidation peak (Ox 1) and an irreversible redox process with reduction peak (Red 2) and oxidation peak (Ox 2). Nucleation loops during the first three cycles were likely due to rearrangements within the PANI film structure and an increase in film thickness resulting in peak separation; b) Characterisation of the film in fresh sulphuric acid confirmed the successful deposition of PANI with the emeraldine redox peaks and corresponding colour change between the green emeraldine salt (Ox 1) and the blue emeraldine base (Red 1).

The potential difference between the respective redox peaks of each process increased with every deposition cycle, but remained constant during the characterisation CVs, shown in figure 73b. The characterisation CVs were conducted in a potential range that did not allow any further aniline polymerisation, in order to avoid changing the PANI film during the characterisation. Consequently, the increasing peak separation during the deposition CVs was attributed to the growing film thickness and the slower kinetics in such a thick PANI film.

A uniform colour change of the film from light yellow to green, and finally to dark blue was observed when the voltage was swept from -0.1 V to 0.4 V, which additionally confirmed that the whole film consisted of electrically conductive PANI. Without application of an overpotential, the PANI films were bright green in colour, but turned dark blue and decreased in conductivity when exposed to air for several hours. It was confirmed that these changes were reversible by cyclic voltammetry of the film in sulphuric acid solution between the potentials -0.1 V to 0.4 V.

6.3.2.2. AFM study of poly-Aniline coated FTO glass

The AFM analysis of the FTO substrate before PANI deposition and of the dried PANI film on the same substrate after the coating, indicated a complete coverage of the surface by PANI, and a decrease in smoothness due to the irregular thickness of the coating across the FTO glass surface. Figure 74 shows a sequence of colour map, 3-dimensional model and line scan tracing for the bare FTO glass surface (a) and the PANI coated substrate (b) respectively.
The PANI coating diminished the distinct FTO particle boundaries, thereby smoothening the surface in sections of nanometres in edge length. However, due to the irregular coating thickness the average surface roughness in a 10 µm x 10 µm section, more relevant for the size of an algae or cyanobacteria cell in terms of adhesion, increased from 39.5 nm to 75.4 nm.

### 6.3.3. Conductive Ti$_2$AlC ceramic allowing customisable electrode shapes

Ti$_2$AlC was investigated as a future replacement for TiO$_2$ ceramics, since its inherent conductivity promised reduced electrode production costs, while retaining the advantages of tuneable electrode geometry according to the replica method. Therefore, the fabrication process tailored for TiO$_2$ ceramics was adapted to suit the sintering conditions for Ti$_2$AlC ceramic. This initially resulted in the so called single coated Ti$_2$AlC ceramics, which were the subject of the electrochemical material investigations and the first pMFC applications. Subsequent improvements in compressive strength of the porous Ti$_2$AlC ceramics by repeated coating of the polyurethane foam template created samples described as double coated Ti$_2$AlC ceramics. Finally, double and single coated Ti$_2$AlC ceramics were compared with respect to their biofilm supporting quality using confocal microscopy.

A compacted Ti$_2$AlC ceramic was produced to complement the data on porous ceramics with information on surface morphology from AFM, surface hydrophobicity through contact angle goniometry and bulk resistivity by means of four terminal sensing.
6.3.3.1. Ti$_2$AlC ceramic preparation for electrochemical investigations

Appropriate adjustment of the water content in the ceramic slip allowed its viscosity to be lowered enough to preserve the porosity of the polyurethane foam template and to permeate its entirety, leaving a coating with uniform thickness (figure 75a). Simultaneously, the viscosity of the slip was still high enough to retain a sufficiently thick slip coating on the template in order to provide the required ceramic robustness for further electrode fabrication steps and handling in experimental setups. The macroporosity of Ti$_2$AlC ceramic was optimised by our collaborators through viscosity measurements of various slip compositions.\[33\]

![Figure 75: a) The template porosity of the polyurethane foam on the left was retained in the Ti$_2$AlC ceramic on the right through slip viscosity optimisation; b) The electrode surface could be increased dramatically as shown by this comparison of a porous and a compacted Ti$_2$AlC ceramic, each made from 3.763 g Ti$_2$AlC precursor powder.](image)

The comparison of a porous and compacted sample made from the same amount of Ti$_2$AlC precursor powder, shown in figure 75b, demonstrates the enhanced surface area and intricacy of structure geometry made available by employing a replication process for the Ti$_2$AlC ceramic fabrication. The prospect of using these qualities for electrochemistry and bio-fuel cell applications was the incentive behind the work on Ti$_2$AlC analysis. The compacted sample was a cylinder of 10.3 mm diameter and 7.1 mm height, while the porous sample was a cube with an edge length of 20.0 cm and a porosity of 10 ppi. This corresponded to a cylinder volume of 0.5945 cm$^3$, compared to a cube volume of 13.48 cm$^3$. An initial mass of 3.763 g was used for each sample; however, following treatment the pellet retained more mass than the porous sample, 3.478 g compared to 3.007 g. This mass loss difference was most likely due to the Ti$_2$AlC slip flaking off when the template foam evaporated during the sintering process.
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6.3.3.2. Compressive strength of single and double coated ceramics

The inclusion of a second coating step in the Ti$_2$AlC ceramic fabrication process minimised the strut fractures and cracks across the surface. As a result, the compressive strength of the porous network was increased more than 19 times, as shown in figure 76.

![Figure 76: a) Single coated Ti$_2$AlC ceramic with cracked surface and partially broken struts; b) Double coated Ti$_2$AlC ceramic with smoother surface and higher fraction of intact struts; c) Compressive strength of single coated compared to double coated Ti$_2$AlC ceramic.](image)

Repeating the coating with a less viscous ceramic slip after the first coating had dried caused less clogging of the template pores than earlier trials involving only a single coating of higher viscosity. The method showed that a uniform coating throughout the volume of larger templates, as well as great mechanical strength of the resulting ceramic, can be achieved by reducing the viscosity of the ceramic slip with higher water content and increasing the number of coating steps.

6.3.3.3. Electrochemical stability of Ti$_2$AlC

Light induced redox reactions were not observed in either of the electrolytes, as a result of this any data obtained that differed within the applied light intensity alone was treated alike.

The magnitude of current response differed between NaNO$_3$ and KCl background electrolyte; the current reached greater magnitudes in 0.1 M KCl than in 0.1 M NaNO$_3$, as shown in figure 77.
Figure 77: CVs with 50 mV s$^{-1}$ using the same Ti$_2$AlC ceramic WE in either 0.1 mol L$^{-1}$ KCl or 0.1 mol L$^{-1}$ NaNO$_3$, showing reproducibly differing current response for the respective background electrolyte.

An aqueous 0.1 M KCl solution should be electrochemically stable in this potential range, but these larger currents indicated additional redox reactions on the electrode, which could have been due to electrolyte impurities, or electrochemical reactions involving the electrode material. The KCl concentration was increased to 1 mol L$^{-1}$ and the resulting CV exhibited more pronounced Faradaic currents than those seen with 0.1 M KCl. Figure 78 shows even greater current increases at potentials positive of 0.6 V in 10 times larger KCl concentration. This was evidence for the participation of the KCl electrolyte in an electrochemically triggered redox reaction. A redox system around -0.38 V became apparent. The lack of potential separation between oxidation and reduction peaks inferred that the redox response was either taking place in the electrode material, or a surface immobilised species was measured.
Cyclic voltammetry on other working electrodes in the same solution did exclude the possibility of impurities in the 0.1 mol L\(^{-1}\) KCl background electrolyte. The analysis of the observed peaks on a platinum foil electrode did not reveal any electrolyte impurities, however a small reduction current was observed at -0.4 V using a glassy carbon electrode, as shown in figure 79.\textsuperscript{[34-36]}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure79.png}
\caption{CV analysis with 50 mV s\(^{-1}\) of the 0.1 mol L\(^{-1}\) KCl background electrolyte with a platinum WE (red) in combination with Pt net CE did not indicate any impurities in the background electrolyte.\textsuperscript{[34-36]} However, using a glassy carbon WE (black) revealed a small reduction current at -0.4 V, which was the same potential that caused a reduction reaction at the TiAlC ceramic.}
\end{figure}
Further investigation on the Ti$_2$AlC ceramic with cyclic voltammetry revealed a scan rate dependence of the observed Faradaic process. When the scan rate was changed from 50 mV s$^{-1}$ to 10 mV s$^{-1}$ the scan from higher to lower potentials produced greater currents than the reverse scan. This effect was repeatedly observed between -0.2 V to 0.7 V vs. Ag/AgCl reference, as depicted in figure 80. The arrows on one cyclic voltammogram at 10 mV s$^{-1}$ illustrate the counterintuitive increase of the current magnitude on the scan from positive to negative potentials as opposed to the reverse scan.

![Cyclic Voltammetry Graph]

**Figure 80:** CVs in 1 M KCl of the same Ti$_2$AlC electrode at 10 mV s$^{-1}$ (solid line) compared to a scan rate of 50 mV s$^{-1}$ (dashed lines) reveal a scan rate dependent material turnover. Arrows on the 10 mV s$^{-1}$ CV in dark represent the current evolution over time observed in all CVs at this scan rate and lower scan rates.

A similar signal response (black line) was not observed with any of the other tested electrode materials and suggested that the Ti$_2$AlC electrode material was involved in the redox reaction.

With this electrochemical setup, the scans at 10 mV s$^{-1}$ in the potential range of -0.5 to 1.2 V exhibited such high current that the readings went beyond the maximum measurable current of 0.4 A between the potentials of 0.5 to 1.2 V. Therefore, a scan in a smaller potential range of -0.5 to 0.7 V (solid black line in figure 80) was used to show the scan rate dependence of the material turnover.

Samples from Ti$_2$AlC ceramics before and after the application of overpotentials were compared using X-ray diffraction crystallography (XRD). XRD investigations of Magnuson *et al.*, Wang *et al.* and Zhu *et al.* were used to identify impurities which
were due to the ceramic fabrication process, so that the material change resulting from the application of overpotentials could be determined.\textsuperscript{[15,17,37]}

The X-ray diffraction pattern of a Ti$_2$AlC ceramic directly after sintering (figure 81a) mainly exhibited impurities of Ti$_3$AlC$_2$ and Ti$_3$AlC, which formed due to sintering above 1300 °C. Residual intermediate products of the sintering process, such as TiC and Ti$_3$Al, were also present.

The ceramic was also studied by X-ray diffraction after exhaustive cycling at low scan rates. Taking the impurities in the sintered sample into account, the application of potentials in the range of -0.3 to +0.7 V mainly caused the generation of $\alpha$-Al$_2$O$_3$ and TiO$_2$, while the intensity of Ti$_2$AlC reflections decreased, as shown in figure 81b.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure81.png}
\caption{Comparison of Ti$_2$AlC XRD data with XRD investigations of Magnuson et al. (*), Wang et al. (†) and Zhu et al. (‡) allowed assigning of the reflections of: a) X-ray diffraction pattern of Ti$_2$AlC ceramic after sintering to mainly Ti$_2$AlC phase with impurities of Ti$_3$AlC$_2$ and Ti$_3$AlC due to sintering temperature over 1300 °C; b) XRD pattern of Ti$_2$AlC ceramic after the application of potentials in the range of -0.3 to +0.7 V vs. Ag/AgCl reference electrode with increased content of $\alpha$-Al$_2$O$_3$.}
\end{figure}
6.3.3.4. Exhaustive Ti$_2$AlC oxidation through continuous cyclic voltammetry

The main changes in current response with number of CV cycle were observed at potentials of +0.7 V and +0.3 V vs. the Ag/AgCl reference electrode when scanning from positive to negative potentials. Figure 82 illustrates this result through the comparison of an initial scan with a distinct oxidation current response (blue solid line with blue filling) to one of the later scans in this experiment with a much lower oxidation current (red dashed line). Hence, the current magnitude at these potentials was used for quantitative analysis of trends in the redox process.

[Graph showing current vs. voltage with max at scan 11 at 1 mV s$^{-1}$ and min at scan 145 at 1 mV s$^{-1}$]

**Figure 82:** The change of CV shape throughout the 169 scans of the Ti$_2$AlC ceramic in 0.1 mol L$^{-1}$ KCl solution with 1 mV s$^{-1}$. The current response was subjected to the greatest fluctuations in current magnitude with cycle number at 0.7 V and 0.3 V on the scan from positive to negative potentials, as could be seen by comparison of the maximum oxidation current response (blue line with blue filling) to the minimum oxidation current response at this scan rate (red dashed line).

The generation of a white gelatinous substance from the electrode surface was observed while continuously cycling between -0.3 V and +0.7 V vs. the Ag/AgCl reference electrode. Figure 83 shows this substance on other Ti$_2$AlC electrodes that underwent cyclic voltammetry under the same conditions.
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Figure 83: a) gelatinous substance as it is generated from Ti$_2$AlC during CVs between potentials of -0.3 to 0.7 V in KCl background electrolyte; b) Ti$_2$AlC electrode after drying of the generated substance; c) XRD diffraction pattern of the dried gel (red line) overlaid with the diffraction pattern of KCl (green line).

The gel only formed on the external protrusions of the ceramic structure during repeated cyclic voltammetry in KCl, not on the interior surface, as depicted in figure 83a. Figure 83b shows this substance after drying on another Ti$_2$AlC electrode; a white powder localised primarily around the pointed features of the external structure where the electric field was the strongest.

The XRD analysis of the white powder, shown as the red line in figure 83c, was inconclusive, since the diffraction pattern did not show a reliable line pattern. The green line pattern superimposed onto the red line measurement of the gel represents the expected diffraction of KCl, which gave the best fit to the experimental data. However, in order to identify this amorphous substance, other types of analysis would have to be employed, which are referred to in the future work section 6.5.3.

This substance generation was accompanied by slow emission of gas from the electrode, which ultimately removed the gelatinous substance, leading to its precipitation. Considering the potential range and low amounts of gas produced from the comparably large electrode surface area, it is unlikely that this gas stemmed from water splitting. The gas emission only caused the sporadic release of gas bubbles and it is therefore unlikely that the larger currents resulted from increased convection of the surrounding solution. Pictures of the greatest build up of this white gelatinous substance (during stage b) in comparison to its appearance during the first (stage a) and the last CV (stage c) at this scan rate are shown in figure 84 and are related to the respective CVs at these stages in figure 85.
Figure 84: Visual appearance of the Ti$_2$AlC ceramic was divided into three stages: (a) initial stage with measureable Ti$_2$AlC material turnover, but no visible surface change; (b) generation of a white gelatinous substance from the electrode while high current to potential ratios indicated a large electrode resistance; and (c) removal of the gel caused by gas emission from the electrode.

The CV corresponding to stage a (black line in figure 85) exhibited larger currents on the scan from positive to negative potentials, strongly indicating Ti$_2$AlC material turnover, while optically no change of the electrode surface was observed. During successive scans from negative to positive potentials (forward scans) the current magnitude continued to increase until the maximum gel buildup stage b was reached (red line in figure 85). However, the reverse scan current magnitude consistently aligned with the previously observed magnitudes from 0.3 V down to lower potentials. This was accompanied by a slight shift of the oxidation peak potential by approximately 0.1 V to more positive potentials.

The removal of the gel layer from the surface was attributed to the gas emission from the electrode. Both the current owing to electrode capacitance, and the current caused by material turnover decreased, as shown by the CV for the end stage c (blue line in figure 85). The current response in CVs during the end stage did not indicate an increase in electrode resistance due to the oxidation process.
Figure 85: CV response recorded in 0.1 M KCl with 1 mV s\(^{-1}\) in relation to the three stages of visual appearance of the Ti\(_2\)AlC ceramic: (black line a) initial stage with a large difference between the lower current during the forward scan and the larger current during the reverse scan current at positive potentials indicate slow Ti\(_2\)AlC material turnover; (red line b) the greater incline of the forward scan current and only slightly increased currents during the reverse scan suggest faster Ti\(_2\)AlC oxidation during the generation of a white gelatinous substance from the electrode; and (blue line c) end stage with lower currents during the reverse scan showed that the material turnover is limited after the formation of gel had stopped.

The lack of generation of white gelatinous substance and consistently low currents during the last scans at 1 mV s\(^{-1}\) indicated that the oxidation process was exhausted.

The observation of changing current magnitude with number of CV scans at the potentials of greatest change in current response, +0.3 V and +0.7 V, confirmed the features of material change observed visually during CVs. During the first 11 scans an increase in current was observed at +0.7 V as well as at +0.3 V to almost double the values of the initial scan, indicating an increase in electrochemical oxidation of the electrode with each consecutive scan. The currents at +0.7 V remained high for a further 45 scans, whilst the backward scan current at +0.3 V diminished in subsequent scans. A sudden drop in current at both potentials after scan 56 was followed by a recovery of the previous current magnitude during the following 25 scans. This can only be explained by an encumbrance of the diffusion of one reactant to the reaction site, which was progressively restored. However, two more drops in current magnitude without recovery after 113 and 141 scans led to a series of repeatedly low currents. Figure 86 depicts the current development at the potentials of interest for the whole experiment at 1 mV s\(^{-1}\).
Figure 86: Current at 0.7 V (black line with dots) and 0.3 V (blue line with triangles) wrt. Ag/AgCl during the scan from positive to negative potentials showing the decline of these currents during repeated cycling with 1 mV s\(^{-1}\). The scans were conducted in 0.1 M KCl at 1 mV s\(^{-1}\).

The experiment at 1 mV s\(^{-1}\) was discontinued following this, since it was believed that the low currents signified a complete exhaustion of the oxidation process, or at least one of the required reactants.

However, with the lower scan rate of 0.5 mV s\(^{-1}\) it was possible to observe that the oxidation process was still taking place at a lower turnover rate, shown in figure 87.
Figure 87: CVs in 0.1 M KCl at a reduced scan rate of 0.5 mV s\(^{-1}\) revealed that the oxidation process had not been exhausted at 1 mV s\(^{-1}\). The CV overlay depicts the initial Ti\(_2\)AlC oxidation during scan 2 (black line), its reduced magnitude halfway through the experiment (red line) and the absence of Ti\(_2\)AlC oxidation by the end of the experiment (blue line).

Equally to the previous experiment at 1 mV s\(^{-1}\), the CV scans were repeated until the oxidation currents became negligible. During scan 202 in figure 87 the current at 0.3 V was 0.14 mA and the material turnover was considered insignificant. Therefore, a continuation of the experiment at even lower scan rate was not conducted.

6.3.3.5. **Scan rate dependence of electrochemical Ti\(_2\)AlC oxidation**

All scan rates between 500 mV s\(^{-1}\) and 5 mV s\(^{-1}\) resulted in typical charging currents without any observable Faradaic process (figure 88a). Oxidation currents were only observed at 1 and 0.5 mV s\(^{-1}\) (figure 88b). The current at the oxidation peak potential, 0.3 V, was more than twice as high when measured with the slower scan rate of 0.5 mV s\(^{-1}\). This suggested slow kinetics of the oxidation process and a lower maximum scan rate for the observation of the oxidation than seen in earlier CVs of the same sample.
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Figure 88: (a) Cyclic voltammetry with a Ti$_2$AlC ceramic electrode in 0.1 M KCl at scan rates of 500 through to 5 mV s$^{-1}$ did not cause detectable oxidation between -0.3 to +0.7 V vs. Ag/AgCl reference electrode. (b) Higher oxidation currents during scans from positive to negative potentials were measured the slower the scan rate was.

It should be noted that the initial CV analysis of the electrochemical Ti$_2$AlC stability (6.3.3.2) already showed pronounced oxidation currents with a scan rate of 10 mV s$^{-1}$, while only non-Faradaic current was observed down to 5 mV s$^{-1}$ during this analysis process. This discrepancy was due to the long-term CV analysis between both investigations, which further exhausted the Ti$_2$AlC oxidation and is additional evidence that the oxidation process is limited to a certain amount of material. The scan rate, which was required for the oxidation to happen, decreased with the increasing amount of oxidised material, suggesting that the already oxidised material slowed down further oxidation.

The reason for testing the influence of scan rate after exhausting the oxidation with continuous CVs was that initial cyclic voltammetry revealed significant changes of oxidation currents with scan number. If the scan rate effect would have been measured during the first few scans, then two variables would have had an influence on the current response and information about the scan rate effect would have been inaccessible. Instead, the influence of scan rate was investigated when the change of the current response with cycle number was negligible.

6.3.3.6. Potential window of Ti$_2$AlC ceramic electrodes

Applying a potential range of -0.3 V to 0.7 V resulted in the typical Ti$_2$AlC oxidation signal (figure 89a, black curve with horizontal line pattern). The reverse scan (from positive to negative potentials) featured larger oxidation currents than the forward scan at potentials around 0.3 V vs. Ag/AgCl reference. Reducing the potential window to -0.2 V to 0.6 V also reduced the magnitude of the oxidation current (figure 89a, red curve with red fill). Despite this, oxidation still took place, which was recognised by the otherwise unusually similar forward and backward scan currents around 0.3 V. CVs in the two potential ranges that caused an oxidation current were overlaid in figure 89a. The red filled curve in figure 89a was shown as
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a black curve with vertical line pattern in figure 89b, in order to illustrate the difference between the faint oxidation in a potential window of -0.2 V to 0.6 V compared to a CV which did not show any Faradaic processes (blue curve with blue fill) in a potential window of -0.1 V to 0.5 V.

Figure 89: Electrochemical Ti$_2$AlC ceramic oxidation dependence on applied potential window. (a) Oxidation took place in potential windows of -0.3 to 0.7 V (black curve with horizontal line pattern) as well as -0.2 to 0.6 V vs. Ag/AgCl reference electrode (red curve with red fill). (b) The application of potentials in a range of -0.1 to 0.5 V did not cause Ti$_2$AlC oxidation (blue curve with blue fill).

The rise in current with increasing overpotential in the CV from -0.1 V to 0.5 V was also seen in CVs from 0.0 V to 0.4 V and can be attributed to non-Faradaic processes.

6.3.3.7. Analysis of Ti$_2$AlC ceramic oxidation based on charge flow

The translation of the experimentally gained information into cumulative charge allowed clearer presentation of a vast amount of data and also served as a proof of concept for further studies on Ti$_2$AlC ceramics.

Figure 90 summarises the outcome of the analysis process described in 3.5.1.5. The cumulative charge resulting from the acquired data was plotted as solid lines and the respective extrapolation as dashed lines. The residual sum of squares between each data set and its fitting function was indicated as similarly coloured $R^2$ values next to the extrapolations in the graph. Supplementing the cumulative redox charge flow (red line for oxidation and blue line for reduction) is the cumulative charge flow due to non-Faradaic processes (green line), in order to illustrate the reasons for fitting the cumulative absolute charge (black line) with an exponential function that changes into a linear function.
Figure 90: The cumulative charge that was transferred at 1 mV s\(^{-1}\) scan rate overall (black line) was corrected for non-Faradaic charge flow (green line) to find the charge that was required to oxidise (red line) and to reduce (blue line). With the information from this, the data could be extrapolated (R squared values indicating respective goodness of fit) to predict how many more CVs would have been required to completely exhaust the Faradaic processes (red dashed curve) of Ti\(_2\)AlC in 0.1 mol L\(^{-1}\) KCl electrolyte.

The gradient of oxidation charge transferred per CV (red line) was predicted to exponentially decline, so that the curved progression of the cumulative absolute charge (black line) adopted the linear increase of the cumulative background charge flow (green line) as the oxidation charge gradient approached zero. This was translated into a redox process, which initially was at maximum rate, then decreased exponentially in reaction rate until it stopped and only non-Faradaic charge transfer contributed to the overall charge flow.

Therefore, the exponential fit of the cumulative oxidation charge (red dashed line) predicted an asymptotic approach to a maximum anodic charge transfer, \(Q_{\text{ox,max}}\), of 1185 C. This was 226 C more than the sum of charge transferred for oxidation by the end of the experiment at 1 mV s\(^{-1}\). The number of CV scans necessary to exhaust the oxidation process to certain levels was analysed from the first derivative of the fit functions in figure 90. After 158 scans, the average oxidation charge increment was already lower than the background charge increment. This made the evaluation from the CV plot of current vs. potential so ambiguous that the experiment was stopped after 166 scans. To exhaust the oxidation process to an extent that causes the corresponding charge flow to be 10% of the background charge flow, 358 CV scans would have been necessary, 192 more than conducted.
However, the achieved information was enough to model the data sufficiently to draw unambiguous conclusions.

One advantage of plotting the cumulative charge transfer was that the sums of charge flown to exhaust an irreversible process were comparable, even if CVs had been measured at different scan rates. Therefore, the data from the experiment at 0.5 mV s\(^{-1}\) could be used to confirm the conclusions drawn from the experiment at 1 mV s\(^{-1}\).

Analysing the data in the same manner as described for the cyclic voltammetry at 1 mV s\(^{-1}\) above, resulted in the cumulative charge plot shown in figure 91.

![Cumulative charge plot](image)

**Figure 91:** The cumulative charge that was transferred at 0.5 mV s\(^{-1}\) scan rate overall (black line) was corrected for non-Faradaic charge flow (green line) to find the charge that was required to oxidise (red line) and to reduce (blue line). Analysis of this data and its extrapolation (dashed lines with respective R squared values indicating goodness of fit) confirmed the conclusions drawn from the previous experiment at 1 mV s\(^{-1}\).

It can be seen from figure 91 that the oxidation process was exhausted during the cyclic voltammetry at 0.5 mV s\(^{-1}\). In addition, all curve progressions were measured as they were predicted on the basis of the data recorded at 1 mV s\(^{-1}\).

6.3.3.8. **Bulk resistivity of compact Ti\(_2\)AlC ceramic**

Directly after sintering, high bulk resistivities of 194 ±0.0829 MΩcm were measured on the surface of the in-house made Ti\(_2\)AlC ceramic. However, a polished cross section of the same sample exhibited a ten magnitudes smaller resistivity of 13.0
±0.334 mΩcm. The sample of pure Ti<sub>2</sub>AlC from Kanthal only allowed cross checking of the latter result, since it was delivered polished on all sides. Its bulk resistivity of 9.02 ±0.629 mΩcm was in agreement with the high conductivity of the Ti<sub>2</sub>AlC bulk material produced in our laboratories. Optically, the polishing did not change the Ti<sub>2</sub>AlC surface (see figure 92a and b).

![Figure 92: Ti<sub>2</sub>AlC surface of: a) our sample directly after sintering, exhibiting large bulk resistivity; b) our polished sample with low bulk resistivity; c) Kanthal sample after CVs in ferricyanide corroded the surface and increased the bulk resistivity; d) Kanthal sample after electrochemically corroded surface had been polished reducing the bulk resistivity again.

Four point probe measurements following the application of our Ti<sub>2</sub>AlC ceramic as working electrode (WE) for CVs with potentials ranging between -0.2 to 1.0 V in 0.3 mM potassium ferricyanide (K₃[Fe(CN)₆]) showed high resistivities of 194 ±0.0346 MΩcm, similar to the resistivity before the polishing. Furthermore, the surfaces of both samples showed signs of corrosion after the cyclic voltammetry, as shown in figure 92c, which suggested the deposition of Fe₂O₃. Polishing the corroded surfaces decreased the bulk resistivity of both samples by eight magnitudes to 1.41 ±0.0264 Ωcm in our samples and 8.51 ±0.0725 Ωcm in the Kanthal sample. All measurements throughout different stages of Ti<sub>2</sub>AlC ceramic production and application are summarised in table 11.

**Table 11:** Resistivity measurements of Ti<sub>2</sub>AlC ceramic after certain stages in its production and use as electrode material. Results indicated passivation during sintering and after cyclic voltammetry in ferricyanide solution.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Bulk resistivity ( r_\infty ) of Ti&lt;sub&gt;2&lt;/sub&gt;AlC from our labs (Ωcm)</th>
<th>Bulk resistivity ( r_\infty ) of pure Ti&lt;sub&gt;2&lt;/sub&gt;AlC from Kanthal (Ωcm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural surface directly after sintering</td>
<td>1.94E+08 ± 8.29E+04</td>
<td></td>
</tr>
<tr>
<td>Polished surface directly after sintering</td>
<td>1.30E-02 ± 3.34E-04</td>
<td>9.02E-03 ± 6.29E-04</td>
</tr>
<tr>
<td>Rinsed surface after CVs from -0.2 to 1.0 V in 0.3mM K₃[Fe(CN)₆]</td>
<td>1.94E+08 ± 3.46E+04</td>
<td></td>
</tr>
<tr>
<td>Polished surface after CVs from -0.2 to 1.0 V in 0.3mM K₃[Fe(CN)₆]</td>
<td>1.41E+00 ± 2.64E-02</td>
<td>8.51E+00 ± 7.25E-02</td>
</tr>
</tbody>
</table>
**6.3.3.9. Atomic force microscopy of a compacted Ti$_2$AlC ceramic**

Atomic force microscopy was performed on the compacted Ti$_2$AlC ceramic sample to complement the electrochemically achieved results on porous Ti$_2$AlC ceramics with information on the roughness of the biofilm support. The observed surface morphology of the compacted sample was confirmed with scanning electron microscopy (SEM) on porous samples at a similar magnification, in order to validate that the study on the compacted sample is representative for porous Ti$_2$AlC ceramics.

The common surface characteristics observed during AFM imaging were the partition of the surface into scales with comparatively large cavities between them. Many scales exhibited lamellar morphology. Figure 93 illustrates the subdivision of the surface into segments with a sequence of line fitted colour map, 3D model and SEM confirmation in the corresponding magnification for a 50 µm and a 16.3 µm section respectively.

![Figure 93](image)

**Figure 93**: AFM micrographs of the compacted Ti$_2$AlC ceramic surface morphology compared to SEM images of a porous Ti$_2$AlC ceramic, which were recorded with the same magnification: a) 50 µm section and b) 16.3 µm section, both showing the partition of the surface into layered segments. The orientation of the layers was similar within a segment, but often differed between segments.

The SEM micrographs of a porous Ti$_2$AlC ceramic, produced by single coating of a foam template and sintering via setting method 1 according to 3.4.6.2, confirmed the scaled surface morphology and identified the scattered sub-micrometer structures as more conductive (brighter in the SEM image) particles.
Consequently, the average surface roughness was comparatively large, ranging from $344 \pm 99$ nm in a 10 $\mu$m area to $870 \pm 86$ nm in a 50 $\mu$m area.

Figure 94 shows that the appearance of a lamellar morphology on certain scales could not be an imaging artefact resulting from dragging of the sample, since the lamella were often differently orientated on separate scales. SEM imaging confirmed that the lamella resulted from layered crystallites.

![Figure 94: Confirmation of the lamellar Ti$_2$AlC ceramic structure with SEM images of the same dimensions revealed that the layers appear along the edges of crystallites: a) 21.2 $\mu$m section demonstrating the varying orientation of the layers and b) 8.14 $\mu$m section showing the fine structure of the lamellae.](image)

Depending on the orientation of the crystallite, the AFM would either show the smooth surface of one layer, or could measure the layered structure on the lateral faces of the crystallite. Thus, the distance between layers observed with AFM was dependent on the angle between the planes of measurement and lateral face of the crystallite.

### 6.3.3.10. SEM of porous Ti$_2$AlC ceramic before and after oxidation

SEM was performed on Ti$_2$AlC ceramic in order to analyse changes in surface morphology and elemental composition before and after electrochemical oxidation as well as to complement the AFM data. This yielded a more 3-dimensional insight into the Ti$_2$AlC ceramic surface availability to exoelectrogenic cells.
Figure 95 shows the typical geometry of one pore in a 10 ppi Ti$_2$AlC ceramic, including the strut cross sections that form across the faces of a Ti$_2$AlC ceramic during the sintering process.

![Figure 95](image.png)

**Figure 95:** SEM micrographs and dimensions of a) one Ti$_2$AlC ceramic pore with cross sections of the hollow struts, which connected it to further pores; b) a Ti$_2$AlC strut cross section showing the cavities left behind after the pyrolysis of the template foam. The ceramic in both micrographs resulted from a single coating of the template and was sintered according to setting method 1 as described in 3.4.6.2.

As illustrated in figure 95b, the pyrolysis of the polyurethane foam template leaves a cavity of approximately triangular cross-sectional shape along all struts of the porous Ti$_2$AlC scaffold with cracks forming at the triangle corners. Gas, which did not escape through such cracks, can get entrapped in the viscous ceramic slip thereby forming spherical voids, as shown in figure 95b. Five analysed strut cross sections had an average diameter of 512 ±73 µm and a wall thickness of 208 ±65 µm, which equates to a Ti$_2$AlC ceramic fraction of 83 ±12 % of the cross section area. The large size variations resulted from the differences in cross section position with regard to the closest strut nodes.

Simplifying the strut geometry to a cylinder of roughly 2 mm length with a triangular volume inside it illustrates the practical implication of the remaining 17 % of hollow strut cross sectional area. The external strut area of 3.2 mm$^2$ was extended with 1.7 mm$^2$ surface area of the internal strut cavity, which was accessible to micro-organisms, such as green algae and cyanobacteria, as well as to electron mediators through cracks and strut cross sections.

Strut cross sections also gave first insights into how exhaustive oxidisation through cyclic voltammetry changed the Ti$_2$AlC ceramic surface morphology and showed how thick the layer of electrochemically modified material is. As illustrated by the comparison between the strut cross sections of a single coated Ti$_2$AlC ceramic directly after sintering (figure 96a) and then following long-term cyclic voltammetry in a range of -0.3 V to 0.7 V vs. Ag/AgCl in an aqueous solution of 0.1 mol L$^{-1}$ KCl (figure 96b), the strut interior was unaffected by the oxidisation process.
In both cases the interior strut morphologies matched the angular crystallite shape, which was observed on the surface of Ti$_2$AlC ceramic directly after sintering. A detailed study of morphological features at the edge to the exterior of the same strut as shown in figure 96b is presented in figure 97. The strut exterior, seen on the left side of the centre image, was encrusted in flocculent structures (figure 97a and b) of smaller dimensions than the typical Ti$_2$AlC crystallites (figure 97c to e). This crust was only several micrometers thick, but formed an almost continuous film over the whole surface of the oxidised ceramic.
Figure 97: Comparison of the exterior and interior features of an oxidised Ti₂AlC ceramic strut cross section. The top centre image shows the whole strut cross section at 150 times magnification and the image below it is a 2000 times magnification of the outer strut layer including strut surface. a-b) Flocculent features on the surface of electrochemically oxidised Ti₂AlC ceramic; c-e) The original layered structure of Ti₂AlC ceramic, as it is usually seen on the surface of non-oxidised Ti₂AlC samples, was only observed in the interior of oxidised samples. The colours of the image frames respectively match the colours of the sections on the lower magnification image.

The formation of a russet film in the vicinity of the electrical contact between the stainless steel wire and Ti₂AlC ceramic was detected during the long-term cyclic voltammetry. A comparison between optical appearance and SEM micrographs is given in figure 98.

Figure 98: Oxidised area around the electrical contact between stainless steel wire and Ti₂AlC ceramic resulting from long-term cyclic voltammetry in a potential range of -0.3 V to 0.7 V vs. Ag/AgCl in an aqueous solution of 0.1 mol L⁻¹ KCl: a) Dried electrode section around wire contact; b) SEM micrograph showing the more flocculent morphology of the oxidised film as compared to the angular structure of Ti₂AlC ceramic, which only remained uncovered in the right bottom side of the picture; c) fine structure of the oxidised film.
The morphology of the surface structures matched the flocculent features seen across the rest of the oxidised electrode; however it was interspersed with flocculent structures of smaller scale. This was in contrast to the remaining electrode surface and indicated the involvement of a material which was incorporated in the electrical contact or its insulation. The russet film and the smaller flocculent structures were only seen in the vicinity of the electrical contact. SEM and optical appearance of the oxidised Ti<sub>2</sub>AlC therefore suggest that the larger flocculent features do not originate from the electrical contact or its insulation. Similarities in shape probably result from the amorphous nature of both films.

Cross sections of electrochemically oxidised Ti<sub>2</sub>AlC ceramics were analysed regarding phase and elemental composition with SEM in backscattered electron composition imaging (BEC) mode. Samples of porous ceramics were embedded in resin to stabilise the structure during the lapping. Material was removed with gradually decreasing grain size to yield representative strut cross sections.

A layer of up to 70 µm thickness formed on the surface of the oxidised Ti<sub>2</sub>AlC ceramic and differed from the bulk material in composition, density and conductivity. The layer only advanced into such large depths if it was composed of multiple phases, as shown in figure 99.

![Figure 99](image.png)

**Figure 99:** Cross section SEM of electrochemically oxidised Ti<sub>2</sub>AlC ceramic showing the formation of three layers on the strut surface. BEC in combination with elemental mapping revealed four phases: 1) a dense outer phase of 10-20 µm thickness with lower conductivity. This phase exhibited high contents of Al and O, and no Ti, suggesting Al<sub>2</sub>O<sub>3</sub> formation; 2) a less dense and more conductive phase of 25-28 µm thickness with high Ti content between two phases of lower conductivity; 3) a phase similar to phase 1 that formed a second 10-20 µm thick layer approximately 50 µm below the ceramic surface; 4) a loosely packed interior strut that exhibited similar composition and conductivity as observed for phase 2, but with a lower density.

The strut surface was consistently composed of a phase with lower conductivity and high aluminium as well as oxygen content (phase 1) in all samples. Although not measured directly, the lower conductivity of phase 1 compared to other phases in the material was suggested by its darker appearance in the SEM image. The elemental composition of phase 1 indicated that an Al<sub>2</sub>O<sub>3</sub> layer had formed during
the oxidation of the Ti$_2$AlC ceramic. Layers of up to 70 μm thickness were only observed if a phase with similar composition and conductivity had formed at a distance of 25-30 μm from phase 1. This phase was called phase 3 in figure 99, although its characteristics suggest that phase 1 had formed in two layers. More detailed investigations are required to confirm this assumption and the phases 1 and 3 are therefore treated separately within this work. Phases 1 and 3 each formed layers of 5-20 μm thickness and surrounded a phase with lower Al and O content, but with comparatively higher Ti content (phase 2). The brightness of phase 2 indicated a higher conductivity compared to the phases 1 and 3, and it exhibited less dense packing. Its similarity to the bulk phase (phase 4) in composition and conductivity suggests that this was Ti$_2$AlC ceramic. Phase 2 was therefore only distinguished from the bulk phase by its denser packing, which could have resulted from either the formation of the phases 1 and 3, or from the sintering process.

It should be noted that the false colour images in figure 99 are an indication of the relative elemental composition and that the elements Ti, Al, C and O were present in all phases. This is illustrated by the relative carbon content, which was so large in the background that the intensity of carbon emission from the ceramic could not be shown.

The Al and O rich phase was often observed as a single layer in direct contact with the bulk phase, as depicted in figure 100.

![Figure 100: Cross section SEM of electrochemically oxidised Ti$_2$AlC ceramic showing a single layer on the strut surface. The layer was 5-10 μm thick and denser than the interior of the ceramic. It was characterised by lower conductivity as well as high contents of Al and O, which indicated a surface passivation of the Ti$_2$AlC ceramic resulting from Al$_2$O$_3$ formation.](image)

A somewhat denser packing of phase 4 was seen adjacent to phase 1; however, it did not form a layer as thick as shown by phase 2 in figure 99. This suggested that it was indeed the formation of the Al and O rich phase which caused the denser packing of Ti$_2$AlC.
6.3.3.11. Contact angle goniometry of compacted Ti₂AlC ceramic

For biofilm formation crucial surface hydrophilicity of the in house produced Ti₂AlC ceramic was evaluated and compared to the company sample from Maxthal with contact angle goniometry. An average Milli-Q water contact angle of 73.41 ±4.60° was achieved from measurements in various locations on the in-house produced Ti₂AlC ceramic (figure 101a), while the company sample presented a slightly lower average water contact angle of 69.51 ±3.90° (figure 101b).

![Figure 101: Contact angle of Milli-Q water on a) the natural surface of the in-house produced, compact Ti₂AlC ceramic and on b) polished Ti₂AlC produced by Maxthal.](image)

This slight difference in contact angle was attributed to the polished state of the company sample as opposed to the non-polished surface of the in-house produced Ti₂AlC ceramic. Our sample was kept non-polished, since this was more representative of the unpolished surfaces of the porous Ti₂AlC bio-anodes applied in MFCs. However, both average contact angles indicated a hydrophilic surface independent of the ceramic surface roughness.

6.3.3.12. CV and SWV of differently fabricated Ti₂AlC ceramics in culture

Cyclic voltammetry (CV) studies of the exoelectrogenic interaction of the green algae culture *C. vulgaris* with Ti₂AlC ceramic resulted in the reproducible identification of a cathodic peak when single coated Ti₂AlC ceramic was employed, however this peak was not seen with double coated or compacted Ti₂AlC. Furthermore, an oxidation peak was not observed during the CV on any of the ceramic electrodes. As a result, square wave voltammetry (SWV) was employed in order to elucidate the reasons for the varying CV responses and to identify the oxidation potential of the redox-active substance in culture.

The limited Ti₂AlC oxidation at positive overpotentials, described in 6.3.3.4, was exhausted with 345 CVs in the potential range of 0.0 V to 0.7 V, in order to assure that all redox currents could be attributed to the CVu culture. During this procedure the CV shape changed from the form depicted in figure 102a to the one in figure 102c.
Figure 102: Electrochemical oxidation of the porous, single coated Ti$_2$AlC ceramic through cyclic voltammetry with 5 mV s$^{-1}$ in the potential range 0.0 V to 0.7 V wrt. Ag/AgCl was characterised by: a) partially larger currents on the scan from 0.7 to 0.0 V, which declined with number of scans; b) an initial rise in maximum CV current for the first five scans, followed by an exponential decline; c) reproducible CVs presenting the charging current of the electrode at the end.

Initially, the CVs presented large anodic currents and partially larger currents on scans from higher to lower overpotentials, elaborated on in 6.3.3.2. These features intensified throughout the first five CVs and then diminished exponentially, as illustrated on the example of the maximum anodic current in figure 102b. The continuous cyclic voltammetry was stopped when the CV shape and consistently low currents indicated that only charging current was observed in the KCl electrolyte.

13 days after incubation in a CVu culture the single coated Ti$_2$AlC ceramic showed the cathodic peak current at -0.15 V during CVs in a potential range of -0.3 V to 0.4 V. Figure 103a shows that variation of the scan rate only influenced the magnitude of the charging current, but not of the reduction peak current. However, if the CV was measured in a reduced potential range of -0.3 V to 0.1 V, then the reduction peak disappeared at all scan rates, as seen in figure 103b.

Figure 103: The reduction peak current measured at the single coated Ti$_2$AlC ceramic after 13 days of incubation in a C.vulgaris culture: a) Was independent of scan rate and b) did not arise if the potential range was reduced to -0.3 to 0.1 V. The disappearance of the reduction peak was reversible by extending the potential range to 0.4 V again.

Extension of the potential range to 700 mV recovered the reduction peak again, indicating that the redox-active substance was present in its reduced form and had to be electrochemically oxidised in the potential range of 0.1 V to 0.4 V before it could be detected through reduction at -0.15 V. This phenomenon was consistently
observed during the following 26 days of analysis, as illustrated in figure 104a. Reducing the charging current with slower scan rates did not reveal an oxidation peak down to 2 mV s\(^{-1}\). Therefore, the detection limits were augmented with SWV and an oxidation peak was found at 0.0795 V. Figure 104b shows both peaks as measured with SWV, the reduction peak (black line), which was measured at a frequency of 12 Hz and an amplitude of 2 mV, and the oxidation peak (blue line), which was detected at a frequency of 100 Hz and an amplitude of 40 mV.

Figure 104: a) The reduction peak current and CV shape of *C. vulgaris* on the single coated porous Ti\(_2\)AlC ceramic, here presented for a scan rate of 10 mV s\(^{-1}\), were observed over weeks; b) An oxidation peak was identified in culture at 0.0795 V with SWV at a frequency of 100 Hz and an amplitude of 40 mV.

In order to detect the oxidation peak, the SWV had to be performed at considerably larger amplitude than it was necessary for the reduction peak. Consequently, this signal amplification caused the oxidation peak to broaden and to exhibit larger peak current than the reduction.

Oxidation peaks measured at less ideal conditions of 80 Hz and 40 mV, as well as reduction peaks recorded at 8 Hz and 5 mV, are summarised in figure 105, in order to allow comparisons between data from early experiments with later experiments over time.

Figure 105: SWV of Ti\(_2\)AlC ceramic in *C. vulgaris* culture revealed: a) increasing oxidation currents over time at a frequency of 80 Hz and an amplitude of 40 mV; b) reduction peak shift to more positive potentials and decrease in magnitude at 8 Hz and 5 mV.
Although the oxidation peak current was found to increase over time, more data is required to draw a conclusion on the type of current increase. Additionally, a shift of the reduction peak potential was observed, which was attributed to changes in the reversibility of the redox reaction.

In contrast, samples of double coated porous ceramic and compacted ceramic did not show a redox current during cyclic voltammetry examinations in culture at any tested scan rate, as shown in figure 106.

The absence of Faradaic current was confirmed with SWV measurements at a range of frequencies and amplitudes, including the settings used previously for single coated Ti$_2$AlC ceramics.

6.3.3.13. Biofilm support of single and double coated Ti$_2$AlC ceramics

Confocal microscopy was used to elucidate the differences between the electrochemical responses of single and double coated Ti$_2$AlC ceramic bio-anodes, and was assessed as a technique for further studies.

The initial lambda measurement identified the maximum fluorescence intensity at 684 nm, which is the typical chlorophyll a fluorescence wavelength. Overlaying the images of chlorophyll a fluorescence with bright-field microscopy allowed imaging of photosynthetic cells, and the relation of biofilm coverage with the curved structure of the porous Ti$_2$AlC ceramic underneath. Incubation of the single and double coated ceramics in the same C.vulgaris culture for over three weeks resulted in a minuscule cell adherence to the double coated sample, while a continuous biofilm formation was observed on the single coated sample, as seen by comparing figure 107a with figure 107b and c.
**Figure 107**: Overlay of bright-field and confocal micrographs of *C. vulgaris* biofilms on double coated Ti$_2$AlC ceramic (a) and single coated Ti$_2$AlC ceramic (b-c) showing better cell adhesion to the surface of the single coated ceramic. The chlorophyll a fluorescence was recorded at 684 nm and is not visible across the whole surface of the struts, since the curved form of the struts precluded focusing on all cells in one image.

The focal plane in figure 107b was adjusted to show the dimensions of biofilm and strut, which prevented the collection of fluorescence light from the centre of the round strut and therefore caused darkening in this region. However, variation of the focal depth confirmed that this strut was covered in a continuous, photosynthetic biofilm of approximately 37 μm thickness.

**6.3.4. Flat ITO plastic and FTO glass bio-anodes in an air-cathode pMFC**

Continuous flow cells were used to acquire controls for each pMFC solution component and to examine the long-term performance of ITO plastic and FTO glass anodes as well as of hot-pressed air cathodes from activated carbon cloth and Nafion®. Two preliminary short-term experiments confirmed the pMFC sustainability and the effects of ferri-/ferrocyanide mediator addition to a *C. vulgaris* culture respectively, followed by a long-term investigation in which the mediator was added before the culture was measured.

The overall power output of the continuous flow cells was inferior to batch systems due to larger diffusion lengths and solution volumes. However, the constant solution exchange, which reduced toxin buildup and supplied fresh nutrients in the flow cells, facilitated long-term culture viability, resulting in biofilm growth and yielded the potential to conduct multiple investigations on one single biofilm.

**6.3.4.1. System sustainability**

Preliminary studies into the system suitability for long-term measurements began with the simultaneous addition of all later separately tested solution components,
the growth medium 3N-BBM+V, the redox mediator couple ferricyanide and ferrocyanide at concentrations of 2.5 mmol L⁻¹ and the green algae culture CVu. A decrease in power generation by an average of 81% in all flow cells throughout the first day of operation indicated an initial redox reaction of non-biological solution components and was confirmed to be a regular response to the addition of either growth medium, or mediator during the later long-term experiment. The photoresponse in all flow cells confirmed sufficient sensitivity of this system to measure biological responses after one day and exhibited greater power generation whilst under illumination. A strongly fluctuating signal, which resembled the cultural growth phases (lag-, exponential-, stationary and death phase) was observed during the first 13 days and continued into a much less fluctuating power generation at lower magnitude, as shown in figure 108a.

![Figure 108: The flat pMFC system sustainability tests were characterised by: a) A strong initial drop in power generation to an average of 19% of the starting output within 0.5 days, followed by an average power generation profile equal to a growth curve over the subsequent 12 days, ending in a constantly declining power generation at small magnitude; b) Lower average power generation of ITO plastic anode 1 and photo-response magnitudes proportional to the average power generation.](image)

The comparatively lower power generation of ITO plastic anode 1 was explained by the larger internal resistance of this pMFC, which resulted in the rewiring of all four electrodes after this experiment was finished. Figure 108b depicts the photoresponse after day 13 in greater detail and shows a continuously decreasing average power generation and amplitude of photo-response. The second addition of fresh CVu culture to the stock culture after 21 days of operation did not change the positive photo-response under illumination within one day, although further data would be required to confirm this over a longer time period.

Following the second preliminary experiment, cell adhesion of CVu onto FTO glass as well as ITO plastic anodes was confirmed, as presented in figure 109.
6.3.4.2. FTO glass anodes compared to ITO plastic anodes

The power generation and photo-response from all flow cells did not indicate significant differences between the ITO plastic and FTO glass as a pMFC anode material. Instead, differences could be determined as a result of the type of media, which facilitated the growth of differing cultures. Biofilms appeared to be thicker and denser on FTO glass after equal operation periods; however this was not reflected in the electrical response from the pMFCs.

6.3.4.3. Impact of the ferricyanide/ferrocyanide redox mediator couple

The second preliminary experiment investigated the effect of the redox mediator couple, ferricyanide and ferrocyanide, and revealed an amplification of both power generation and photo-response amplitude in the presence of an electron mediator. The resulting power output difference was smaller in ITO plastic anode containing flow cells, shown in figure 110a, than in pMFCs with FTO glass anodes, illustrated in figure 110b.
The second addition of fresh CVu culture after six days of operation did not affect the photo-response and long-term trends in average power generation differed between the flow cells. This implied that the voltage signal measured included the response from the CVu culture at all times of the experiment.

The approximately 600 times larger average power generation of the FTO glass pMFC with mediator (as opposed to without) did not arise from fabrication differences between the two flow cells, since the same equipment did not exhibit such discrepancies during the following long-term investigation. Furthermore, the power generation of the ITO plastic and FTO glass anode pMFCs without mediator was almost superimposable on each other, which showed the operational reliability of the FTO glass pMFC without mediator. The results therefore indicated that the power generation is generally lower without mediator.

The photo-response was positive under illumination in all flow cells, and its amplitude was proportional to the average power generation. Therefore, CVu cells were generating more electrical power under illumination and the electron mediator was either enhancing the CVu based power generation, or was leading to greater output by means of independent redox reactions. The long-term investigation was performed by sequential addition of growth medium and electron mediator as well as by measuring each solution for several days, in order to clarify this issue.

Operating the pMFCs with only growth media (and no algal cells) resulted in an exponentially decreasing power generation, which indicated positive photo-responses under illumination in all flow cells after six days. Following nine days of media only operation the power output became so low that a further investigation into these faint photo-responses would have contained too much error. Based on the low daily decline in power generation after nine days, the growth media levels were regarded as stable and the mediator mixture of ferricyanide and ferrocyanide was added, each at a concentration of 2.5 mmol L\(^{-1}\). As shown in figure 111a, this
resulted in an instant increase in power generation of the ITO plastic anode and BG-11 media containing flow cell, as opposed to the decrease in power output that was measured in the three other flow cells.

Figure 111: a) A photo-response with greater power generation in the light than in the dark was observed upon the addition of the ferricyanide/ferrocyanide redox mediator mix; b) The addition of mediator caused a polarity reversal in flow cells with FTO glass anodes and an increase in average power generation during the following days, which was more pronounced in BG-11 containing pMFCs.

A polarity reversal was observed in the two pMFCs with 3N-BBM+V media upon mediator addition, as seen in the plot of voltage versus time in figure 111b. Considering that only the anodic environment was changed, the drop in power generation was therefore likely to have been induced by the reduction of ferricyanide on the anode. The resulting signal trend differed between the BG-11 and 3N-BBM+V containing flow cells, although, a positive photo-response under illumination as well as reduced signal noise were exhibited by all flow cells after mediator addition.

6.3.4.4. Photo-response indication for culture growth

Although a photo-response was seen directly after the addition of electron mediator, changes in its amplitude and type coincided with the visual confirmation of biofilm growth in the flow cells and cannot be attributed to electron mediator alone.

All flow cells and tubing were disassembled, washed with detergent and following re-assembly, the flow cells were flushed in sequence with ethanol and water for 2 days respectively before the long-term experiment was launched. However, after 27 days of operation the growth of green biofilms was visually confirmed in all flow cells, although no culture had been added. Contamination through the air was excluded, since the only connection to the surrounding air was established through the stopper foams in the stock bottle caps, which were especially designed to filter out contaminating cells. Moreover, the stock bottle solutions remained colourless even when the green biofilms had already been confirmed inside the flow cells.
previous experiments involved the green algae culture *Chlorella vulgaris* (CVu) only. However, considering its unplanned growth, further possible contaminations were accounted for by considering the biofilm a photosynthetic mixed culture and the investigation was continued due to the regular use of mixed culture biofilms in other pMFCs experiments.

Four days before the visual conformation of a biofilm the type of the photoresponse changed simultaneously in both 3N-BBM+V containing pMFCs from greater power generation under illumination to larger outputs in the dark, as indicated in figure 112a.

![Figure 112: a)](image)

**Figure 112: a)** A change in photo-response from high power generation under illumination to larger readings in the dark was observed in both pMFCs containing 3N-BBM+V media simultaneously and this was followed by an increase in photo-response amplitude in each of the pMFCs; **b)** The photo-response of one pMFC reversed this response, which was also followed by an increase in photo-response amplitude.

This reversal in photo-response type was coupled to an increase in photo-response amplitude with each following 12 hour cycle over up to 10 days. A second photo-response reversal was seen for the FTO glass and 3N-BBM+V containing flow cell approximately 117 days after the first one. The second reversal was preceded by a continuous decrease in photo-response amplitude and was followed by another gradual increase in photo-response amplitude, as depicted in figure 112b.

Similar photo-response changes were observed in the flow cells containing BG-11 medium, although this occurred later in the experiment and was implemented over longer time scales, as shown for the ITO plastic anode flow cell in figure 113.
Figure 113: a) The photo-response in flow cells filled with BG-11 medium changed from larger power production in the light to greater values in the dark as well; however, this occurred later than seen for 3N-BBM+V medium containing pMFCs and not concurrently with the BG-11 partner cell; b) A reversal to the original photo-response was seen in both BG-11 pMFCs within one month.

The reversed photo-response in these flow cells only lasted for an average of 20 days.

It should be noted that the presence of a source for larger power generation in the dark was indicated in measurements before the photo-response reversal was detected. The alternating 12 hour periods of light and dark were interrupted during polarisation curve measurements and one lighting condition was kept constant until the polarisation curve was completely recorded. Periodic fluctuations in power generation that repeat every 12 hours were observed despite the constant lighting condition. The amplitude of these fluctuations declined with time at constant lighting. As shown in figure 114a by the dashed sections, during continuous illumination the power generation decreased whenever a light period would have taken place under normal operation conditions and increased when the following dark period had been due.

Figure 114: a) Application of continuous illumination revealed a photo-response with greater power generation in the times when light would have usually been switched off, although the photo-response change was not recorded for BG-11 containing pMFCs up to day 28; b) The photo-response change observed for the 3N-BBM+V filled pMFCs was accompanied with the detection of biofilm formation in all flow cells. This biofilm was still rich in chlorophyll containing cells after the experiment had ended over 250 days later. During constant illumination the minimum power generation was larger than the previous maximum power generation under standard operating conditions. This indicated
that the average power generation was facilitated by at least two sources, of which one was generating greater electrical power in the light and the other in the dark. The source for greater power output in the light was constantly contributing under constant light conditions and the other source only added to the power generation when the dark cycles had normally taken place and this addition decreased each time a following dark cycle was applied.

The growth of a photosynthetic biofilm was confirmed in all flow cells prior to the investigations in constant light and following the disassembly of the flow cells at the end of the experiment, as illustrated in figure 114b.

In the five days between photo-response change in the 3N-BBM+V filled flow cells and the confirmation of green biofilms, the yellow colour caused by the redox mediator mix disappeared.

6.3.4.5. Growth media comparison of 3N-BBM+V and BG-11 for mixed cultures

Flow cells with the same growth medium exhibited more similar power generation and photo-response pattern than flow cells with the same electrode material. This was particularly the case for flat electrode materials, such as ITO plastic and FTO glass, where the type of growth medium was crucial for the selection of exoelectrogenic culture. As shown in figure 115, the 3N-BBM+V filled pMFCs supported the growth of the culture responsible for greater power output in the dark, while the growth of this culture was more limited in BG-11.

Figure 115: Chronological overview of the various media addition to each flow cell and the observed type of photo-response.
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The partitions in figure 115 represent the times when one type of photo-response was dominant over the other, but do not necessarily mean that the source for the reversed photo-response was not present.

6.3.4.6. Minimum equilibration times

The equilibration time following solution addition was investigated to evaluate the representativeness of readings in fresh solutions achieved with flow cells as well as batch cells. During the first 9.5 days of operation with only growth medium as anodic solution, the initial power generation dropped exponentially by 90 to 92 % in the ITO plastic anode containing pMFCs and by 99.8 to 99.98 % in the FTO glass containing pMFCs. The addition of redox mediator to the media solution without organisms was performed following a waiting period for the quasi equilibration of the growth media signal. Voltage fluctuations were observed in all flow cells in response to the mediator addition, which led to a temporary polarity reversal in the FTO glass anode containing pMFCs, as described in 6.3.4.3. Using the periods of exponential change in voltage as an indication, the addition of ferricyanide and ferrocyanide at concentrations of 2.5 mmol L⁻¹ interfered with the long-term readings for at least one day.

The effect of replacing the whole stock bottle with a fresh solution of redox mediator in growth medium is depicted for 3N-BBM+V medium in figure 116a and for BG-11 medium in figure 116b.

Figure 116: The addition of fresh mediator and media to the pMFC culture causes greater interference than the mediator would on its own in the case of both 3N-BBM+V (a) and BG-11 (b) filled pMFCs and entailed a polarity reversal in all flow cells.

Biofilm growth was confirmed visually and by photo-response reversal before this solution addition was performed. Photosynthetic organisms were therefore present, contrary to the first addition of mediator only.

A polarity reversal was observed in each case and the voltage fluctuation magnitude as well as decay time was greater than seen after the addition of redox
mediator alone. While the fluctuation magnitude was larger with BG-11 medium than with 3N-BBM+V media, the decay time was approximately six days in both cases and coincided with the decay time following the start of the long-term experiment when the flow cells only contained media.

6.3.4.7. Light utilisation efficiency

The light utilisation efficiency was calculated by dividing the power generation by the incident light energy in the area of the pMFC anode chamber. It should be noted that the power production of a whole day was divided by the incident light energy, although light was only provided for 12 hours per day. This calculation is therefore accurate during light cycles, but neglects that the power production in the dark originates from metabolic products that were generated during light reactions. Biomass production through anabolic metabolism also utilises light, but is not reflected in direct electric power generation. The light utilisation was therefore actually more efficient than indicated through this power based parameter. The data was presented in this manner to preserve the information contained in the photo-response and in the polarity reversal.

In case such detail is not required in the data, then an average light utilisation efficiency could be calculated that accounts for the continuing power production in the dark. This is achieved by averaging the power generation in light and dark and then doubling this value before relating it to the incident light energy, in order to account for the fact that light was only provided during half of the power generation time.

Furthermore, it should be considered that the incident light was monochromatic with the wavelength 625 nm and the light utilisation values were therefore only an indication of the pMFC performance at this wavelength. However, the data was presented in this form to allow comparisons between various types of solar energy technologies.

Maximum light utilisation was observed in each flow cell after a photosynthetic biofilm had been visually confirmed and a change in photo-response type had occurred. The photo-response amplitude was generally at a maximum in the periods when maximum power outputs of the respective flow cells were recorded. Both ITO plastic anode containing flow cells achieved maximum power values in dark cycles during the same time period, as shown with an indication of the last measured values before mediator addition (dashed lines) in figure 117.
Figure 117: The maximum power generation of both pMFCs containing ITO plastic anodes was seen from day 76 to day 90 and was characterised by larger energy generation in the dark than in the light. a) The 3N-BBM+V media containing cell turned $8.5 \times 10^{-5}$ % of the incident light energy into electrical power during illumination and $2.6 \times 10^{-4}$ % during in the dark; b) The cell filled with BG-11 media turned over $2.0 \times 10^{-4}$ % during illumination and $1.4 \times 10^{-3}$ % in the dark.

The maximum power output in 3N-BBM+V media was less than seen for the BG-11 filled flow cell with the same electrode material. Power generation did not drop to zero after a dark cycle, but fell to a 24-hour-minimum and increased slowly throughout the following light cycle.

In contrast to the ITO plastic anode flow cells, both FTO glass anode flow cells had already exhibited the second photo-response reversal back to greater power generation in the light when the largest power outputs were recorded, as depicted in figure 118.

Figure 118: Both FTO glass anode containing pMFCs produced more power during illumination than in dark in their respective periods of maximum power output. a) The 3N-BBM+V filled pMFC exhibited a polarity reversal upon each change in lighting conditions; b) The light utilisation of the BG-11 filled pMFC dropped from $4.0 \times 10^{-4}$ % during illumination to $2.0 \times 10^{-4}$ % in the dark.

Figure 118a shows that the power generation of the FTO glass anode and 3N-BBM+V containing flow cell dropped after each light cycle and rose to a smaller maximum during the following dark cycle, which resulted from a polarity reversal during each change in lighting conditions. While the light cycle power generation only started dropping upon ending the illumination, the dark cycle power output already decreased shortly before each light cycle. The power generation of the
equivalent BG-11 filled pMFC did not drop to zero after a light cycle and its decline became smaller throughout the dark cycle.

6.3.4.8. Polarisation curves

Polarisation curves were measured in constant light in response to the detection of biofilm growth in the flow cells. Figure 119 therefore describes the pMFC including growth media, mediator and photosynthetic organisms shortly after the culture had started influencing the photo-response.

The photosynthetic organisms had developed at an external load of 10 kΩ, but the largest power outputs were achieved with the BG-11 filled pMFCs at an external resistance of 50 kΩ. Considerably lower maximum power outputs were achieved with the 3N-BBM+V containing flow cells, which had already exhibited a change in photo-response type before the polarisation curve measurement. The resistance at the maximum power point (optimal external resistance/load) for these flow cells varied between 100 kΩ and 400 kΩ.

The equilibration time after switching to another resistance was in the range of hours, which became problematic considering that the lighting conditions had to be kept constant for the duration of the polarisation curve measurement. Additionally, the data suggested a continuing photo-response even at constant lighting conditions, and possible culture death from too long illumination or dark periods. For these reasons, further polarisation curves were not conducted to reduce the risk of impairing the whole experiment.
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6.3.5. Power generation of various anodes in one multi-channel pMFC

The influence of anode surface roughness and hydrophobicity on power generation in a pMFC was investigated using atomic force microscopy (AFM) and contact angle goniometry, followed by the application of all anodes in a multichannel pMFC with equivalent conditions in each channel. Polyaniline (PANI) coating of FTO glass and the subsequent AFM characterisation were already presented in 6.3.2. AFM results of the three further test materials, indium tin oxide coated polyethylene terephthalate sheets (ITO plastic), platinum coated carbon fibre paper (Pt coated C-fibre paper) and 304 stainless steel are quoted, together with the contact angle goniometry data, in table 12.

Table 12: Summary of average surface roughness values and water contact angles on all anode materials, which were tested in the multichannel pMFC.

<table>
<thead>
<tr>
<th>Material</th>
<th>Average surface roughness $S_a$ (nm)</th>
<th>Water contact angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt coated C-fibre paper</td>
<td>$10 \cdot 10^3$</td>
<td>139.1 ±8.5</td>
</tr>
<tr>
<td>PANI coated FTO glass</td>
<td>128.3 ±75.0</td>
<td>65.5 ±8.5</td>
</tr>
<tr>
<td>304 stainless steel</td>
<td>20.1 ±3.6</td>
<td>45.0 ±7.7</td>
</tr>
<tr>
<td>ITO plastic</td>
<td>4.0 ±0.1</td>
<td>66.1 ±1.1</td>
</tr>
</tbody>
</table>

*Estimates for platinum coated carbon fibre paper were adapted from Bliznakov et al. [39]

Pt coated C-fibre paper could not be measured with AFM equipment in our laboratories due to its loosely packed fibre structure, so its roughness values were approximated by our collaborators according to Bliznakov et al. [39]

Following inoculation with *P. limnetica*, photo-responses with greater power output in the light were recorded on all bio-anodes without the use of artificial electron mediators, while the abiotic control did not exhibit a photo-response. The variation in chlorophyll concentration between the biofilms indicated differing growth rates before the polarisation curve measurements. Obtained currents were therefore normalised to the chlorophyll concentration at the time of the examination and are reported per anode area, in order to establish comparability between the channels. The polarisation and power curve measurements in the light identified ITO plastic as the most effective anode material in this pMFC, as shown in figure 120.
Figure 120: a) Polarisation curves of ITO plastic, stainless steel, polyaniline coated FTO glass and carbon fibre paper in light were normalised per anode area and per chlorophyll content to account for differences in biofilm growth; b) Power curves calculated from the polarisation curves revealed the maximum power outputs of the pMFC with each anode material and were indicated with dashed lines.

The optimal external resistances for maximum power generation of ITO plastic, 304 stainless steel, PANI coated FTO glass and Pt coated C-fibre paper were $305 \pm 52 \, \text{k}\Omega$, $834 \pm 86 \, \text{k}\Omega$, $444 \pm 100 \, \text{k}\Omega$ and $773 \pm 67 \, \text{k}\Omega$ respectively. Arranging the anode materials according to increasing power density in the dark revealed an exceptionally large light power density of stainless steel compared to the results of the polarisation curves in light, as shown in figure 121.
Figure 121: Average power densities in dark (purple columns) and light (blue columns) with the respective percentage increase in power density from dark to light conditions for each anode material tested in the multichannel pMFC.

The photo-response amplitude of stainless steel was also the largest during the 100 minute cycles of light and dark, while the remaining materials exhibited proportionality between average power generation and photo-response amplitude. The maximum power generation of stainless steel resulting from polarisation curve measurements in the dark was the lowest amongst the tested anode materials. The trend in maximum power generation between the remaining materials was maintained in the dark, as illustrated in figure 122.
Figure 122: a) Polarisation curves in the dark with dashed lines indicating the potentials at the respective maximum power output; b) corresponding power curves with dashed lines indicating the values of the maximum power output. The polarisation curves recorded in the dark showed a lower maximum power output of stainless steel, but the trend seen with all other anode materials in the light was maintained.

As previously shown with the light polarisation curves, ITO plastic displayed the largest power densities amongst the multichannel pMFC anode materials.

Arranging the polarisation curve data according to increasing maximum power densities in light, as illustrated in figure 123a, showed a similar proportionality in maximum power densities in the dark. However, a relation to the optimal external resistance was not seen. The material surface characteristics, average surface roughness and water contact angle, revealed an inverse proportionality between both factors and the maximum power density in light, if the anode materials were arranged in the same way, shown in figure 123b.

Figure 123: Correlation between pMFC power generation and anode surface roughness and hydrophobicity: a) Ordering the materials according to increasing maximum power generation in the light (orange columns) revealed the same dark power generation build-up within the error (purple columns), but no apparent proportionality to the optimal external resistance (green columns); b) An inverse proportionality between maximum power generation and the two factors, average surface roughness and hydrophobicity, could be identified (scale was not adjusted to average surface roughness of Pt coated carbon fibre paper in order to maintain the visibility of its relation to the other anode materials).
6.3.6. Macroporous ceramic and carbon foam anodes

Macroporous ceramics were fabricated from TiO$_2$ and Ti$_2$AlC by means of the foam replica method, with the aim of utilising the resulting reticulated anode shape and natural ceramic roughness for enhanced biofilm growth as well as more efficient volume use in pMFCs. A third material, reticulated vitrified carbon foam, was employed as a non-ceramic alternative in pMFCs, since it could also be produced in reticulated shapes with defined porosity. All of these materials were investigated in continuous flow pMFCs to examine if the respective material qualities could serve to overcome problems with fluorine doped tin oxide coated glass (FTO glass) and indium tin oxide coated polyethylene terephthalate (ITO plastic) anodes.

6.3.6.1. TiO$_2$ ceramic anodes in air-cathode pMFCs

TiO$_2$ ceramic anodes had shown promising results during short-term studies in stagnant solution pMFCs, reported by Thorne et al.$^{[21]}$ and were therefore investigated in continuous flow pMFCs over longer periods as next step towards application. The confirmation of the media response was first followed by the addition of photosynthetic culture and then by artificial redox mediator, in reaction to the identification of a photo-response from the artificial redox mediator during the long-term experiments on FTO glass and ITO plastic anodes in 6.3.4. Ferricyanide and ferrocyanide were added at concentrations of 0.25 mmol L$^{-1}$ and 34 days later in the 10 times larger concentration, which was equal to the mediator concentration used in 6.3.4.

Following the addition of both the cyanobacteria culture, *Synechocystis PCC6803* (SCy), and the green algae culture, *Chlorella emersonii* (CEm), an increased power generation up to 0.17 nW cm$^{-2}$ as well as a photo-response were observed. Each photo-response exhibited greater power generation in the light than in the dark; however, the amplitude was marginal compared to the signal noise. The CEm power generation gradually decreased five days after addition and was negligible after three further days, while the SCy power generation remained stable between 0.05 and 0.09 nW cm$^{-2}$ for 30 days. Two more culture additions of CEm and SCy respectively were performed after 18 days and 26 days of this 30 day period, but did not affect the power generation or photo-response.

The addition of 0.5 mmol L$^{-1}$ redox mediator to the CEm cultures caused an instantaneous increase in power generation to approximately 1 nW cm$^{-2}$ and a photo-response with greater power generation in the light, which gradually decreased to previous levels within eight days. Although no further modifications were done to the system, nine days after the mediator addition the power generation rose to 3 nW cm$^{-2}$ within 1.5 days and remained at these levels for four
days before dropping to previous power levels again. The addition of the 10 times larger mediator concentration to the CEm flow cells induced the sustained power output between 1 and 3 nW cm⁻². Figure 124a and figure 124b show an overlay of 14 day periods with the corresponding mediator conditions in CEm and SCy filled pMFCs, respectively.

Figure 124a and 124b show an overlay of 14 day periods with the corresponding mediator conditions in CEm and SCy filled pMFCs, respectively.

As shown in figure 124b, SCy filled pMFCs responded differently to the respective mediator concentrations than the CEm flow cells. The lower mediator concentration did not considerably influence the power generation, however, the larger mediator concentration caused a permanent drop in average power output by one order of magnitude. It should be noted that the lower magnitude CEm power outputs were similar to the highest SCy power outputs, which resulted in a three magnitudes larger power generation by CEm than by SCy in the presence of 0.5 mmol L⁻¹ mediator.

Flow cytometry of samples from the anode chambers indicated that the CEm growth was unaffected by the presence of 0.5 mmol L⁻¹ mediator, while the SCy cell density substantially decreased, as illustrated in Figure 125.
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times larger concentration following day 71. Culture additions to confirm that signal response were biologically caused within this test period were denoted with green dashed lines and circles.

More culture was inoculated in the pMFCs before the addition of the higher mediator concentration to confirm biological signal responses. This only resulted in the expected elevated cell density in the SCy culture, but caused a strong decline in CEm cell density. The culture addition did not affect the power generation within six days, which was the reason for continuing with the addition of the larger mediator concentration. Flow cytometry following the last mediator addition indicated a toxic effect of the ferricyanide and ferrocyanide at a concentration of 5 mmol L⁻¹ on CEm as well as SCy, in which the CEm cell density declined by 68.4 % and the SCy population by 87.9 %.

6.3.6.2. Porous Ti₂AlC ceramic as bio-anode in an immersed Pt cathode pMFC

A mixed culture of green algae and cyanobacteria was used in a continuous flow pMFC with a single coated porous Ti₂AlC ceramic anode and a platinum foil cathode separated by a Nafion® membrane, as an initial examination of Ti₂AlC ceramics for flow cell applications. Due to the sustainable power output over 80 days of operation and the detection of a photo-response to the laboratory lighting, the experiment was continued under diurnal light and dark cycles and additional investigations on optimal external resistance and light conditions were conducted. The results indicated that the high light intensity of the 625 nm LED exhibited an inhibitory effect on pMFC power generation and that the long-term application of external resistances of 80 kΩ and larger resulted in a decline in power generation over several weeks. Photo-responses were observed without the use of artificial redox mediators throughout the operation of this pMFC. Ti₂AlC ceramic in only BG-11 growth media did not exhibit catalytic abilities in light, so that all photo-responses were attributable to exoelectrogenic, photosynthetic organisms.

The 1 W LED was initially positioned 2 cm from the glass wall of the anodic chamber, in order to provide as much light intensity throughout the volume of photosynthetic culture as possible. However, shining the LED onto the mixed culture stock bottle next to the pMFC resulted in a more than six-fold increase in power generation, as shown by the power generation comparison before and after the blue dashed line in figure 126a.
Following the change in lighting conditions the pMFC was still exposed to 12 hour long light cycles, albeit from diffuse light of lower intensity. The reproducible photo-response of rising power output throughout the whole dark cycle and sharp power drops at the beginning of the subsequent light cycle altered following the reduction in light intensity. For approximately two months the photo-response was characterised by a declining power generation throughout the first third of a light cycle, which turned into increasing output over the remaining two thirds and resulted in larger power generation at the end of a light cycle than at its beginning. However, the power generation now diminished during the dark cycles, which caused the average power generation to decline, as illustrated in figure 126b. Following the minimum in power generation after 273 days a photo-response reversal to larger power output in the light than in the dark was observed and the average power generation rose to higher values than seen during the period of high light intensity cycles.

Comparing the results on light intensity to the outcome of the investigation on the long-term effect of external resistances revealed that the gradual decline in power generation after 220 days could have also been a long-term consequence of increasing the load to 80 kΩ. Within similar time frames of application, the power generation at 10 kΩ and 80 kΩ was commensurate, while the values at 1 kΩ were one magnitude lower, as shown in figure 127a.
Figure 127: a) Power densities were similar during phases at 10 kΩ (black) and 80 kΩ (blue), but remained low at 1 kΩ (red); b) The magnitude of the power generation as well as the photo-response at 80 kΩ external resistance declined in the long-term, which was not indicated at any other external resistance.

However, figure 127b shows that the application of 80 kΩ external resistance caused a long-term decline in average power generation and photo-response amplitude to similar magnitudes as measured at 1 kΩ. The power output did not recover from this decline until the light intensity was lowered 90 days later, which indicated that the observed signal change was due to a permanent adaptation of the photosynthetic culture to the larger external resistance. The comparatively large photo-response amplitudes of the initial phase at 80 kΩ were not recovered when the light intensity was lowered, showing that the biological capacity behind the large power outputs during dark cycles had degenerated between day 100 to day 200 as well. Consequently, the application of 80 kΩ could have affected the long-term power generation after lowering the light intensity by removing the culture’s ability to utilise the Ti$_2$AlC ceramic as final electron acceptor during dark periods.

Polarisation curves measured during illumination continuously resulted in optimal external resistances between 80 to 100 kΩ during the phases when 10 kΩ or 1 kΩ were applied. Following the actual application of 80 kΩ the polarisation curves indicated optimal external resistances of 200 to 400 kΩ, as illustrated by the periods at each resistance (red dashed lines) in relation to the optimal external resistance (black line with squares) in figure 128a.
Figure 128: a) The measured optimal external resistance (black line with squares) compared to the three phases at the respective applied resistances (red dashed lines) shows that applying the optimal external resistance after 85 days resulted in elevated optimal external resistance values during the following measurements; b) The maximum power generation and average open circuit potential declined during the long-term operation at the larger external resistance. The inclusion of the lighting conditions into this consideration (blue dashed lines in (a) and partition into direct and diffuse light in (b)) revealed that the long-term decline in power generation after decreasing the light intensity could have also been caused by the large external resistance.

Figure 128b shows that the maximum power generation as well as the open circuit potential (OCP) during the operation at 1 kΩ were larger than during the period at 80 kΩ, contrary to the average power generation. The most ideal power output characteristics amongst the three tested external resistances were therefore exhibited during the operation at 10 kΩ. The maximum power output and OCP values after 90 days of operation under reduced light intensity were again lower than during the direct light phase at the same resistance of 80 kΩ. This suggested that the superior performance during the operation at 10 kΩ was not caused by the lower light intensity of the ambient light and that the observed pMFC performance trends were determined by the applied external resistance.

Polarisation curves were also measured with three different lighting conditions in one run, in order to minimise the influence of culture adaption to certain operating conditions. In particular, this was done to distinguish between responses to external resistance and to lighting conditions, since the switch from 1 kΩ to 80 kΩ and the change from ambient lighting to diurnal cycles were performed at similar times. The power generation was largest in the dark, lower if the pMFC was irradiated with diffuse, white fluorescent light and lowest under illumination from the 625 nm LED in a distance of 2 cm from the anodic chamber, as illustrated in figure 129.
Figure 129: a) Polarisation curves were measured while the pMFC was either illuminated by direct light from a 625 nm LED diode (red), or by diffuse white light (blue), or kept in the dark (black) and readings at the respective maximum power output indicated with dashed lines in the corresponding colour. All readings were taken during the phase with diurnal light and dark cycles at an external resistance of 80 kΩ; b) the maximum power generation gradually increased from LED diode over white light to dark, indicating that the light intensity at the particular wavelength 625 nm had an inhibitory effect on photo-microbial power generation; c) The optimal external resistance under both types of illumination was 200 kΩ and diminished in the dark to 100 kΩ.

Figure 129c shows that the optimal external resistance was the same in both types of lighting, but was only half the value in the dark, which suggested separate sources for power generation in light and dark within the mixed culture. These results were in accord with the above elucidated long-term observation of declining power outputs in the dark in response to the application of a large external resistance.

Contrary to other flow cell experiments, nutrients were supplied by complete replacement of the stock bottle content with fresh BG-11 growth media only, in order to promote the survival of cells with a greater predisposition to form biofilms as opposed to cells that remain in solution (planktonic). Evidence for the success of this technique with regard to the exoelectrogenic species in the mixed culture was achieved with non-invasive measurements of the power generation. Early media replacements were consistently followed by a drop in power generation and loss of photo-response until both recovered several days later, as shown using the example of one media exchange in figure 130a. The period of time at diminished power generation is likely to be indicative of the lag phase of the exoelectrogenic components of the mixed culture.
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Figure 130: a) The first three exchanges of the stock bottle content with fresh BG-11 media (green dashed line) resulted in power drops over several days until the output recovered; b) following media replacements indicated that the main contribution to the power generation came from a biofilm on the Ti$_2$AlC ceramic anode, since power drops due to a lack of cells in solution were not observed.

Figure 130b shows the response to later media replacements after the formation of a green biofilm on the Ti$_2$AlC ceramic anode had been visually confirmed. The continued photo-response and maintenance of power output, despite the lower cell densities in solution, indicated that the power generation was mainly based on a photosynthetic, exoelectrogenic biofilm.

The visual nature of the biofilm after 300 days of pMFC operation is shown in figure 131a and confirmed the biocompatibility of single coated Ti$_2$AlC ceramic as well as its support of cell adhesion under continuous flow of solution.

Figure 131: Mixed culture biofilm on a single coated porous Ti$_2$AlC ceramic after 300 days of operation in a continuous flow pMFC: a) A thick, green biofilm on the ceramic showed long-term biocompatibility of Ti$_2$AlC with photosynthetic organisms; b) Confocal images revealed that the ceramic surface was not continuously covered in chlorophyll containing cells; c) The photosynthetic cells were similar in size (in the range 1.3-2.4 μm) and due to their dimensions likely to be eukaryotic.

The pMFC was subsequently disassembled and the chlorophyll autofluorescence was utilised in a confocal microscopy examination of the biofilm composition. As illustrated in figure 131b and figure 131c, chlorophyll containing cells densely populated the ceramic surface, but did not form a continuous biofilm. Due to the shading of the ceramic, light microscopy could not be used to clarify whether the photosynthetic cells were separated by extracellular matrix, dead cell fragments, or
another culture of non-photosynthetic organisms. This suggested that flushing of the chambers with cross linking agents while the pMFC is still assembled could be beneficial for biofilm investigations with SEM following the completion of future experiments. At four times greater magnification the photosynthetic cells appeared similarly sized in a range from 1.3 to 2.4 \( \mu \text{m} \), which also indicated that these were eukaryotic. It can therefore be assumed that exoelectrogenic green algae determined the photo-response and that these were either the sole source for the pMFC power output, or that these worked in combination with an exoelectrogenic non-photosynthetic organism.

6.3.6.3. Porous \( \text{Ti}_2\text{AlC} \) ceramic in an air-cathode pMFC

Four double coated \( \text{Ti}_2\text{AlC} \) ceramics were examined in the same continuous flow pMFCs with air cathodes, which were used for the investigations on porous \( \text{TiO}_2 \) ceramics in 6.3.6.1, for over 220 days. In addition to the \( \text{Ti}_2\text{AlC} \) ceramics with a porosity of 10 ppi, two reticulated vitreous carbon (RVC) foam electrodes, one with a porosity of 10 ppi and the other with 20 ppi, were also tested under equivalent conditions. All pMFCs were operated without artificial redox mediator and utilised the same mixed culture as was applied in experiment 6.3.6.2 on a single coated \( \text{Ti}_2\text{AlC} \) ceramic.

Photo-responses were observed in three of four \( \text{Ti}_2\text{AlC} \) ceramic pMFCs several weeks after culture addition, while single coated \( \text{Ti}_2\text{AlC} \) ceramic instantly showed a photo-response following the addition of the same mixed culture in 6.3.6.2. Lowering the light intensity of the diode array caused no effect on power output or photo-response for 90 days, so photo-inhibition as seen in 6.3.6.2 was unlikely to have caused a reduction in average power generation. A repetitively seen feature of the photo-response was a fast dropping power output from the beginning of a light cycle, which switched to an equally fast rising power output approximately six hours later, as seen from day 60 to 65 in figure 132a.

**Figure 132:** a) The mixed culture photo-response on double coated \( \text{Ti}_2\text{AlC} \) ceramics often included dropping power outputs throughout the first half of the light cycle and rising power generation for the rest of the light
cycle as seen from 60 to 65 days. A discrepancy between the timeline of data and light cycles was not the reason for this as seen during the days before and after this period; b) Photo-response trends could change multiple times within a light condition, indicating various exoelectrogenic processes overlaying each other while changing in rate.

The photo-responses of four days before and after this period demonstrate that the changing trend in power generation was not a result of a shift in time allocation between power recording and light cycles. Furthermore, this effect was observed in several pMFCs in differing time periods. The results therefore suggest the presence of multiple power sources with varying turnover rates at specific times within a diurnal cycle. More complex diurnal patterns in power generation were observed and demonstrated the sensitivity of double coated Ti$_2$AlC ceramic to metabolic activity of exoelectrogenic organisms, as illustrated in figure 132b.

It was also possible to generate power from photosynthetic organisms with RVC foam anodes, although at lower magnitudes than achieved with Ti$_2$AlC ceramic. The interaction with at least two sources of light sensitive power generation was confirmed with a change in photo-response type approximately eight days after adding fresh BG-11 media and culture to the 10 ppi RVC foam pMFC. Figure 133a shows the 14 day period, which starts three days after culture addition and exhibits the change to increasing power generation in the dark following day 124.

![Figure 133](image_url)

**Figure 133:** a) Two kinds of photo-responses measured on 10 ppi C-foam proved a successful interaction of this material with the respective sources for greater power generation in the light as well as in the dark; b) The photo-response on the 20 ppi C-foam was barely visible, due to the low signal to noise ratio.

As demonstrated in figure 133b, the pMFC with the 20 ppi RVC foam anode showed a photo-response as well, however, the low power output from this pMFC also decreased the signal to noise ratio so far that reliable investigations were not possible.

Polarisation curve measurements, shown in figure 134, confirmed the superior power generation of double coated Ti$_2$AlC ceramics compared to RVC foams at a range of external resistances and identified comparatively low current flow as a reason for the lower power generation with RVC foam anodes.
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Figure 134: a) Maximum power generation per electrode area of both RVC-foams (black and blue) was inferior to all double coated Ti$_2$AlC ceramic pMFCs (red, green, magenta, orange) with the same mixed culture; b) Polarisation curves showed an optimal external resistance of approximately 6 kΩ for Ti$_2$AlC anode pMFCs, while this was 100 kΩ and 300 kΩ for the 10 ppi and 20 ppi C-foam anode pMFCs respectively; c) Low power generation of C-foams resulted from comparatively low current generation, which indicated impeded interaction between exoelectrogenic organisms and RVC-foams (abbreviated further as C-foam).

All flow cells were supplied with culture from the same stock bottle, so similar cell densities were present in the anodic solution of each pMFC. The lower currents therefore suggest that the exoelectrogenic organisms interacted less effectively with the RVC-foams, since the high conductivity of RVC-foam and equal pMFC designs also exclude that a larger internal resistance compared to the Ti$_2$AlC pMFCs could have been the reason.

6.3.6.4. Solution pH to photo-response relation of a biofilm on Ti$_2$AlC ceramic

A single coated Ti$_2$AlC ceramic was incubated in a Chlorella vulgaris (CVu) culturing flask for 115 days and the resulting biofilm was examined with simultaneous measurements of pH and power generation during 12 hour periods in light and dark. Biofouling was not observed on the pH electrode throughout the 64 days of investigation, which indicated that a pH electrode can be incorporated into pMFCs during long-term measurements in mixed solutions of green algae. Distinct pH shifts were measured in response to the changes in lighting from the first day of the experiment, despite the phosphate buffered 3N-BBM+V media solution. The power output increased in the dark, while the pH decreased, which confirmed that reduced metabolites were released simultaneously with protonated species from the biofilm. However, the amplitude of the pH photo-response was not proportional to the power photo-response and the gradients of both quantities did also not show any proportionality, as shown in figure 135a.
Figure 135: Concurrent pH and power output measurements of a CVu biofilm on single coated porous Ti$_2$AlC ceramic showed that: a) the average power generation shifted, while the average pH remained relatively stable; b) neither the amplitudes, nor the gradients of photo-response and pH changes were proportional, however, both quantities were controlled by photo-sensitive factors.

The long-term power generation demonstrated a stronger variation than the long-term pH change in comparison to the respective photo-response amplitude, which is illustrated in figure 135b. Instant responses of both quantities to changes in light condition demonstrated that there was no measurement lag time between Ti$_2$AlC ceramic and pH electrode due to mass transport of species from the interior of the biofilm to the pH electrode. However, in response to switching the light off the power increased to the maximum output of the respective cycle within two hours, while the pH constantly declined throughout each dark cycle. For the first 40 days of the experiment, light cycles were characterised by continuously increasing pH, while the power output dropped sharply during the first two hours and remained nearly stable for the rest of the light cycle, as shown in figure 135b.

The diurnal development of the pH gradient changed during the latter part of the investigation. During light cycles the pH increase started to exhibit abrupt changes to almost zero, whilst the pH response during dark cycles remained similar to the initial part of the experiment, as shown in figure 135a.

### 6.3.7. Hydrophobicity and surface roughness of carbon based electrodes

Contact angle goniometry with water and AFM microscopy were used to assess the support of cell adhesion on carbon based materials with regard to implementation as bio-anodes in pMFCs. RVC foam was examined during operation in a pMFC, as presented in 6.3.6.3. The water contact angles and average surface roughness, $S_{av}$, of the remaining carbon materials were compared to the results for Ti$_2$AlC ceramic in table 13.
Table 13: Contact angles and average surface roughness, $S_a$, of carbon based materials compared to Ti$_2$AlC ceramic.

<table>
<thead>
<tr>
<th>Material</th>
<th>Carbon felt</th>
<th>Carbon paper</th>
<th>Carbon plate</th>
<th>Glassy carbon</th>
<th>Ti$_2$AlC ceramic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact angle (*)</td>
<td>107 ±4.1</td>
<td>74 ±1.3</td>
<td>42 ±1.6</td>
<td>58 ±3.5</td>
<td>73 ±4.6</td>
</tr>
<tr>
<td>$S_a$ (nm)</td>
<td>---</td>
<td>29 ±9.7</td>
<td>251 ±18.5</td>
<td>1.7 ±0.06</td>
<td>282 ±66.3</td>
</tr>
</tbody>
</table>

The evaluation of the carbon felt roughness was not possible with AFM, since the spacing between its fibres was in the range of micrometers; however, the carbon felt water contact angle of 107° demonstrated the strongest hydrophobicity amongst the tested materials. The contact angles of the other three carbon materials showed sufficient hydrophilicity to allow cell adhesion whilst still being large enough to ensure that the removal of adhesion proteins by water was unlikely. All carbon materials with sufficient hydrophilicity exhibited lower average surface roughness than Ti$_2$AlC ceramic and did not offer the same level of flexibility in electrode fabrication that could be achieved by using the replica method on ceramics. The only carbon material which allowed similarly intricate electrode shapes as Ti$_2$AlC ceramic was RVC foam and it was therefore also the only carbon material that was tested as an anode in a pMFC.
6.4. Conclusions on bio-anodes

The results emphasised that the most important attribute of an electrode in MFC technologies is its quality as a substrate for biofilm growth. If biofilms are not formed on the electrode, then the biological responses to environmental changes will be more difficult to evaluate and the pMFC power output will be lower, which decreases the signal to noise ratio. Thus, pMFC electrodes have to be produced in shapes that maximise the surface for biofilm formation and the pMFC chamber conditions have to constitute selective factors that favour biofilm formation, despite the lower nutrient availability in a biofilm.

Regularly exchanging the media stock bottle with fresh media was found to help in the removal of cells with a decreased predisposition for biofilm formation during experiments. Light and shear flow were identified as the best tunable selective factors amongst the continuously applied system parameters, and more extensive biofilm formation was observed the higher the intensity of these factors was. However, using high light intensities increased the risk of causing photo-inhibition through oversaturation of the light harvesting complexes (LHC) in the thylakoid membrane, which resulted in low power outputs. In addition to this, the results indicated that weeks of LHC oversaturation caused the selection for cells with truncated antenna systems, which utilised lower light intensities less effectively. In the case of using shear flow as a selective factor for biofilm formation, the electrode surface roughness had to be large enough and the hydrophobicity had to be sufficiently low, so that cells could adhere to the electrode.

The optimum electrode material which met these fabrication and surface requirements was Ti$_2$AlC ceramic and for this reason, this material was investigated more thoroughly than others. An alteration in the fabrication process, from single to double coated ceramics, reduced the surface roughness by sealing a large part of the fractures, which previously resulted from the pyrolysis of the polyurethane template. Long-term investigations in mixed culture pMFCs showed that this increased the required incubation time for biofilm formation and limited the applicable shear flow, but improved the compressive strength of the reticulated structure by 19 times.

The electrochemical investigation into Ti$_2$AlC ceramic demonstrated that this material undergoes an oxidation process in the presence of KCl electrolyte if potentials of 0.5 V wrt. Ag/AgCl reference were applied for sufficiently long time to allow for the slow kinetics of the oxidation process. This material characteristic would not interfere in pMFC applications, since the voltages and currents are usually several magnitudes too low to cause any significant Ti$_2$AlC ceramic oxidation. Furthermore, the deliberate amplification of the Ti$_2$AlC oxidation showed that it is

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most likely limited through a surface passivation process and can therefore also be exhausted. Hence, interference from the oxidation currents can be avoided through the application of oxidation potentials prior to the application of Ti$_2$AlC ceramic electrodes. Investigations before and after exhausting the Ti$_2$AlC oxidation revealed that the sensitivity to photo-responses from the biofilms as well as the electrode resistance were not significantly affected by the surface passivation. Thus, Ti$_2$AlC remained the most promising anode material for pMFC applications.

### 6.4.1. Electrochemical coating of bio-anodes

The electrochemical deposition of Prussian blue on FTO glass showed good coverage with a lasting film that was comparable to the Prussian blue film polymerised onto TiO$_2$ ceramics. This showed that both surfaces were suitable substrates for a fatigue endurable Prussian blue coating. However, the electrochemical coating would have to be adjusted towards a less continuous coating for future testing in pMFCs, to ensure that Prussian blue islands would attract biofilm formation without forming an insulating layer on the surface of the FTO coated TiO$_2$ ceramic.

Polyaniline coating of FTO glass was shown to improve the electrode surface roughness in 6.3.2.2, which is beneficial for cell adhesion in continuous flow applications as indicated by the comparison between single and double coated Ti$_2$AlC ceramics in 6.3.3.13. However, the pH dependent conductivity and low durability of the polyaniline coating made it unfavourable for long-term pMFC applications, which require reliable photo-responses and a constant electrode surface area for the correct interpretation of the signal.

### 6.4.2. Microbial fuel cell design

Several pMFC designs were tested and adapted to investigate the variously shaped anode materials, including batch cells with adjustable anodic volume, flat Perspex pMFCs for a maximum light harvesting surface and minimal diffusion lengths, and even the adaptation of airtight food containers for bulky electrodes.

The main practical issue was the presence of leakages following pressure changes, which resulted from the unequal release and uptake of gas from the microbial organisms. The pressure was adjusted in a sterile manner through the stopper foams in the bottle caps, but the pressure equalisation response time through the tubes was too long to cope with changes in the gas release rate. Leakages changed the cell density inside the pMFC and required unscheduled additions of fresh media, which interfered with the investigations. This issue was resolved in the pMFCs for porous electrodes through the addition of a pressure overflow in the form of an
additional container, which was connected to the top side of the pMFC with a short tube.

The experiments on the flat Perspex pMFCs showed that a more homogenous shear flow strength throughout the pMFC volume was required, so that high flow rates could be used as a selective factor for biofilm formation, without risking too strong shear flow in the vicinity of the solution inlet. Extending the solution inlets into the pMFC volume and directing them towards the chamber wall allowed the use of higher flow rates and simultaneously resulted in the covering of more anode surface area in biofilm.

6.4.3. Electrochemical stability of Ti$_2$AlC ceramics

From the data obtained and hence deduced characteristics it was evident that in the presence of the KCl background electrolyte the application of positive potentials wrt. the Ag/AgCl reference electrode triggered an irreversible oxidation reaction of the Ti$_2$AlC electrode. This oxidation could be attributed to a restructuring process of the working electrode, since higher oxidation currents were measured on the scan from positive to negative potentials than on the reverse scan. In view of the fact that the corresponding oxidative currents increased in magnitude with decreasing scan rates, the oxidation process must have been governed by slow kinetics. Hence, it was concluded that ions within the Ti$_2$AlC ceramic had to move to the electrode-KCl interface to react. No effect on the required potential or on the current response was seen between experiments conducted at either fluorescent white light, 625 nm red light from a LED, or complete darkness. Therefore, photoredox catalysis of the Ti$_2$AlC ceramic material change could be excluded. Furthermore, there was no evidence for redox processes in NaNO$_3$ when potential ranges between -1.0 V to +1.2 V were applied, which proved that an electrochemical stability issue of Ti$_2$AlC ceramic as an electrode material was only given in the presence of certain ions, in this case potassium ions, chloride ions or both.

6.4.4. Exhaustive Ti$_2$AlC oxidation through continuous cyclic voltammetry

The repeated cycling with 1 mV s$^{-1}$ confirmed three important aspects of the Ti$_2$AlC oxidation process. Firstly, it was exhaustible, since the magnitude of the reverse scan current declined over time. Secondly, resulting from the first observation and supported by the lack of any reduction peak, it can be concluded that this oxidation process was irreversible. Finally, in conjunction with the fact that the oxidation only occurred in KCl background electrolyte, this oxidation process took place at the surface of the electrode and was governed by the slow kinetics of ion movement.
through the interior of the electrode. Taking into account that there was no evidence for further redox reactions, it is unlikely that the emergence of this effect is dependent on the scan direction. It is believed that the oxidation currents were of greater magnitude during the scan from positive to negative potentials because the slow kinetics of the ion movement led to a delay of the oxidation reactions. The actually decisive factor for the occurrence of Ti$_2$AlC oxidation was the time spent at sufficiently large positive potentials. Therefore, higher currents were measured when the positive overpotentials were already being decreased.

The initial scans showed gradually increasing current at potentials larger than 0.0 V on the forward scans and down to 0.3 V during reverse scans until the formation of a thick gel layer was visible. A diminishment of electrode resistance during these scans could be excluded as reason for the increasing current, since the current magnitude in response to lower potentials remained unchanged. Therefore, the larger forward scan current during the gel buildup stage had to be attributed to an increased oxidation rate of Ti$_2$AlC.

The shift of the oxidation peak potential by approximately 0.1 V to more positive potentials was likely due to a rise in WE capacitance, which could have been caused by either an increase in electrode resistance or electrode surface area. The greater currents as compared to initial CVs made the first possible explanation highly improbable. The extension of the electrode surface area could have been caused by an augmented porosity of the electrode, which would have been a result of the material loss involved in the formation of the gel around the electrode.

Both, irreversible oxidation response in the CVs and visual confirmation of gel build up confirmed that electrode material was being turned over if Ti$_2$AlC ceramic was cycled between -0.3 V and +0.7 V. In addition, the experiments at 0.5 mV s$^{-1}$ demonstrated that the electrode material could be oxidised further if the scan rate was reduced.

The results suggested two practical conclusions with regard to the application of the Ti$_2$AlC ceramic as electrode material.

Firstly, Ti$_2$AlC ceramic could be used directly after sintering in NaNO$_3$ background electrolyte, as long as it was ensured that no other components of the electrolyte would react with Ti$_2$AlC at the applied potentials. This approach would have required screening and establishing an extensive library of substance responses in order to define the applicability of Ti$_2$AlC ceramic to certain experimental environments.

Furthermore, the exhaustion of the irreversible Ti$_2$AlC ceramic oxidation in the presence of KCl electrolyte led to a promising current response during the last CVs.
which were recorded at 0.5 mV s⁻¹ and are represented by the blue line in figure 87. Further studies were conducted on the characterisation of the irreversible Ti₂AlC ceramic oxidation process, following proof that the current response obtained from the material became more reliable once the oxidation process had been exhausted. Thus, the investigations were focused on acquiring enough information about the Ti₂AlC oxidation to assess the feasibility of an electrochemical treatment prior to its use as an electrode. The analysis of the Ti₂AlC ceramic oxidation based on charge flow was performed for this purpose.

The SEM comparison of the Ti₂AlC ceramic strut cross sections before and after the exhaustive electrochemical oxidation revealed an almost continuous coverage of the electrode surface in flocculent structures of smaller size than the typical angular Ti₂AlC crystallites. This, in conjunction with the fact that these flocculent structures only formed a several micrometer thick layer on top of the otherwise unchanged strut morphology, proved that the oxidation was a surface bound process. In case Ti₂AlC decomposition at the applied potentials had taken place, a material change throughout the whole volume of the strut would have been observed. Since this was not the case, it can be assumed that the Ti₂AlC ceramic material itself was electrochemically stable and only reacted with further chemicals on its surface, if sufficiently high overpotentials were applied.

This was confirmed with SEM and EDX of polished strut cross sections following the electrochemical oxidation of the Ti₂AlC ceramic. A 5-10 μm thick layer of an aluminium and oxygen rich phase was observed on the surface of all samples. This indicated that the application of potentials positive of 0.5 V wrt. Ag/AgCl resulted in a surface passivation through the formation of a dense layer of Al₂O₃. Wang et al. studied the high-temperature oxidation behaviour of Ti₂AlC ceramic at temperatures of 1000-1300 °C in air for up to 20 hours and also analysed the resulting samples with cross section SEM. Their results were remarkably similar, showing a discontinuous outer layer of TiO₂ followed by a continuous α-Al₂O₃ layer, which passivated the surface and stopped further oxidation of the Ti₂AlC bulk material. Wang et al. analysed the transport processes during the high-temperature oxidation and described the formation of the protective Al₂O₃ layer with the reaction mechanism:

\[
4\text{Ti}_2\text{AlC} + 13\text{O}_2 \xrightarrow{\Delta T} 8\text{TiO}_2 + 2\text{Al}_2\text{O}_3 + 4\text{CO} (g)
\]

On the basis of similar SEM results on layer formation and composition, it can be assumed that the results of Wang et al. on the high-temperature oxidation are comparable with the electrochemical oxidation of Ti₂AlC ceramic. Consequently, the discontinuous TiO₂ scale observed by Wang et al. was produced in the form of an amorphous white gel during the cyclic voltammetry, which is supported by the
fact that its desiccation resulted in a white powder residue. The slow gas emission during the electrochemical oxidation can be attributed to the formation of CO from Ti$_2$AlC, which stopped as soon as a continuous Al$_2$O$_3$ layer had formed.

6.4.5. Scan rate dependence of electrochemical Ti$_2$AlC oxidation

The extensive number of CVs in between the rough initial characterisation from 6.3.3.3 and the systematic scan rate investigation resulted in a clarification of the effects involved in the Ti$_2$AlC material change and yielded useful information about the nature of the underlying oxidation process.

Results showed that a standard scan rate, which would always be low enough to trigger the Ti$_2$AlC oxidation process could not be stated. The required scan rate was rather continuously decreasing as the irreversible oxidation process was being exhausted. This in turn, meant that the oxidation kinetics were becoming slower as the number of CVs increased.

Taking this into account, achieving higher oxidation currents at 0.3 V with 0.5 mV s$^{-1}$ instead of 1 mV s$^{-1}$ was expected. The kinetics of the Ti$_2$AlC oxidation had become so slow that the slower scan rate allowed more material to react, since it provided more time at sufficiently positive potentials.

Accordingly, the results shown here supplement the model described in 6.3.3.7 for fixed scan rates. The amount of transferred oxidation charge had to diminish with increasing number of scans at a fixed scan rate, since the oxidation kinetics became slower with increasing amount of oxidised Ti$_2$AlC.

This decline of the oxidation rate constant with increasing amount of oxidised material meant that the oxidation products were not transported away from the reaction site over the weeks of the conducted cyclic voltammetry. Consequently, the reason for the diminishment of the rate constant was either increasing mass transport length between reactants and reaction site, products blocking access to the reaction site, or both. Considering that the results of 6.3.3.3 proved the influence of the type of electrolyte on the Ti$_2$AlC oxidation, the reaction site had to be the electrode surface. This meant that a surface change of the Ti$_2$AlC ceramic electrode could be expected, if it was subjected to sufficiently positive potentials. The time period at these potentials was at least one limiting factor for the thickness of the resulting oxidised layer, since slow diffusion of reactants from the electrode volume to its surface can be assumed. A further limiting factor could be the possibility of surface passivation by this oxidised layer.
6.4.6. Potential window of Ti$_2$AlC ceramic electrodes

According to the results presented in 6.3.3.3, the Ti$_2$AlC ceramic electrode could be used in NaNO$_3$ background electrolyte, in order to prevent the electrode material itself from undergoing redox processes with the electrolyte and thereby assuring a reliable response. However, further possible interactions with other chemicals were unknown, so exhausting the oxidation process of Ti$_2$AlC with chronoamperometry at sufficiently positive potentials was considered to be the most effective option to achieve an inert electrode.

The identification of an oxidation potential was complicated by the slow kinetics of the Ti$_2$AlC oxidation. The diminishment of the reverse scan oxidation current below the magnitude of the forward scan oxidation current after reducing the positive vertex potential from 0.7 V to 0.6 V was due to the reduced time period at oxidising overpotentials. This could have resulted in a misinterpretation of the lack of an oxidation peak as absence of an oxidation reaction. However, the reverse scan current was of almost the same magnitude as the forward scan current, which made it implausible to assume that the observed current was only due to capacitive charging of the electrode. The oxidation current became lower than the charging current because the scan rate was very fast compared to the mass transport of reactants to the electrode surface. Considering this issue in the context of previous evidence for the decrease of reactant mass transport rate with number of scans (described in 6.4.5) made the determination of a specific oxidation potential with the data of this experiment inconclusive. Accordingly, the result of this experiment was that Ti$_2$AlC ceramic was indubitably oxidised when subjected to 0.6 V vs. Ag/AgCl reference in 0.1 mol L$^{-1}$ KCl background electrolyte and that these conditions can be used for a monitored exhaustion of the Ti$_2$AlC oxidation with chronoamperometry.

Although the oxidation current dependence on slow mass transport of reactants hampered the analysis of an exact oxidation potential, it made clear that sufficiently fast scan rates can be used to avoid the problematic interference from the oxidation of the Ti$_2$AlC electrode. During the initial analysis of the Ti$_2$AlC ceramic working electrode (6.3.3.3), when the Ti$_2$AlC oxidation rate was fastest, a scan rate of 20 mV s$^{-1}$ was already so fast that an oxidation of electrode material could not take place. Experiments carried out at this scan rate or faster could therefore already employ an untreated Ti$_2$AlC ceramic electrode.

As can be seen in 6.3.3.4, after the oxidation process had been promoted with 158 CV scans at 1 mV s$^{-1}$ the average oxidation current was lower than the background charging of the electrode. Hence, such a treatment made the Ti$_2$AlC sufficiently
Biofilm supporting anode materials

inert for most electrochemical investigations, since oxidation currents of such low magnitude would not affect the current response significantly.

6.4.7. Analysis of Ti$_2$AlC ceramic oxidation based on charge flow

The previously presented data on the Ti$_2$AlC oxidation had only shown certain CVs, or currents at specific potentials, which involved the risk of accidently analysing outliers. Reviewing all of the data in a continuous and comprehensive manner was made possible through the presentation of cumulative charge flow as a function of CV number.

Two characteristics of the cyclic voltammetry on Ti$_2$AlC were illustrated clearly in the plot of cumulative charge transfer against scan number. The oxidation of Ti$_2$AlC was exhaustible and it was not reversible. Both conclusions were obtained from the change of an exponentially declining amount of absolute charge transfer per CV to a constant amount in conjunction with the cathodic charge transfer remaining three orders of magnitude smaller than the anodic charge transfer.

While the exponential fit of the CVs at 1 mV s$^{-1}$ projected a further requirement of 226 C of anodic charge to completely exhaust the oxidation process, a cumulative charge of 282 C was measured and predicted to a maximum of 292 C during the investigations at 0.5 mV s$^{-1}$. Accordingly, more material was oxidised at a scan rate of 0.5 mV s$^{-1}$ than was predicted in the model based on the CVs at 1 mV s$^{-1}$, which added evidence to the conclusion that the Ti$_2$AlC oxidation was governed by kinetics that become slower with the amount of turned over material. This conclusion was explained with the reaction product forming a blocking layer between the reactants and the reaction site, which was found to be an $\alpha$-Al$_2$O$_3$ layer by SEM cross section analysis of the Ti$_2$AlC struts, as described in 6.4.4.

Analysing the flow of charge aided determination of how much material had been turned over in a Ti$_2$AlC ceramic electrode as soon as the oxidation reaction mechanism was known. Putting this into relation to mass and surface of several samples enables prediction of a ratio of charge to electrode mass, which was required before the oxidation process was exhausted. This ratio could then be applied to Ti$_2$AlC ceramics of differing mass and surface to volume ratios. Ideally, the required flow of charge to completely exhaust the electrochemical Ti$_2$AlC oxidation is then known by determining the weight and average porosity of the sample. Thus, the advantage of knowing when the oxidation has been exhausted as opposed to reading it from the CV measurements is that a simplified treatment for Ti$_2$AlC ceramics prior to their application as electrodes can be developed. Furthermore, charging currents increase with electrode surface area and can cover Faradaic signals. Considering that the purpose of the Ti$_2$AlC studies was to yield
porous, large surface electrodes, analysing the oxidation currents was an issue worth investigating.

**6.4.8. Bulk resistivity of Ti$_2$AlC ceramics**

The results suggested a passivation of the Ti$_2$AlC ceramic during the sintering as well as during the cyclic voltammetry experiments.

Wang and Zhou observed the formation of a continuous $\alpha$-Al$_2$O$_3$ and a discontinuous outer TiO$_2$ layer, if Ti$_2$AlC was exposed to air at 1000-1300 °C.$^{[17]}$ Samples investigated here were sintered in a constant argon flow to avoid such a high temperature oxidation reaction. However, as the flow rate of argon was kept low in order to maintain the required high temperatures, leakage of air into the sintering chamber cannot be excluded.

One minute of polishing the surface with a SiC abrasive sheet by hand, equal to a removal depth in the range of 10-500 $\mu$m, was sufficient to restore good conductivity even after days of continuous cyclic voltammetry. The Ti$_2$AlC material change therefore did not take place in the bulk of the material. Rather, it suggested that the underlying process was either passivation, or electroplating onto the working electrode.

The surface passivation caused such high resistances that an application of Ti$_2$AlC ceramic as electrode material would have been out of the question. Polishing of the porous Ti$_2$AlC ceramics was not feasible due to their intricate geometry. However, the electrochemical analysis on porous Ti$_2$AlC ceramics did not reflect the high resistances observed for the non-porous samples. If the electrical resistance of a porous ceramic had actually been this large, then a more linear increase of current with voltage according to Ohm’s law would have been expected. Additionally, the corrosion after cyclic voltammetry in ferricyanide, shown in figure 92c, was not observed for porous Ti$_2$AlC ceramics. These anomalies could only be explained with an inherent material difference between porous and non-porous Ti$_2$AlC ceramics.

Our collaborator’s sintering procedure for Ti$_2$AlC tablets of 13 mm diameter and 2 mm thickness had to be adapted for the larger porous samples by extending the dwelling period at maximum temperature from three to five hours to yield similarly pure Ti$_2$AlC phase. Likewise, it can be assumed that the cylinder used in this investigation, which had a 224% larger volume than such a tablet, would require an even longer dwelling period at 1400 °C to be of equal Ti$_2$AlC content as a porous sample, since the greater diffusion lengths would otherwise lead to an incomplete material turnover. Although intended to maintain consistency throughout the experiments, sintering conditions should be optimised according to sample geometry and scrutinised with XRD rather than kept constant for all samples in
future investigations. Furthermore, the application of inadequate sintering conditions and the corresponding impurities in the ceramic explain the discrepancy between the resistivity of the polished surface of our sample (13.0 ±0.334 mΩcm) and the results of Hettinger et al. who measured Ti$_2$AlC resistivities in the region of 0.36 μΩcm at room temperature.$^{[40]}$

The presented four terminal sensing study identified problems for electrochemical applications of Ti$_2$AlC ceramic arising from impurities in the Ti$_2$AlC phase, as a result of suboptimal sintering conditions. Insufficiently long dwelling periods at high temperatures had been proven by our collaborator to result in stronger TiC contamination, as shown in figure 136.

Figure 136: The argon stream caused a temperature gradient during the sintering, which could lead to TiC contamination in the colder volume (I) of the furnace tube if the argon stream had been too strong, or the sintering temperatures not high enough. Extending the dwelling period at 1400° to five hours allowed maintenance of sufficient conditions to ensure consistent yield of higher contents of Ti$_2$AlC ceramic (II).

TiC itself has a comparatively low electrical resistivity of 30 to 80 μΩcm.$^{[41]}$ If, and how its presence can lead to a passivation process will have to be determined in future experiments, in order to adjust the sintering conditions as well as the slip composition correctly. Conversely, the electrochemical studies on porous Ti$_2$AlC ceramics confirmed that the presented sintering process had been sufficiently optimised for the actually intended geometry of the electrode, since none of the anomalies seen in non-porous samples were reflected in the CV data.

6.4.9. Exoelectrogenic activity of investigated cultures

The photo-response from pMFCs was used to confirm exoelectrogenic activity in this study, as elaborated in 6.4.15. Long-term investigations, which included media controls before the addition of culture, were chosen exclusively as indications for biological electron transfer to the electrode, since the investigations often involved electrode materials with unknown responses to light. It was observed that not only the type of culture, but also the electrode quality as a biofilm substrate and final electron acceptor in the metabolic cycle of the culture determine the success of the biological power generation in a pMFC. Mohan et al. have already proven the
greater power generation efficiency of a biofilm compared to cells floating in solution, but have not researched the available factors to facilitate biofilm growth.\textsuperscript{[25]} The experiments in the presence of toxic mediator, shown in 6.3.6.1, could further evolve their results by revealing that the power generation from an exoelectrogenic culture could be enhanced by the solution toxicity, which acted as an environmental stimulus to form a biofilm on the pMFC electrode. A culture with strong exoelectrogenic activity, but less tendency to form a biofilm, could therefore still result in lower power outputs than another culture with lower exoelectrogenic activity and a natural predisposition to form biofilms.

Exoelectrogenic activity was confirmed between TiO\textsubscript{2} ceramic anodes and the green algae culture, \textit{Chlorella emersonii} (CEm), as well as with the cyanobacteria culture, \textit{Synechocystis PCC6803} (SCy), in the continuous flow pMFC investigation described in 6.3.6.1. In the presence of 2.5 mmol L\textsuperscript{-1} ferricyanide and 2.5 mmol L\textsuperscript{-1} ferrocyanide, the CEm power generation was three magnitudes larger than the power generation of SCy, while the CEm cell density was three magnitudes lower. This elevated power generation per cell indicated that CEm either possesses a greater capacity for exoelectrogenic activity, or was more compatible with TiO\textsubscript{2} ceramics.

6.4.10. Porous TiO\textsubscript{2} ceramic, Ti\textsubscript{2}AlC ceramic and RVC foam power generation

The continuous flow air-cathode MFCs were successfully employed to generate long-term data on artificial mediator-free application of porous anode materials, such as 20 ppi TiO\textsubscript{2} ceramics, 10 ppi double coated Ti\textsubscript{2}AlC ceramics and 10 ppi as well as 20 ppi RVC foams. An initial decline in power generation over several days was measured with all anode materials, which stabilised at lower power output levels for the amount of time it took the culture to form an exoelectrogenic biofilm on the electrode. Ensuing photo-responses resulted in elevated power generation, but were at least one magnitude lower than the output during the first days of operation, showing that long-term performance cannot reliably be estimated from experiments within this initial period.

The length of time between output stabilisation and the start of photo-response varied between the materials and is assumed to be dependent on the anode surface roughness, hydrophobicity and on the magnitude of the external resistance. The anode surface features impacted the cell adhesion with greater significance the stronger the shear flow was and the corresponding results are discussed in more detail in 6.4.12. External resistance did not affect the formation of a biofilm, but the results of 6.3.6.2 indicated that it determines its content of exoelectrogenic
organisms and therefore the pMFC long-term power output, which is elucidated in 6.4.20.

TiO_2 ceramic anodes exhibited photo-responses in the presence of the cyanobacteria SCy and the green algae CEm, which confirmed that exoelectrogenic activity between both cultures and TiO_2 ceramic is possible. Photo-responses were observed instantly after culture addition, however, only the SCy pMFC retained the photo-response and an average power generation of 700 nW m\(^{-2}\), whilst both of these declined in the CEm pMFC within five days. Hence, TiO_2 ceramics presented poor biofilm substrates and the power generation was poor compared to Ti_2AlC ceramics.

The double coated Ti_2AlC ceramics gave photo-responses two weeks after the addition of culture at the earliest, which suggests that only photosynthetic organisms in a biofilm utilised the electrode as an electron acceptor. It should be noted that the mixed culture in the Ti_2AlC ceramic investigations did not contain SCy bacteria and that other mechanisms of electron transfer with a shorter range could have been employed by the photosynthetic organisms. The most efficient double coated Ti_2AlC pMFC exhibited long-term power generation of 20-160 \(\mu\)W m\(^{-2}\), which was multiple magnitudes higher than power output achieved with TiO_2 ceramics.

Single coated Ti_2AlC ceramics represented a predecessor stage to double coated ceramics in the development of fabrication methods, and differed mainly in a much more fractured structure that resulted in cracks across the whole surface. As a result of the rougher surface, the single coated ceramic facilitated approximately 37 \(\mu\)m thick CVu biofilms, while only sparse colonies formed on the double coated ceramic in the same culture (6.3.3.13) and a photo-response was measured only three days after adding mixed culture to the pMFC (6.3.6.2). The short-term results on the enhanced cell adhesion support of single coated ceramics compared to double coated ceramics therefore indicated that a superior pMFC performance could be expected. These differences, and the fact that the same mixed culture was employed in the investigations on each Ti_2AlC ceramic, justified a performance comparison despite the application in different pMFC designs. Following the elimination of the source for photo-inhibition, the single coated Ti_2AlC pMFC generated power in the range of 1-7 \(\mu\)W m\(^{-2}\).

RVC foams demonstrated an inferior performance compared to double coated Ti_2AlC ceramics under the same conditions. Best results were achieved with RVC foams with a porosity of 10 ppi, which exhibited power outputs of 1-6 \(\mu\)W m\(^{-2}\) for approximately 100 of the 230 day long examination period. However, the power generation during the initial 100 days of operation did not exceed 4 nW m\(^{-2}\) and high levels of noise rendered studies of the photo-response more difficult than with
TiO$_2$ or Ti$_2$AlC ceramics. The 20 ppi RVC foam featured power outputs of only 0-2.5 nW m$^{-2}$ with higher levels of noise than the 10 ppi sample.Neither of the RVC foams showed visual signs of biofilms when the pMFCs were disassembled, which indicated that the smooth surface of RVC foams did not allow cell adhesion. These conclusions are in agreement with the results of the multichannel pMFC experiment described in 6.3.5, in which the carbon fibre paper exhibited low maximum power densities compared to PANI coated FTO glass and ITO plastic in combination with a large water contact angle of 139°. Consequently, RVC foams could only be used in conjunction with cultures which do not require biofilm formation for exoelectrogenic activity.

The results of polarisation curves for RVC foams (20 and 10 ppi) and single and double coated Ti$_2$AlC containing pMFCs that employed a certain mixed culture are compared in figure 137.

Figure 137: Polarisation curves of porous anode materials in the presence of the same mixed culture after long-term application in pMFCs: a) The maximum power generation of double coated Ti$_2$AlC ceramic was seven times larger than of single coated Ti$_2$AlC ceramic, 31 times and 158 times larger than 10 ppi and 20 ppi RVC foam respectively; b) Comparatively low current densities of single coated Ti$_2$AlC ceramic were responsible for its inferior performance.

The comparatively large errors in data from RVC foam experiments were a result of the permanent signal fluctuations during the application of this electrode material.

6.4.11. Sensitivity of biofilms on flat electrodes to shear flow

Examination of the biofilms following preliminary tests on the air-cathode pMFCs for ITO plastic and FTO glass anodes discussed in 6.3.4.1 illustrated the dependence of biofilm thickness on shear flow if smooth substrates are used. As a result of the low roughness of these anode materials, the biofilm thickness was diminished to nonexistent near the solution inlet, which caused a less effective utilisation of the available anode surface. However, the importance of shear flow for the removal of dead cell material, which could otherwise also reduce the effective use of available anode surface area by forming an insulating layer on it, was shown in 6.3.4.1 as well,
and is discussed in detail in 6.4.14. The practical conclusion is therefore that pMFC anodes with appropriate roughness or reticulated geometry have to be found, in order to support cell adhesion at sufficiently strong flow rates to prevent cell debris from increasing the internal pMFC resistance. The presented application of the reticulated foam replica synthesis method on the inherently conductive Ti$_2$AlC ceramic is an attempt to realise such a pMFC anode.

6.4.12. Anode morphology and surface energy impact on power generation

Our collaborator’s bright-field microscopy of single and double coated Ti$_2$AlC ceramics showed a less cracked surface as a result of the second coating, which improved the compressive strength of the porous ceramic structure. However, confocal microscopy confirmed that *Chlorella vulgaris* only sparsely adhered to the surface of the double coated sample, while the same culture formed an approximately 37 µm thick biofilm on the single coated ceramic. The square wave voltammetry investigations on single and double coated Ti$_2$AlC ceramics only showed a redox system with the single coated ceramic electrode, which indicates that only cells adhered to the electrode surface actually use it as an electron acceptor for reduced metabolites. These results also suggest that the cracks across the surface of a single coated ceramic facilitate biofilm formation by allowing cells to settle in environments with less shear flow, which then support the adhesion of further cells outside the cracks by development of an extracellular matrix. However, the sparse colonies on the double coated samples prove that cell adhesion is also possible on the smoother surface of these ceramics. It is possible that biofilm formation would have just taken a longer incubation time, or a less intense agitation from the orbital shaker, which is supported by the results of the long-term study on double coated Ti$_2$AlC ceramics discussed in 6.3.6.3. In this investigation four double coated Ti$_2$AlC ceramics were used in pMFCs with the same mixed culture that had been applied in a pMFC with a single coated Ti$_2$AlC ceramic, which was elucidated in 6.3.6.2. The observation of a photo-response in pMFCs with double coated Ti$_2$AlC ceramic anodes showed that photosynthetic exoelectrogenic organisms could also utilise these smoother surfaces as final electron acceptor. This required several weeks of incubation with the mixed culture, contrary to the immediate photo-response from single coated Ti$_2$AlC ceramic under the same conditions. It can therefore be concluded that the absence of cracks on the Ti$_2$AlC surface slowed the biofilm formation down, but did not preclude it. Furthermore, this experiment served as evidence that the improved mechanical strength of double coated Ti$_2$AlC ceramics can be harnessed in future studies on pMFCs, if the flow rate is sufficiently low and the operation time long enough to allow biofilm formation.
The comparison of several electrode materials with varying surface roughness and hydrophobicity under equal conditions in a multichannel pMFC, presented in 6.3.5, showed that several electrode surface requirements have to be met simultaneously, in order to enable exoelectrogenic cultures to utilise the electrode as final electron acceptor.\textsuperscript{[29]} Large surface areas of reticulated electrodes can only be accessed, if the surface hydrophobicity is low enough, so that the solution can permeate the whole structure and allow cells to adhere. Thus, the strong hydrophobicity of platinum coated carbon fibre paper, which was reflected in its large water contact angle, is likely to be the reason for the low average power generation and photoreponse amplitudes. Its fibrous structure caused it to have large surface roughness values; however, each individual fibre still shares the comparatively smooth surface that is common to carbon materials. The electrode surface roughness showed little significance overall in this specific investigation, as the experiments were conducted in a stagnant solution. Hence, the power densities are more indicative of how well \textit{P.limnetica} could use the respective material as an electron acceptor. However, it is likely that industrial as well as sensing applications of the pMFC technology will work under continuous flow conditions where the electrode surface roughness has a greater impact on biofilm formation.

It should be noted that definite conclusions cannot be drawn, since the conductivity of the test materials varied between each other and was not examined in this experiment. This affected the PANI coated FTO glass especially, since the PANI conductivity varies with pH and biologically caused pH changes are the basis for pMFC power generation.

\textbf{6.4.13. Limiting factor to reduction peak current of \textit{C.vulgaris} on Ti$_2$AlC}

A limiting factor to the magnitude of the reduction peak current was identified during the cyclic voltammetry of a \textit{C.vulgaris} culture with a single coated Ti$_2$AlC ceramic in 6.3.3.12. The peak current did not increase quadratic with scan rate according to the Randles-Sevcik formula for dissolved species, nor did it increase linearly, as it would be expected for surface immobilised species such as biofilms.\textsuperscript{[42]} Conversely, the results indicated that the reduction peak at -0.15 V was due to the algae or a metabolite produced by the algae, since it was only measured on electrodes which were incubated in culture and was not observed with non-incubated Ti$_2$AlC ceramics in controls with the appropriate growth medium. Therefore, it was likely that the peak current was limited by an additional factor which could be explained in two ways.

Firstly, it was observed that an electrochemical oxidation had to be performed at approximately 0.08 V, or a reduction would not take place. Therefore, the
magnitude of the reduction peak could have been limited by the amount of substance oxidised in each CV. Alternatively, it is also possible that the magnitude of the reduction peak current was limited by the turnover rate of the algae. Since an initial electrochemical oxidation was required, the algae metabolite had to be in its reduced state, which is common for metabolic end products. The production of reduced metabolites depends on the metabolic turnover rate inside the cell. Hence, it is assumed that either the metabolic turnover rate or the amount of electrochemically oxidised substance limited the magnitude of the reduction peak.

6.4.14. Biofilm changes in long-term pMFC applications

The system sustainability examination of the air-cathode pMFC for flat anode materials, discussed in 6.3.4.1, gave evidence that the power generation magnitude of the initial two weeks of pMFC operation cannot be expected throughout long-term operation. These results are also of concern for the representativeness of batch cell data for long-term pMFC operation, since the setup of batch cell experiments does not usually provide confirmation data beyond two weeks. The background to this conclusion is that the power generation resembled a full growth curve, including death phase, and ended in the average power generation during the long-term investigations on the same system, presented in 6.3.4.4 to 6.3.4.6.

In the experiment described in 6.3.4.1, the green algae culture, *Chlorella vulgaris* (CVu), was added together with its growth medium, 3N-BBM+V, and with the redox mediator couple ferricyanide and ferrocyanide at concentrations of 2.5 mmol L⁻¹ directly from the start of the experiment. Following the initially strong drop in power generation due to equilibration of ionic strength throughout the first day, a further, less intense decline with simultaneously decreasing photo-response amplitude (difference between light and dark power production) was observed over three days. This suggests a lag phase of the organisms during which the culture acclimatised to the new environment, since the photo-response within this period already indicated sufficient sensitivity for such measurements. A rise in photo-response amplitude and average power generation was observed following the adaptation of the organisms, which remained constant for approximately six days and was followed by a drop in power output in all four pMFCs, which resembles exponential-, stationary- and death phase of a growth curve. In this comparison, the death phase of the culture would have begun 11 days after addition of all solution to the pMFC. However, the power generation did not drop to zero and a photo-response remained visible at a continuous lower power level, which was not immediately affected by the addition of fresh culture eight days after death phase. This could be explained by the initial biofilm formation on the electrode, which partially covered the electrode in insulating material in the form of either dead cells
after cell necrosis, or extensive production of non-conductive extracellular matrix as a protective mechanism against the toxic electron mediator. Both materials would diminish the available electrode surface area for the exoelectrogenic activity of living cells and have to be accounted for in every long-term application of pMFCs. Larger flow rates might help to provide sufficient shear flow to transport dead cell material away from the electrode. The overproduction of extracellular matrix can be addressed by reducing the toxicity of the biofilm environment, which is another motivation for the development of pMFCs without artificial redox mediators.

It should be noted that the data analysis showed a slight increase in photo-response amplitude and average power generation on the day after the culture addition. However, with the knowledge at the time this was considered insignificant and this phase of the experiment was ended in favour of an earlier start of the next experimental phase without gathering more data on the long-term effects of culture addition. Although solution additions in later experiments on the same pMFC setup showed responses within several minutes, the continuation of this experiment for only 1.5 days after solution addition might have been too short to state with full certainty that the addition of fresh culture had no effect.

6.4.15. Interpretation of pMFC photo-responses

The long-term studies of a mixed culture in air-cathode pMFCs with ITO plastic and FTO glass anodes, presented in 6.3.4.4, indicated that photosynthetic cultures can exhibit different types of photo-response and that restructuring processes within a biofilm are accompanied by changes in photo-response amplitude.

This experiment confirmed that the redox mediator couple ferricyanide and ferrocyanide shows a photo-response with constant amplitude and greater power generation under illumination from a 625 nm LED array. However, several photo-response characteristics were observed which could not be explained with a photo-chemical reaction of the mediator and therefore allowed the elucidation of the metabolic photo-response of microbial organisms. A photo-chemical reaction should be initiated as soon as the appropriate light energy is provided and continue for as long as reactant and light are available. All evidence for biological photo-responses is therefore based on signal deviations from this behaviour seen during pMFCs investigations and pH-photo-response experiments in culture.

A biologically caused photo-response with greater power generation in the dark was verified when the semi-diurnal switchovers between light and dark were replaced with constant light. The power generation reached the levels from previous light cycles within a few minutes as soon as the light was turned on and remained above these levels throughout the entirety of the continuous light period.
The biologically caused photo-response became visible in the form of additional power generation during the times when a dark period had usually taken place. As soon as the dark period would have begun, the power output gradually increased for about six hours and then decreased with the appropriate rate to reach the power level of the previous light cycle at the time when the next light cycle would have usually started. This behaviour was observed every 12 hours with decreasing photo-response amplitudes of each following cycle and can only be explained with a biological rhythm that was tuned to the previous semi-diurnal light and dark cycles.

Photo-response reversals from greater power generation in light to larger power outputs in the dark or vice versa were immediately followed by an increase in photo-response amplitude. A photo-chemical reaction of the artificial redox mediator, which consumed reactants over 12 hours, is unlikely to exhibit larger photo-response amplitudes in the following 12 hour light cycle. It is therefore assumed that from cycle to cycle increasing photo-response amplitudes had to be of biological origin. Taking into account that this phenomenon was observed for photo-responses with greater power generation in the light as well as in the dark, it can be concluded that either one exoelectrogenic culture with shifting intensities of photosynthetic and respiratory metabolism was measured, or separate cultures of photosynthetic and non-photosynthetic organisms were using the electrode as electron acceptor in the respective lighting conditions.

The first photo-response reversal with following periodic amplitude increase was observed 14 days after the addition of redox mediator in the 3N-BBM+V filled flow cells and in this time the photo-response type as well as amplitude remained constant, so it is unlikely that the reversal or change in amplitude was a photo-chemical reaction of the mediator. The chronological appearance of this effect was also more related to the composition of employed growth media than to the electrode material, which adds to the evidence that these signal changes were biologically linked. Hence, the repeated increase in photo-response amplitude following the second photo-response reversal suggests that a second culture with a positive photo-response under illumination was present. This conclusion directly opposes the argumentation that photo-response reversal and amplitude changes resulted from alterations in the metabolism of a single culture. However, the amplitude changes occurred gradually over several days, which is beyond the life time of a single green algae or cyanobacteria cell, and therefore the conclusion of multiple cultures with particular photo-responses was drawn. Consequently, the overall power production would have resulted from all cultures together and the type of photo-response would have only depended on the dominant culture.
6.4.16. Photo-response differences between pH and power generation

The simultaneous measurement of the power generation from a CVu biofilm and of the pH in the solution around it in 6.3.6.4 showed that both quantities were controlled by photo-sensitive sources. The lack of proportionality between gradients and amplitudes of the photo-responses from the pH and from the power readings suggested that at least two independent mechanisms governed the respective quantities. The differences in pH and power output gradients were unlikely to have resulted from any detection lag time between Ti$_2$AlC ceramic and pH electrode, since responses to changes in light intensity were measured almost instantly on both electrodes. A decline in pH is generally seen as the driving force behind the current generation and the fact that the power output reached a maximum, while the pH kept decreasing, could be explained with a limiting factor in the pMFC equipment, which constrained the rate of the electron injection. However, during light cycles the pH was continuously increasing, whereas the power output reached a minimum after two hours and remained stable for the rest of the cycle. A technical component could not have limited the power generation to a minimum output and the main limiting factor for maximum power outputs, the ion-selective membrane, was not implemented in this basic pMFC. It is therefore more likely that maximum and minimum power generation were determined by mechanisms in the biofilm. Thus, employing pH measurements in future experiments could help to understand the different factors that determine the electron injection into the Ti$_2$AlC ceramic and the rate of production of protonated species.

6.4.17. Using light utilisation efficiency as performance indicator

The interpretation of pMFC power generation through light utilisation efficiency has resulted in a practical approach for the evaluation of pMFC data. Light sources and incident light energy are rarely reported, but are important for the practical relevance of the reported results and for attempts to compare various pMFC systems. The efficiency of electricity generation under specified lighting conditions makes the reported values more transparent for the consideration of appropriate environment and expected yearly revenue of a solar energy technology. However, in the form used here the parameter does not consider the increased material and fabrication costs of miniature devices against their improved light utilisation efficiency resulting from their minimised diffusion lengths and resistances. The presented porous Ti$_2$AlC ceramics with their large surface areas would result in inferior light utilisation efficiencies, although such a structure enables better use of the anode chamber volume. Therefore, further improvements to this parameter are
required, although it already contains more relevant information than mere power generation values.

6.4.18. Choice of growth medium for exoelectrogenic cultures in pMFCs

The long-term investigations on pMFCs containing either 3N-BBM+V or BG-11 growth media, discussed in 6.3.4, highlighted the importance of growth media choice for the selection of exoelectrogenic cultures with large electron-donating capacities. As shown in 6.3.4.7, maximum light utilisation efficiencies always occurred in association with the largest photo-response amplitudes and these are likely to be a consequence of culture growth, as elucidated in 6.4.15. It is therefore imperative for the power generation efficiency of pMFCs to find the growth media which either favours the growth of the most exoelectrogenic culture, or stimulates cultures generally to make use of an external electron acceptor in the form of the pMFC anode.

From the relative times of specific photo-response types, shown in figure 115, it can be concluded that the growth of an organism with positive photo-response in the dark was more favourable in 3N-BBM+V growth media than in BG-11, while BG-11 media supported the development of an organism which caused greater power generation in the light. Taking into consideration that the 3N-BBM+V medium is usually used to facilitate the growth of eukaryotic fresh water cultures and that the BG-11 medium supports cyanobacteria growth, it can be speculated that these are the cultures responsible for the respective photo-response types, although further analysis of each culture would be necessary to prove this.

Photo-responses with greater power generation in the dark resulted in maximum values of 1.7 nW cm$^{-2}$ in a flow cell filled with 3N-BBM+V and of 10 nW cm$^{-2}$ in the flow cell containing BG-11 medium. The maximum readings during periods of photo-responses with greater power output in the light were approximately 2.1 nW cm$^{-2}$ in the 3N-BBM+V filled flow cell and 3.0 nW cm$^{-2}$ in the BG-11 filled pMFC. Hence, although the periods with positive photo-responses in the dark were much shorter in BG-11 than in 3N-BBM+V, the peak power generation was achieved with BG-11 in the dark. It should also be noted that BG-11 containing flow cells also produced more electrical power on average throughout the 190 days of this long-term experiment, which was one of the reasons for supplying all flow cells in later experiments on porous ceramics with BG-11 medium.

Voltage fluctuations were observed following each addition of growth media or redox mediator and probably resulted from the associated change in ionic strength of pMFC solution. Magnitude as well as decay time of the fluctuation would therefore be proportional to the solution tonicity and should be considered in
future experiments, in order to minimise signal disturbances and the risk of misinterpretations. Changes to tonicity could be kept to a minimum by replacing media more often than necessary to nurture the organisms.

Additionally, the results demonstrated the importance of a minimum waiting time after the launch of each pMFC before recordings could be used as representative for long-term application. Using data before the system had enough time to reach steady state conditions would only result in measurements of the ionic strength of a medium, but would not provide any performance characteristics of the tested pMFC system. The necessary equilibration times will differ between pMFCs depending on flow rate, membrane and electrode areas as well as solution volume and should be determined for each system before data analysis.

6.4.19. Utilisation of the redox mediator couple ferricyanide and ferrocyanide

Although improving the average power generation of pMFCs, the implementation of artificial redox mediators is not representative for actual applications of the pMFC technology and should therefore only be seen as an investigative tool.

The results presented in 6.3.4.3 showed that photosensitive redox mediators can falsify photo-response results, which ultimately hampers the analysis of the inherent exoelectrogenic activity of a biofilm. Furthermore, the toxic nature of many artificial mediators has biological consequences, such as the overexpression of an insulating extracellular matrix or the selection of cultures that may be more resistant to mediator toxicity, but lead to reduced power generation in the pMFC. Consequently, subsequent experiments were conducted without artificial redox mediators, so that the ability to utilise the electrode as final electron acceptor has more impact on the selection process and with the aim of measuring faint biological signal responses more accurately.

A simultaneously conducted investigation, shown in 6.3.6.1, indicated greater tolerance to this mediator couple in the green algae culture, *Chlorella emersonii* (CEm), than in the cyanobacteria culture, *Synechocystis PCC6803* (SCy). Following the addition of ferricyanide and ferrocyanide at concentrations of 0.25 mmol L⁻¹, the CEm cell density continued to grow, while a population decline was observed in the SCy culture. During this stage, the CEm power generation was at least equal to the SCy power output and sporadically increased to 40 times larger power outputs over several days. The cell densities of both cultures decreased after the addition of the 10 times larger concentration of mediator, however, the CEm culture now continuously exhibited 40 times larger power outputs of approximately 2 nW cm⁻², while the SCy power output dropped to 0.002 nW cm⁻². These trends in power generation indicated that the decline in CEm cell density had a different reason.
than the one of the SCy culture. Considering that the SCy cell density diminished as soon as the mediator was present, it can be assumed that SCy did not possess any protective mechanism, nor did it develop an adaptation to the mediator toxicity, which resulted in cell death. However, the increasing CEm cell density at lower mediator concentrations verified that CEm at least has a protective mechanism against the mediator toxicity. Furthermore, the elevated power output at concurrently decreasing cell density in the presence of higher mediator concentrations suggests that the CEm culture reacted with intensified biofilm formation. The greater solution toxicity demonstrated a selective factor, which promoted the growth within the protective extracellular matrix of a biofilm. Hence, the solution cell density was reduced, while increased cell density on the electrode interface caused greater power generation. Although more data is required to confirm this specific relation, the experiment showed how the introduction of selective factors to promote biofilm growth on the electrode could further improve pMFC power generation and help to screen for suitable exoelectrogenic species. It has already been shown that exoelectrogenic species contribute more efficiently to the power generation in the form of a biofilm on the electrode than from solution.\(^{[25]}\) However, the nutrient availability to cells in a biofilm is lower than in solution, so harsh environmental factors, such as shear flow, luminous intensity, toxicity, or the necessity for symbiotically working microbial mats due to the lack of certain nutrients are required to make biofilm formation favourable.

6.4.20. Benefit of keeping external resistances of pMFCs lower than “optimal”

The long-term investigations on the continuous flow pMFC, described in 6.3.6.2, suggested that the application of external resistances, which provided maximum power outputs during polarisation curve measurements, is disadvantageous for the long-term sustainability of the power generation. Optimal external resistances were found to always be larger than the applied resistances, since the cultures evolved away from utilising the electrode as final electron acceptor as the external resistance was increased. Such a culture evolution towards less exoelectrogenic activity effectively increased the internal resistance of the pMFC and therefore also the optimal external resistance. Hence, the initial power outputs following the application of the optimal external resistance increased, while the adaptation of the photosynthetic culture over numerous generations resulted in decreasing outputs and photo-response amplitudes in the long-term. These results clarified that the pMFC design has to be primarily focused on enhancing the interaction between microbe and electrode rather than maximising the power generation, since the microbes cannot be treated as a system component with a fixed set of parameters. The long-term application of large external resistances was therefore identified as an obstructive factor for the growth of exoelectrogenic cultures in pMFCs.
Proof for this conclusion was achieved through lowering the light intensity 100 days later. The removal of photo-inhibition resulted in an increase in average power generation, but the photo-response amplitude remained similar. Thus, the application of 80 kΩ probably made the electrode equivalent to an insulator for the source of elevated power output in the dark and so it regressed. The initially high power outputs after lowering the light intensity could therefore not be maintained and the power generation dropped until the culture had adapted to the new conditions two months later, which was signified by the photo-response reversal to larger power outputs in the light and a subsequent rise in average power generation. Short circuited MFCs have already been found to maximise the microbial turnover rate in the research field of wastewater treatment.\(^{43}\) It is therefore not surprising that the application of increasing external resistances diminishes the interaction between biofilm and electrode and selects for non-exoelectrogenic species during long-term applications.

On the contrary, the power generation at an external resistance of 1 kΩ was permanently as low as the later power outputs at 80 kΩ. A definite conclusion on the best external resistance can therefore not be stated and the results furthermore indicate that a single resistance cannot serve to optimise the power output from all mixed culture components. However, it has been shown in long-term and short-term experiments that the respective sources for light and dark power generation require differing external resistances to make the electrode a beneficial final electron acceptor for the organisms. The above elucidated long-term effects at 80 kΩ indicated that the source for power generation in the dark required lower external resistances than the power generation mechanisms during the light cycles, since large power outputs in the dark could not be recovered by excluding the photo-inhibition from the high LED light intensities. This was confirmed by the polarisation curves, which resulted in an optimal external resistance of 200 kΩ during illumination with white, fluorescent light as well as with the 625 nm LED, but only 100 kΩ in the dark. Thus, applying different resistances according to the lighting condition could improve the overall power generation from pMFCs. Furthermore, polarisation curve measurements only proved to be indicative of short-term optimal external resistances, since the change of resistance according to one polarisation curve resulted in a larger optimal resistance during the following measurements. A polarisation curve should therefore only be used to describe the momentary pMFC output capacity, considering that the maximum power generation declined during the long-term application of these larger resistances.
6 Biofilm supporting anode materials

6.4.21. Imaging techniques for studies of biofilms on macroporous structures

Confocal microscopy allowed the imaging of certain regions of interest (ROI) for longer than it would have been possible with the high energy electron radiation of scanning electron microscopy (SEM) and featured the possibility of insights into the 3-dimensional structure of the biofilm through z-stacking. Furthermore, the autofluorescence of chlorophyll allows the instant imaging of samples, since it renders the sample preparation with dyes unnecessary and also enables the identification of photosynthetic cells in a mixed culture biofilm. Utilising the automatic component extraction mode allows multiple fluorescence sources to be distinguished in one image. This has the potential to identify and separate various photosynthetic cultures in a mixed culture biofilm, since the chlorophyll fluorescence differs between organisms. However, only the photosynthetic cultures of a mixed culture biofilm are visible and samples have to be imaged before drying changes the natural shape of the biofilm, or fixation agents become necessary.

SEM always involves the application of fixation agents and therefore the risk of changing the natural structure of the biofilm. However, it provides the resolution required to analyse the extracellular network between exoelectrogenic cells and can image such chlorophyll-free structures. In combination with energy dispersive spectroscopy the imbedding of toxic compounds into the polysaccharide matrix of cells and the influence of electrode material composition on biofilm formation can be examined.
6.5. Prospects for bio-anode investigations

6.5.1. Targeting exoelectrogenic biofilm formation through coatings

The successful coating of FTO coated glass and TiO$_2$ ceramics with Prussian blue has proven that robust Prussian blue films can be created on FTO coated ceramics as well. The next step of this investigation would involve incubation of FTO-TiO$_2$ ceramics respectively coated and uncoated with Prussian blue in green algae and cyanobacteria cultures under load from an external circuit. Such a setup selects for micro-organisms which require external electron acceptors for their metabolic cycle and would reveal if providing an iron containing surface could enhance this selection process. Supporting the biofilm formation of these organisms presents a cost-effective alternative to genetic engineering of ideal electron donating algae, and involves less environmental risks if these organisms are released into natural waters.

6.5.2. Exploration of further Ti$_2$AlC processing routes

While the replica processing of ceramics allows for the greatest permeability, other fabrication techniques, such as the sacrificial template method, can yield more durable ceramics with only slightly reduced permeability. The release of gas within a ceramic suspension or ceramic precursor followed by setting and sintering also enables the formation of a porous network within the ceramic. The ceramics resulting from these processing routes vary in morphology as well as the corresponding fluid dynamics and may exhibit beneficial biofilm support qualities.

6.5.3. Study of biofilm adaptation to MFC anode materials with SEM

Whilst much progress has been made on MFC materials research and reactor geometry, the interaction of the mixed cultures used in the majority of studies, and the selection processes occurring during the operation of the MFCs, is largely unexplained. DNA barcoding for the identification of certain green algae and cyanobacteria strains in a mixed culture biofilm is possible; however, it is a very intricate and time consuming procedure due to the multitude and genetic similarity of the investigated organisms. Consequently, new reactor designs and electrode materials are often tested by incubating a mixed culture in a working MFC for a certain period and then gathering results on this unidentified composition of organisms. This is carried out without knowledge about which of these organisms is contributing to what extent to the measurement and hence, this introduces a high
factor of uncertainty into the investigations and hampers reproduction of supposedly superior MFC systems, if the respective culture is unavailable.

Long-term investigations on the natural selection of organisms in an operating MFC are therefore required. Cryogenic SEM is a method which yields less detailed information about a biofilm, but allows comparatively fast qualitative analysis of green algae versus cyanobacteria due to cell size discrepancies and of the biocompatibility of electrode materials according to cell shape and extent of extracellular matrix. The greater resolving power of an electron microscope compared to a light microscope also makes it possible to study the connections between cells, which make the contribution of filament based exoelectrogenic activity beyond the first layer of cells on the electrode possible. Furthermore, the fixation of cells with glutaraldehyde and embedding them within resin allow the analysis of biofilm layers in their original shape by cross section SEM, which would not be possible with any other method of similar resolution.

6.5.4. EDX analysis of MFC bio-anode cross sections before and after operation

Insights into the driving force for exoelectrogenic organisms to adhere to an MFC anode surface can be gained from understanding what characteristics of the anode surface the cell senses and utilises. The common use of metal-reducing bacteria as electron donors in MFC technologies is based on the fact that these organisms require an external electron acceptor to re-oxidise their metabolites. In case a preference of specific organisms to certain elemental anode compositions was found, then this could help to improve the power generation in MFCs by matching the donor and acceptor potentials for a minimum power loss. Additionally, the identification of such potentials could improve our understanding of the currently unclear electron transfer mechanisms between organism and electrode surface.

Therefore, energy dispersive X-ray spectroscopy (EDX) of a bio-anode cross section before and after long-term implementation in MFCs might indicate which elements certain organisms particularly interact with. The presented fabrication method for porous ceramic electrodes allows inclusion of a wide range of metals into the slip mixture, which could be compared in equivalent MFC systems.

6.5.5. Biofilm growth on double coated Ti$_2$AlC ceramics

Double coating of Ti$_2$AlC ceramics has resulted in higher pMFC power densities; however, the start-up times for biofilm formation became longer due to the smoother surface compared to single coated Ti$_2$AlC ceramic. Optimising the biofilm growth conditions for double coated Ti$_2$AlC therefore represents the most crucial research target to utilise the promising benefits of this new electrode material.
Enhanced mechanical strength of porous double coated Ti$_2$AlC ceramic would be beneficial for its application as an electrode material. The brittleness of single coated Ti$_2$AlC ceramic made the application of electrical contacts difficult, and the fracturing of the porous ceramic network after determination of its surface area decreased the certainty of power density calculations. However, the results of confocal microscopy, cyclic voltammetry and square wave voltammetry on single and double coated Ti$_2$AlC ceramics after similar incubation periods in a Chlorella vulgaris (CVu) culture suggested that the latter are inferior biofilm supports.

The sparse CVu cultures on the surface of the double coated ceramics, which were shown in 6.3.3.13, would have made an application in a microbial fuel cell ineffective. These samples were constantly agitated with 120 rotations per minute from an orbital shaker, which might have created a shear flow that was too strong for cell adhesion on the smoother surfaces compared to single coated Ti$_2$AlC ceramics.

Application of double coated ceramics in continuous flow cells with less shear flow, described in 6.3.6.3, resulted in biofilm growth on the electrode following at least two weeks of incubation. However, once a biofilm had formed, the power density was one to two magnitudes higher than the power density of the single coated ceramic pMFC, despite using double coated ceramics with larger surface area. It is hypothesised that these higher power densities resulted from a less cracked electrode structure with consequentially lower internal resistance, which is supported by the measurement of lower optimal external resistances of double coated compared to single coated ceramics.

Therefore, an investigation into the optimal shear flow and illumination conditions for biofilm formation on double coated Ti$_2$AlC ceramics would benefit the application of this new electrode material. Confocal microscopy has proven to be a reliable and quick method to measure the biofilm formation of photosynthetic cells. Furthermore, variations in the type of culture and culturing conditions for better biofilm formation should be investigated.

**6.5.6. Background electrolyte**

Although it was beyond the scope of this work, the screening of various electrolytes for their effects on Ti$_2$AlC WEs could elucidate the type of ions and mechanism involved in the Ti$_2$AlC material change and also set an application range for this material. Such screening would involve cyclic voltammetry of Ti$_2$AlC WEs over broad potential ranges and at differing scan rates, similar to the approaches that were made in 3.5.1.3 and 3.5.1.4 in KCl background electrolyte.
6.5.7. Analysis of material segregation during Ti$_2$AlC CVs

The XRD analysis of the material that is initially segregated in gelatinous consistency from the Ti$_2$AlC electrode, shown in 6.3.3.4, only revealed that it is an amorphous substance and therefore not suitable for identification by XRD. The identification of this substance could contribute considerably to the elucidation of the underlying Ti$_2$AlC oxidation process. If the stoichiometry of the oxidation was clarified, the amounts of material turned over in the electrochemical reaction could be calculated, which would simplify the oxidation treatment of Ti$_2$AlC by chronoamperometry.

Alongside traditional elemental analysis, RAMAN microscopy could be used as a promising technique, since it does not require any sample preparation and therefore allows the analysis of the sample in its natural form. This is especially useful for fragile samples such as gels. Considering that this gel formation resulted from an unknown process, the additional information on molecular structure and phase composition that this technique yields could be very helpful in the exploration of the reaction mechanism. The coupling with high resolution microscopy enables pinpointing of the site of analysis on the sample, which would make RAMAN mapping of sample cross sections attractive, especially since the Ti$_2$AlC oxidation has been proven to be restricted to the electrode surface within this work.

Furthermore, gas chromatography of the gas emitted from the Ti$_2$AlC electrode during the application of sufficiently positive potentials, described in 6.3.3.4, could add additional insight into the oxidation mechanism. In order for such an analysis to work, the electrolyte and atmosphere of the reaction vessel would have to be purged with nitrogen gas first and kept in a controlled atmosphere.

6.5.8. Scan rate dependence of electrochemical Ti$_2$AlC oxidation

The range of tested scan rates proved the existence of a scan rate minimum to prevent the occurrence of an oxidation process and that a correlation between decreasing scan rate and increasing Faradaic current can be assumed below this minimum. In order to improve the confidence in this conclusion, the scan rate dependency should be measured with at least four scan rates of 1 mV s$^{-1}$ and lower. This would help to increase the oxidation current to charging current ratio and allow enough time for the slow mass transport of reactants.

The scan rates should preferably be squares of the previous scan rate (e.g. 0.7 mV s$^{-1}$ to 0.49 mV s$^{-1}$, 0.24 mV s$^{-1}$ to 0.0576 mV s$^{-1}$, if the potentiostat allows it), in order to simplify the analysis of the proportionality between peak current and
scan rate. This could add evidence that the Faradaic process is based on Ti$_2$AlC ceramic turnover on the surface in cases where the peak current $i_p$ is directly proportional to the scan rate $v$ according to:

\begin{equation}
    i_{p,\text{surface}} = \frac{n^2F^2\Gamma Av}{4RT}
\end{equation}

where $n$ is the number of electrons transferred per redox reaction, $F$ is the Faraday constant, $\Gamma$ is the surface coverage, $A$ is the electrode surface area, $R$ is the ideal gas constant and $T$ is temperature.\[^{42}\]

However, it could also serve to clarify if the electrolyte is involved in the Ti$_2$AlC turnover by limiting the peak current $i_p$ to a square root dependency on the scan rate according to the Randles-Sevcik equation:

\begin{equation}
    i_{p,\text{solution}} = 0.4463nFAc_RD \sqrt{\frac{nFD_Rv}{RT}}
\end{equation}

With the additional factors diffusion coefficient $D_r$ and concentration $c_R$ of the redox active species.\[^{45}\]

### 6.5.9. Potential window of Ti$_2$AlC ceramic electrodes

Testing a range of potential windows at a slow scan rate directly after a Ti$_2$AlC ceramic has been sintered, and repeating this procedure after the Ti$_2$AlC oxidation has been exhausted, could provide additional quantitative data on the oxidation effect as well as on the reliability of the Ti$_2$AlC with an oxidised surface as an electrode material. The tested potential windows should be applied to a sample in KCl electrolyte and a sample in a mixture of KCl and a reversible redox couple, such as ferricyanide and ferrocyanide. The former would serve as a control, showing that no further Ti$_2$AlC oxidation is occurring and determining the applicable potential range for passivated Ti$_2$AlC, while the latter would confirm the sensitivity of the electrode in the respective states.
6.5.10. Irreversibility of the Ti$_2$AlC oxidation

The oxidation of Ti$_2$AlC ceramic could be promoted using chronoamperometry at potentials of 0.6 V or higher vs. Ag/AgCl reference. While the conduction of initial investigations with cyclic voltammetry was expedient due to the fact that processes at a whole range of potentials could be monitored constantly, it unnecessarily complicates the measurement of only the Ti$_2$AlC oxidation. Chronoamperometry could directly add further evidence to the conclusion that the Ti$_2$AlC ceramic oxidation is an irreversible process, which has a rate that decreases with the amount of product, without the need for translation of the data into other formats, as was done in 6.3.3.7. Furthermore, it would eliminate uncertainties in the length of time spent at sufficiently high potentials to account for the slow kinetics of the reactant mass transport.

A baseline current could be achieved by starting the measurement in an electrolyte which does not facilitate the Ti$_2$AlC oxidation, such as NaNO$_3$. The subsequent switch to KCl electrolyte would then initiate the Ti$_2$AlC oxidation and the background corrected currents could be used to evaluate the oxidation rate.

6.5.11. Sensitivity

Continuous cyclic voltammetry on a Ti$_2$AlC WE, as described in 6.3.3.4, could be carried out in presence of a reversible redox couple, in order to analyse how the Ti$_2$AlC material change influences the sensitivity of the electrode. Results indicated that the electrode resistivity and capacitance are still promising after the oxidation of the Ti$_2$AlC had been fully exhausted. However, a study of the redox current response throughout the whole material change process would allow a clearer evaluation of the sensitivity quality of the Ti$_2$AlC electrode.

6.5.12. Bulk resistivity

Sheet resistivity measurements of thin Ti$_2$AlC ceramic films could confirm the presented electrochemical results in future studies. Sufficiently thin, non-porous films of Ti$_2$AlC can be achieved by coating an electrically non-conductive substrate that remains inert at 1400 °C with a low viscosity Ti$_2$AlC slip. Such a substrate could be an alumina plate of large enough diameter to avoid edge effects during the 4-point probe investigation. The resulting sample would be more representative for the actual intended application of Ti$_2$AlC as a porous electrode for two reasons. Firstly, the sintering procedure would not have to be changed to accommodate greater sample thickness, so that porous and non-porous sample could be sintered together without the risk of material differences. Secondly, SEM studies of the
hollow Ti$_2$AlC ceramic strut cross sections have shown Ti$_2$AlC wall thicknesses below 500 μm (6.3.3.10), which makes the analysis of sheet rather than bulk resistivity more expedient.

6.5.13. Culture selection

The intentional selection of cultures for the maximum power generation in a certain pMFC system is hampered by the lack of knowledge about the mechanisms behind exoelectrogenic activity. Until we gain a better understanding, culturing and operating conditions have to be adapted to favour the survival of beneficial cultures. As elucidated in 6.4.19, it is essential for large power generation in pMFCs that the exoelectrogenic organisms form a biofilm on the electrode, but this only happens if the environmental conditions require such a protective mechanism. It should also be noted that a culture, which is selectively grown to form biofilms, is likely to regress as soon as the environmental requirement for biofilm formation is no longer present.

The identification of appropriate selective factors, which are economic in long-term applications and continuously support high growth rates of exoelectrogenic cultures during culturing and pMFC operation, would therefore be of immense value for the progress in pMFC research. An agreement on a protocol with a certain set of selective factors, such as shear flow and luminous intensity, would also simplify the identification of pMFC system improvements between research groups. Considering that MFC research is also aimed towards water treatment, using toxic water pollutants to select for resistant cultures could additionally serve to identify novel biological extraction technologies. Furthermore, the investigation of the resulting highly adapted cultures could help to identify the crucial factors behind exoelectrogenic activity.

6.5.14. Culturing Conditions

The culturing conditions outside pMFCs were aimed at imitating the growth conditions of a natural environment, in order to explore the optimal operating conditions and performances that could be expected with naturally grown algae and cyanobacteria. However, it is likely that pMFC and algae biofuel technologies will be combined in future industrial applications, in order to increase the revenue of these systems, which implies that pMFCs will have to be optimised for the cultures that are used to produce high oil yields. The algae biofuel industry has already started to focus on certain cultures through selective culturing and genetic engineering.\(^{[46]}\) Extensive research has been done into bioreactor designs and lighting conditions to maximise the growth rates.\(^{[47,48]}\) Future experiments should
therefore use the progress that has already been made in algae biofuel research by focusing on the implementation of pMFC technology into the already existing culturing conditions. Compromises between the optimisations for either technology could be integrated early in the development, rather than trying to adapt the highly specialised components afterwards, which would shorten the development time and increase the impact of advances in each research field.

6.5.15. Improvements to the study of exoelectrogenic activity by CV and SWV

The reproducible observation of a reduction peak during cyclic voltammetry measurements of a C.vulgaris biofilm on porous single coated Ti$_2$AlC ceramic in 6.3.3.12 proved that this material can be used for the study of microbial exoelectrogenic activity. Square wave voltammetry (SWV) investigations on the same electrode for a period of 26 days indicated an increase in oxidation peak current that related to the growth of the culture, however, more data is required to elucidate these relationships.

Using the findings presented in this study, SWV at the optimal conditions of 100 Hz and 40 mA could be used to monitor the oxidation peak current from the first day of incubation as well as the settings 12 Hz and 2 mV for the reduction peak. An in depth investigation with controls of the Ti$_2$AlC ceramic in the respective growth medium preceding the incubation and paired with each following measurement of the biofilm would further improve the confidence in the resulting conclusions. The examinations could be performed for shorter intervals, since the respective measurements would not have to be conducted in large ranges of multiple factors.

The data from such an experiment would allow elucidation of what kind of factors of a MFC system could be investigated with the novel Ti$_2$AlC ceramic electrodes and how these measurements could be optimised.

6.5.16. Improvements to existing pMFC designs

Over the course of this study several modifications were applied to the pMFC setup, which improved sterility, prolonged operational time and minimised necessity for repairs during the investigations. These advances resulted in the air-cathode pMFC setup that was used in 6.3.6.3 and highlighted further options to improve existing pMFC setups.

Sterile additions and extractions of solution were achieved using long syringe needles through the stopper foams into the stock bottles, which were sealed with a filter outside the bottle. The disadvantage of this setup was the risk of biofouling inside the syringe needle, which required repeated filling and emptying of the
syringe to reduce the probability of falsifying cell density data. Consequently, the resulting hydrodynamic stress at the needle orifice caused extensive cell lysis and presented a further source of error. These problems could be avoided by the inclusion of injection ports into the tubing.

Research into growth enhancing lighting conditions has shown that pulsed light at elevated intensities can be used more effectively by algae than exposing them to the same light energy with constant illumination, which results in enhanced growth rates.\textsuperscript{[47,49]} The illumination times would therefore just be condensed to short periods, while the same amount of light energy is used. Pulsed light would also penetrate further into the solution volume than continuous light due to its higher intensity, which allows the more cost-effective use of materials in larger pMFCs. These advantages can also be used with constant light sources, such as the sun, by applying an additional layer of parabolic lenses onto the top of continuous flow pMFCs, as shown in figure 138.

![Figure 138](image)

\textbf{Figure 138:} \textit{a)} The stream of photosynthetic cells (green spheres) is constantly exposed to light (red) in the pMFC used in this study; \textit{b)} By adding a panel of consecutive parabolic lenses the light can be focused into areas with greater light intensity than before and leaves darker areas. Each cell in the stream will therefore be exposed to alternating periods of higher and lower light intensity, which imitates a flashing light effect.

In these microlensed pMFCs, cells move through alternating regions of high and low light intensity and the frequency of the resulting flashing light effect is determined by the flow rate.

\textbf{6.5.17. Continuous pH measurements in long-term pMFC investigations}

The implementation of continuous pH measurements during pMFC investigations could improve our understanding of how the factors that determine the pH differ from the biological factors that limit the pMFC power generation.

As discussed in 6.4.16, the simultaneous measurement of pH shift and power output from a biofilm indicated that both quantities are controlled by biological mechanisms, which are independent from each other. The photo-responses
confirmed that pH as well as power generation were determined by the photosynthetic organisms, but the power generation quickly reached a maximum level during dark cycles, while the continuously decreasing pH should have facilitated similarly increasing power output. It was also concluded that the maximum power output was most likely biologically limited. A continuous observation of the differences between pH and power output responses to pMFC modifications could therefore help to identify the environmental conditions that govern the responsible biological mechanisms.

Signal interferences from biofilm formation on the pH electrode were not observed during 64 days of operation in a stirred CVu culture. It is therefore believed that the long-term application of pH electrodes in pMFC experiments under sufficiently strong shear flow is feasible. The pH changes could still be measured several centimetres away from the biofilm, and without noticeable delay in comparison to the electrical photo-response. Therefore, the pH electrode could be mounted at an elevated position within future pMFC designs and any potential biofouling could be minimised.

### 6.5.18. Serial connection of pMFCs

The comparability between equivalent pMFCs could be improved and the risk of having to abort the experiment due to premature culture death could be reduced by connecting the pMFCs in series regarding the stock solution flow. This would facilitate a constant exchange of biological material between the pMFCs, which would spread successful random mutations amongst all equivalent cells and would instantly replace unsuccessful mutations with cells from another, more healthy biofilm. The resulting enhanced selection process with concurrent advanced survivability of each biofilm would also accelerate the adaptation process of exoelectrogenic organisms to the pMFC environment. Discrepancies in solution composition originating from developments in the stock bottles would also be equalised more efficiently.

### 6.5.19. Verification of photo-response measurements

The long-term investigations on photosynthetic mixed cultures in air-cathode pMFCs, which are presented in 6.3.4.4, indicated that the difference in power generation between light and dark conditions (photo-response) could be a valuable tool to monitor the viability and exoelectrogenic activity of biofilms in pMFCs. Such a measurement would have the advantage of delivering real-time information in a non-invasive manner; however, without control measurements it could only provide indicative results.
6 Biofilm supporting anode materials

The proposed relation between visual confirmation of biofilm growth in the pMFCs and the change in photo-response type should therefore be confirmed with an additional online measurement. Considering that continuous flow cells are used, an electrochemical \( \text{O}_2 \) measurement could be realised if the stock bottles connection to ambient air was shut.

Alternatively, the tubing could be run through a UV/VIS cuvette and automatic readings taken to simultaneously monitor the appearance of chlorophyll and disappearance of a coloured redox mediator.

6.5.20. Future work conclusions

The experiments within this work have identified double coated Ti\(_2\)AlC ceramic as a promising electrode material, which enables more efficient MFC designs through the possibility to adapt the electrode geometry via versatile ceramic manufacturing routes. MFC technologies currently present a compromise between energy efficiency and resource recovery, which restricts the MFC applications to a niche market. Advances in MFC power density and a better understanding of the biological pathways that lead to the interaction between organisms and electrode are therefore crucial to promote this research field. It has been shown within this work that both can be achieved with Ti\(_2\)AlC ceramic electrodes and that further research into its production and application in MFCs can consequently benefit multiple aspects of MFC research.
6.6. Equations in chapter on bio-anodes

Equation 21: CV peak current of surface immobilised species .................................. 296
Equation 22: CV peak current of solubilised species ...................................................... 296
6.7. References in chapter on bio-anodes


Supporting information
7. Supporting information

7.1. Supporting chapter 6.3.3.7

7.1.1. Background to CV integration

Current is defined as the amount of charge that passes through a certain conductor cross section in a specific time interval.

Equation 23:

\[ i = \frac{dq}{dt} \]

This means that the charge related to a current can be expressed as the integral of this current over time.

Equation 24:

\[ q = \int_{t_i}^{t_f} i \, dt \]

In a chronoamperometry experiment the voltage is held constant and the current is measured over time, so the above expression would be sufficient to calculate the charge. However, in cyclic voltammetry the voltage is continuously varied. This introduces two new variables, the potential, \( E \), and how fast this potential is changed, scan rate \( \nu \). Since scan rate is the change in potential over time, \( \nu = \frac{dE}{dt} \), the change in potential over scan rate is another way of expressing the change in time. Taking this into account, equation 24 can be modified for cyclic voltammetry to give:

Equation 25:

\[ q = \int_{t_i}^{t_f} i \, dt = \int_{E_i}^{E_f} i \, \frac{dE}{\nu} = \frac{1}{\nu} \int_{E_i}^{E_f} i \, dE \]

With this correlation it is possible to determine the amount of charge that has flown in a certain potential range of the cyclic voltammogram. It should be noted that this integral will be negative for negative (reduction) currents, or for the scan
from positive towards negative potentials. Absolute values of potential increment and current have to be used, in cases where the total charge transfer is of interest.

Figure 139: A schematic of a cyclic voltammogram showing how the algebraic signs of the calculated charges depend on the algebraic sign of the current and scan direction.

The trapezoidal rule was used as an approximation for the actual calculation of the integrals. In this method, calculating an area of complex shape is simplified by approximating it as a trapezoid. This approximation is more accurate the smaller the increment between two trapezoids is. In other words, the smaller the potential step size during the measurement of the cyclic voltammogram, the smaller the error in using this approximation. The amount of charge that has flown between the first applied potential, \( E_1 \), and any following potential, \( E_n \), can then be calculated as follows:

Equation 26:

\[
q_n = \frac{1}{v} \int_{E_i}^{E_{i+1}} i \, dE \approx \sum_{l=1}^{n-1} q + \left( E_{n+1} - E_n \right) \cdot \left[ \frac{i_{n+1} + i_n}{2} \right]
\]

With potential steps, \( E_{n+1} - E_n \), for example 2 mV in the measurement with the scan rate of 1 mV s\(^{-1}\). As mentioned previously, the above expression leads to charges cancelling each other out, depending on the scan direction and algebraic sign of the current that they were calculated from. However, absolute values can be applied according to the desired type of analysis. If absolute values of the potential steps are taken, then scan direction becomes irrelevant.
Equation 27:

\[ q_n = \frac{1}{2} \int_{E_i}^{E} i \, dE \approx \left[ (E_{n+1} - E_n) \cdot \frac{i_{n+1} + i_n}{2} \right] \]

Oxidising currents will result in positive charges, while reducing currents will cause negative charges. This is particularly useful to separate and compare the amounts of charge used to either oxidise or reduce. Accordingly, the charge is calculated for each separate CV and not added to the previous generation of charge. For this reason, equation 27 is missing the sum term from equation 26. If the positive and negative charges of a reversible system are compared over a suitable potential range that completely covers its oxidation and reduction potentials, then the difference between these two quantities should be zero.

In case the overall charge flow is of interest, for example in electrode surface studies, the currents should be computed as absolute values as well.

Equation 28:

\[ q_n = \frac{1}{2} \int_{E_i}^{E} i \, dE \approx \sum_{l=1}^{n-1} q + \left[ (E_{n+1} - E_n) \cdot \frac{|i_{n+1}| + |i_n|}{2} \right] \]

7.1.2. Steps of data processing

1) Maximum and minimum currents of the whole experiment at 1 mV s\(^{-1}\) and their respective potentials were identified. This was first done following measurements over the whole potential range. Later the maximum currents were determined for certain characteristic potentials of the CVs. These were in particular the highest expected current of the forward scan at 0.7 V vs. Ag/AgCl and the "loop" at 0.3 V during the backward scan.

2) The currents were integrated over voltage for each CV to summarise and compare the magnitude of redox reactions happening over the whole number of scans. This was done with absolute values of currents and voltage steps initially, so that the overall charge flow could be determined.

3) From all CVs the one with the lowest overall charge flow was determined and used as background for all other CVs. This was done assuming that the lowest charge flow meant that the oxidation process was exhausted, since the corresponding CV was also found to be amongst the last few recorded ones at this scan rate.
4) All charge flow was added up to the "total charge transferred" and after background subtraction according to number of CVs involved the "charge due to Faradaic processes" was found.

5) The currents were integrated again, but only with absolute values for the voltage steps, so that reducing currents lead to negative integrals and oxidising currents to positive ones. Positive and negative integrals were then separated and the respective charge flow added up to identify how much charge had flown to oxidise and how much to reduce in each individual CV.

6) The CV with the lowest oxidising charge flow and the lowest reducing charge flow were found and used as backgrounds for all other CVs, making the same assumption as for the background subtraction before.

7) The positive charge of all background corrected CVs was summed up as "charge transferred to oxidise" and the same was done for the negative charge leading to the "charge transferred to reduce".

8) The difference between "charge transferred to oxidise" and "...to reduce", "Q_{ox}-Q_{red}\'', was used as mutual control for the charges declared as background. The more similar "Q_{ox}-Q_{red}\'' and "non-Faradaic charge up\'' were, the greater the confidence in the choice of background.

9) The different types of current and charge of each CV were added to the ones of previous CVs to visualise the incremental change in current and charge flow over the course of the experiment. These incremental changes were then formatted for use in Origin and plotted against scan number.

### 7.1.3. Data extrapolation

A plot of cumulative charge versus scan number was the clearest option to visualise a supposedly irreversible Faradaic process, which was being exhausted over a multiplicity of scan cycles. Such a plot allowed for all previously flown charge to be accounted for when drawing conclusions from the gradient. This meant that a quantitative prediction of Faradaic charge flow in future cyclic voltammograms became possible.

The model that was used to fit and extrapolate the data was based on two basic assumptions. First, during every scan an equal amount of non-Faradaic charge flow due to double layer capacitance of the electrode could be assumed. The cumulative non-Faradaic charge flow could therefore be represented with a linear function versus scan number. Moreover, the cumulative Faradaic (oxidation and reduction) charge flow was expected to asymptotically approach a maximum value. In other words, the charge flow due to redox processes was assumed to exponentially decline with time at the appropriate potentials. This time dependence was equivalent to the better definable number of scans, since scan rate and potential...
window were kept constant within the fitted data. All the above assumptions were based on the quality of fitting the resulting model to the acquired data, which was expressed in the form of corresponding R squared ($R^2$) values.

The observed oxidation process showed strong indications of being limited to a certain amount of material that could be oxidised. Therefore, it was assumed that the background corrected sums of the respective charges would approach a maximum value and the standard exponential decay fit function (equation 29) was used for oxidation and reduction trend fitting. The $R^2$ values for the oxidation charge flow were 0.99343 at 1 mV s$^{-1}$ and 0.99867 at 0.5 mV s$^{-1}$. Fitting the reduction charge flow resulted in $R^2$ values of 0.99254 at 1 mV s$^{-1}$ and 0.99556 at 0.5 mV s$^{-1}$.

Equation 29:

$$Q = Q_{\text{max}} + A \cdot \exp\left(-\frac{x}{\lambda}\right)$$

$Q_{\text{max}}$ is the respective maximum amount of cumulative charge, $A$ is the amplitude of the decay curve and $1/\lambda$ is the decay constant of the irreversible redox process. The value of charge $Q$ as function of scan number $x$ was determined from the current response as described in section “Steps of data processing”. Accordingly, the possibility to make predictions about the charge transfer required for the exhaustion of an irreversible electrochemical process, $Q_{\text{max}}$, with the least possible variables was the purpose of this fitting function.

Although it had the benefit of simplicity, equation 29 approached a constant function value. This was appropriate for background corrected data, such as oxidation and reduction charge flow, but not for the absolute charge transferred. Since non-Faradaic charge up of the electrode contributed to every CV, the absolute charge transfer could not approach a constant value in a plot against scan number.

In order to solve this problem, the absolute charge transfer data had to be fitted with an exponential decay function which changed into a linear increase as soon as the redox current dropped to zero. Therefore, the proposed function was based on equation 29 and extended with the linear term for non-Faradaic charge increment, $Q_{\text{non-Faradaic}}$:

Equation 30:

$$Q = Q_{\text{max}} + A \cdot \exp\left(-\frac{x}{\lambda}\right) + Q_{\text{non-Faradaic}} \cdot x$$
Consequently, the experimentally determined quantities were $Q$, $x$ and $Q_{\text{non-Faradaic}}$, while $Q_{\text{max}}$, $A$ and $\lambda$ resulted from the iterative optimisation of $R^2$ of the fitting function. In this equation, $Q_{\text{max}}$ represented the charge offset created by the Faradaic processes as well. However, it should be noted that in case of the absolute charge transfer this was a summary of the individual offsets due to each Faradaic process. The reliability of this model was reflected in the excellent $R^2$ values of 0.99652 for the fit of the cumulative absolute charge at 1 mV s$^{-1}$ and of 0.99975 at 0.5 mV s$^{-1}$.

Equation 30 allowed identification of the number of CV scans necessary to exhaust a non-reversible Faradaic process at a given scan rate and potential window without the need to perform them. Deducing from the number of CVs, the required charge flow and, if the reaction stoichiometry was known, the amount of reactants and products could be determined. This was achieved through the comparison of the first derivative of equation 30 for any Faradaic process with the first derivative of the cumulative background charge, $Q_{\text{non-Faradaic}}$. As it can be seen from figure 140a, when the oxidation process related charge transfer increment (red line with triangles) declined to 0 the absolute charge increment (black line with circles) converged with the constant non-Faradaic charge increment (green line with diamonds).

![Figure 140: Increments of fitted charge transfer data recorded from CVs at 1 mV s$^{-1}$ and the corresponding predicted declines of cumulative accretion in the different types of charge (a) were confirmed in subsequent experiment at 0.5 mV s$^{-1}$ (b).](image)

The dashed line in figure 140a divides the measured data at 1 mV s$^{-1}$ from the predicted progression of the respective charge increments. This prediction and the model were confirmed by the data of the subsequently performed sequence of CVs at 0.5 mV s$^{-1}$ (figure 140b). Naturally, the predicted number of scans required to reach a certain extent of charge increment progression differed from the experimentally found value, since the subsequent experiment was carried out at lower scan rate. However, the qualitative comparison between predicted and measured progression still proved that the assumption of an irreversible oxidation process of exponentially declining rate was correct.
7.2. Additional graph to 6.3.3.3

Figure 141: The scan at 10 mV s\(^{-1}\) in the potential range of -0.5 to 1.2 V (red line) exhibited such high current that the readings went off scale and was therefore replaced with the 10 mV s\(^{-1}\) scan in a smaller potential range of -0.5 to 0.7 V (black line) to show the scan rate dependence of the material turnover.

7.3. Experimental to 6.3.3.8

7.3.1. Equipment

A Multiheight Probe from Jandel Engineering Limited with a pair of four point probe heads was used. Both had a tip spacing of 1.00 mm ± 10 μm and a tip radius of 300 μm. The probes were precision ground tungsten carbide with 45° included angle tips and polished tip radii, optically checked from 12.5 to 500 μm by Jandel Engineering. An Ivium CompactStat was used as potentiostat.

7.3.2. General sample preparation

Samples were prepared in accordance with the Multiheight Probe instructions from Jandel Engineering:

1) Dirt and oxidation layers were removed by either etching, or washing and drying.
2) Sample homogeneity had to be considered when choosing the sample.
3) In case it was known that the sample consists of a top layer on a substrate, then the top layer had to be of the opposite conductivity type to the substrate, i.e. electrically insulated from the substrate. Otherwise, the substrate would offer an easier path for the current to flow and the measured resistivity would have effectively been that of the substrate. If the top layer was known to be very thin, meaning sub-micron, puncturing the layer by excessive needle loading through rapid descent velocity of the probes had to be avoided. Minority carriers (less abundant charge carriers) could have been injected through application of excessive currents. All the above effects could have caused some leakage into the substrate, which would have reduced the measuring current in the top layer. As a result, the resistivity measured would have been too low.
4) The sample material had to be able to make ohmic contact.
5) Even at the maximum current (<20 mA to not damage the probes), materials with very low resistivity could only be measured as thin films of 100’s of angstroms up to 1μm thickness.
6) For samples with expected sheet resistance figures of less than 1 Ω sq⁻¹ the SE and RE probes were reconnected to an external high resolution voltmeter according to the schematic shown in figure 142b.
7) The current was restricted to 20 mA to avoid heating effects and excessive current densities at the probe tips, which meant that very blunt tips were desirable for thin films (where high currents are needed).
8) Sheet resistances up to 10⁷ Ω sq⁻¹ could be measured using very low currents of 1 μA or less. However, voltage indications greater than 200 mV had to be avoided.

7.3.3. Common error sources

1) electrical noise due to poor contact conditions
2) thermally induced voltages
3) actinic effects (light induced material changes)
4) offset voltages produced by devices and the current source
5) general leakage in plugs, lead, etc.

7.3.4. Setup and data processing

Depending on the resistivity of the sample, the probes were either connected according to the standard setup shown in figure 142a, or for very conductive
samples according to figure 142b. If the voltage dropped below the measurement range due to low resistivity of the sample, then an external high resolution voltmeter would have been used to read the voltage instead of the Ivium CompactStat. In order to interconnect the ports of the high resolution voltmeter, V1 and V2, with the inner two probes, the SE was plugged into the WE and the RE into the CE.

![Diagram of connections between the four electrode probes](image)

**Figure 142:** Connections between the four point probe head and the working electrode (WE), counter electrode (CE), reference electrode (RE) and sensing electrode (SE) of the Ivium CompactStat for general investigations (**a**). In order to utilise a high sensitivity external voltmeter with its ports V1 and V2 for very conductive samples, SE was plugged into WE and RE into CE (**b**).

Cyclic voltammetry was used to achieve a function of current versus potential for samples with sufficiently high resistivity using the Ivium CompactStat potentiostat. In cases where the resistivity was not high enough, a sequence of increasing currents were held constant with the Ivium CompactStat and the corresponding voltages were read instead.

The achieved data could then be used to calculate two different electrical resistance characteristics of the sample material; bulk resistivity and sheet resistance. A proportionality between voltage and current according to Ohm’s law was assumed in the calculations. The slope of the linear plot of voltage versus current had to be calculated for the determination of each material quality.

The applicability of the corresponding equations in table 14 was dependent on the sample thickness, which is the reason for the description of two types of bulk resistivity. If the thickness of the sample exceeded five times the probe spacing (5 mm), then the sample could not be considered an “infinite sheet” anymore. In this case, the bulk resistivity had to be calculated for semi-infinite volumes, which was an approximation with less than 1% error. Furthermore, the sheet resistance could not be calculated with the possibility of current leaking into the bulk of the sample. A film thickness of less than 0.625 times of the probe spacing (625 μm) was required to use the sheet resistance formula stated in Table 14 with less than 1%
error. Finally, the apparatus constant 4.532 used in these formulas accounted for tip spacing, tip material etc. and was therefore only applicable for the two probe heads described in the Equipment section above.

Table 14: Calculation formulas for bulk resistivity of semi infinite volumes \( \rho_\infty \), sheet resistance \( R_s \) and bulk resistivity \( \rho_{\text{fin}} \) of thin films, taking into consideration the apparatus geometry with the constant 4.532.

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Formula</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk resistivity ( \rho_\infty ) (for a semi infinite volume)</td>
<td>( 2\pi \pi \cdot \frac{V_2 - V_1}{i_2 - i_1} )</td>
<td>( [\rho_\infty] = \Omega \text{cm} )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>s = spacing of probes in cm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>i = test current</td>
</tr>
<tr>
<td></td>
<td></td>
<td>V = measured voltage</td>
</tr>
<tr>
<td>Sheet resistance ( R_s ) for wafers and films</td>
<td>( 4.532 \cdot \frac{V_2 - V_1}{i_2 - i_1} )</td>
<td>( [R_s] = \Omega/\text{sq} )</td>
</tr>
<tr>
<td>Bulk resistivity ( \rho_{\text{fin}} ) for wafers and films</td>
<td>( 4.532 \cdot t \cdot \frac{V_2 - V_1}{i_2 - i_1} )</td>
<td>( [\rho_{\text{fin}}] = \Omega \text{cm} )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t = thickness of film in cm</td>
</tr>
</tbody>
</table>

7.4. Calculation of hollow space in ceramic struts

SEM pictures of ceramic strut cross sections were used to calculate the empty space within the struts and the interior surface area resulting from these cavities. The cavity cross sections were approximated as triangular, so that the area \( A \) could be calculated from measurement of the triangle edge lengths; \( a, b \) and \( c \), using Heron’s formula:

\[
A = \frac{1}{4} \sqrt{4a^2b^2 - (a^2 + b^2 + c^2)^2}
\]

Equation 31:

The cross section area of the cavity was then multiplied by the average strut length to give the average empty space within a strut.
7.5. Equations in supporting information

**Equation 23:** Current as derivative of charge .......................................................... 310
**Equation 24:** Charge as integral of current ................................................................. 310
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