Research article

Assessing hydrothermal liquefaction for the production of bio-oil and enhanced metal recovery from microalgae cultivated on acid mine drainage

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ABSTRACT

The hydrothermal liquefaction (HTL) of algal biomass is a promising route to viable second generation biofuels. In this investigation HTL was assessed for the valorisation of algae used in the remediation of acid mine drainage (AMD). Initially the HTL process was evaluated using Arthrospora platensis (Spirulina) with additional metal sulphates to simulate metal remediation. Optimised conditions were then used to process a natural algal community (predominantly Chlamydomonas sp.) cultivated under two scenarios: high uptake and low uptake of metals from AMD. High metal concentrations appear to catalyse the conversion to bio-oil, and do not significantly affect the heteroatom content or higher heating value of the bio-oil produced. The associated metals were found to partition almost exclusively into the solid residue, favourable for potential metal recovery. High metal loadings also caused partitioning of phosphates from the aqueous phase to the solid phase, potentially compromising attempts to recycle process water as a growth supplement. HTL was therefore found to be a suitable method of processing algae used in AMD remediation, producing a crude oil suitable for upgrading into hydrocarbon fuels, an aqueous and gas stream suitable for supplementing the algal growth and the partitioning of most contaminant metals to the solid residue where they would be readily amenable for recovery and/or disposal.

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1. Introduction

Declining water quality is an issue of increasing importance worldwide. One major cause of this is, water contamination by heavy metals from domestic or industrial sources. Mining operations have long been recognised as one of the major anthropogenic sources of metals to the aquatic ecosystem [1]; acid mine drainage (AMD) in particular causes persistent and severe pollution and affects most countries with historic or current mining industries [2]. Although chemical compositions and pH vary from site to site, AMD tends to contain elevated concentrations of dissolved metals such as Fe, Al, Zn, Sn and Pb [2,3]. Because of the longevity of AMD (many mines in Europe continue to release metals centuries after closure) [4], treatment presents substantial long term liabilities for mine operators and for governments that inherit orphan sites. As a result, there has been a growing interest in more efficient and cost-effective remediation technologies.

Microbial remediation of metal contaminated wastes has gained increasing popularity over the last few years [5]. Algae in particular have been demonstrated to sequester metals via biosorption and intracellular uptake [6]; the uptake of metals is strongly dependent on the provision of adequate light, temperature and nutrients for algal growth [6]. Living algal cells have been found to be particularly efficient at remediating water with low metal concentrations [7,8]. Although these methods are highly effective in lowering metal concentrations in AMD sufficiently, large volumes of secondary waste are created in the form of metal-contaminated biomass and sediments [9]. Use of the biomass as a fuel and recovery of the associated metals from the AMD could relieve this threat, as well as presenting a revenue stream to offset operational costs. A potentially efficient method to recover the metals and produce a biofuel is to process the biomass through hydrothermal liquefaction (HTL).

HTL utilises water at sub-/near-critical conditions (200–374 °C, 50–280 bar) as both the reaction medium and solvent for a host of reactions, converting algal biomass into a bio-oil, alongside an aqueous phase, a solid residue and a number of gaseous products. HTL can be used to process biomass at a concentration of ca. 5–25% with water, with one study estimating that the energy consumption of biomass preparation was reduced by 88% if the input slurry generated is used.
without drying steps [10], HTL processing of algal biomass has been demonstrated to be energy-efficient and potentially scalable [11]. For example, the life cycle performance of laboratory, pilot- and full-scale scenarios demonstrated significant improvements in GHG emissions with respect to gasoline and corn ethanol, and a potential Energy Return on Investment (EROI) of around 2.5 for the full-scale scenario [12], subject to the optimisation of a closed-loop system incorporating energy and nutrient recycling.

HTL comprises hundreds of simultaneous reactions, including the decarboxylation of carbohydrates to sugars and fragmentation to aldehydes, hydrolysis of lipids to fatty acids and subsequently longer-chain hydrocarbons, and depolymerisation and deamination of proteins. In addition, repolymerisation of the reactive fragments into larger oil compounds is also favourable [13]. Most liquefactions under optimised conditions have resulted in bio-oil yields around 30–45% [14,15], regardless of algae strain, although, notably, Li et al. obtained yields of 55% for Nannochloropsis sp. under HTL at 260 °C for 60 min and at 25% total solid (TS) loading, and 82.9% for Chlorella sp. (220 °C, 90 min, 25% TS) [16]. The numerous reactions occurring under HTL conditions lead to a bio-oil containing a diverse range of chemical compounds, the main constituents of which have been found to be C5–C16 cyclic nitrogen compounds, C15–C33 branched and unbranched hydrocarbons, branched oxygenates, aromatic compounds, and heterocycles [16]. Elevated heteroatom (O and N) content with respect to mineral crude oil are typical of algal bio-oils, which give rise to undesirable fuel properties, such as high acidity and viscosity, and the diverse chemical compositions can negatively affect combustion performance, storage stability and economic value [17,18].

The higher heating values (HHV) of the oils usually fall between 25 and 35 MJ kg⁻¹, with higher lipid levels in the biomass corresponding to higher bio-oil HHV. Although this constitutes a significant increase with respect to the starting biomass, it still falls short of the energy content of mineral oil (41–48 MJ kg⁻¹). Although the bio-oil is not suitable for use as a transport fuel without further modification, potentially it can be refined in a similar manner to crude oil to give a range of fuels [19].

The HTL reaction can also be accelerated by metal catalysts and a number of investigations have examined their effect. As algae are complex mixtures of proteins, carbohydrates, lipids and alternative metabolites, additional metals rarely effect the algae uniformly across species [20], though under certain conditions bio-oil yields have been demonstrated to be increased substantially [21].

To create an economical bio refinery it is necessary to consider upstream factors, as well as final product quality. Despite the advantages conferred by HTL, cultivation of algal biomass is still a relatively energy-intensive process, and requires high inputs of water, nutrients and CO₂ [22]. As well as optimising bio-oil yields; maximising carbon efficiency, efficient water/nutrient recycling and ensuring an inexpensive source of CO₂ are crucial to the success of algal biofuel production [23].

In this investigation the suitability of using HTL to process metal contaminated algal biomass was assessed. Firstly, Spirulina (Arthrospira platensis) with representative levels of metal sulphates were processed in a batch HTL system. Finally, two algal cultures cultivated on AMD were converted under the optimal conditions to assess the viability of encompassing a combination of AMD remediation and biofuel production (Fig. 1). Here, we aim to determine how metals affect the yield and composition of the HTL reaction products (the solid, aqueous, oil and gaseous phases) and assess the viability and usefulness of these products for exploitation as a biofuel, metal remediation and for the recycling of nutrients to promote further microalgal growth.

2. Materials and methods

2.1. Materials

Spirulina powder (A. platensis) was obtained from Bulk Powders (Colchester, UK). The dried biomass contained 63% protein, 20% carbohydrate, 6% fat and 11% miscellaneous biochemical content. Metal sulphates (99%+), (FeSO₄.7H₂O, MgSO₄.7H₂O, ZnSO₄.7H₂O, PbSO₄ and SnSO₄) were obtained from Sigma Aldrich and used without further purification. The HCl and HNO₃ (both trace metal grade) were purchased from Fisher Chemicals.

2.2. Methods

2.2.1. Hydrothermal liquefaction (HTL) batch reactions

Batch liquefaction was conducted in accordance with previous literature precedent [24]. The reactor, connected to a pressure gauge, needle valve, and spring-loaded relief valve, contained a total internal volume of ca. 50 ml. The reactor body was heated inside a vertical tubular furnace, with the temperature of the reaction mixture monitored using a thermocouple connected to data logging software. The reactor was loaded with approximately 4,000 g of dry biomass, 0–1500 mg metal sulphates, and 20 ml deionised water, and heated within the furnace, pre-heated to 550 °C, until the specified reaction temperatures were reached, 310 °C (15 min)–350 °C (35 min), then removed from the furnace and allowed to cool to room temperature. Mixing was provided by convection in the reactor; temperature profiles of the reaction are given in the supporting information. In order to determine experimental error and test the repeatability of experimental results, three repeat runs of HTL of pure Spirulina at both 310 °C and 350 °C were used to assess the variation in the experimental set-up. The reaction pressure required for the hydrothermal liquefaction reaction was generated in situ through the expansion of the reactor fluids and partial vapourisation of the water. The reaction pressure varied from 120 bar at 310 °C to 180 bar at 350 °C.

2.2.2. Gas analysis

After cooling, gaseous products were released via the needle valve into an inverted, water-filled measuring cylinder to measure the volume of the gaseous fraction. Gas phase yields were calculated using the ideal gas law, assuming an approximate molecular weight of 44 g mol⁻¹ (the molecular mass of CO₂, which makes up approx. 96–98% of the gaseous product). A sample from each gas phase was separated and analysed using a gas chromatograph (Agilent 7890A) containing an HP-Plot-Q capillary column (using helium as the carrier gas), and fitted with an Agilent 5975C MSD detector. The samples were loaded at 35 °C, hold time 7 min, ramped to 150 °C at 20 °C min⁻¹, hold time 0 min, ramped to 250 °C at 15 °C min⁻¹, hold time 16 min.

2.2.3. Aqueous phase analysis

The aqueous phase was decanted from the reactor contents and filtered through a 0.22 μm filter. The dissolved product yield in the
water phase was determined gravimetrically from a 2.5 ml aliquot, dried at 60 °C for 12 h. The concentration of ammonium ions in the water phase was determined spectrophotometrically using a Random Urea analysis test kit (Merck, Millipore). The sample was diluted with deionised water to a concentration of 1 vol% prior to analysis. Subsequently 10 μl of sample was reacted for 5 min with 1000 μl of a urease reactant, followed by the addition of 200 μl of sodium hypochlorite solution to induce the colour change. After 10 min, sample absorbance was measured at 600 nm and urea concentration calculated relative to a standard solution. From this, ammonium concentration was calculated. Total nitrogen content analysis was carried out using a Merck-Millipore Spectroquant Total Nitrogen Cell Test kit and photometer, based on the Koroleff method of persulphate digestion to transform organic and inorganic N compounds into nitrate. Each sample was diluted to 0.1% prior to analysis. 10 ml of diluted sample was digested for 1 h at 120 °C, then allowed to cool to room temperature and reacted with a benzoic acid derivative form a nitro compound.

Phosphate concentration in the aqueous phase was determined using the Merck-Millipore Spectroquant test kit and photometer system. Prior to analysis, each sample was diluted by a factor of 5–1000, depending on estimated phosphate content, and reacted with the reagents provided. Aqueous samples (6 ml), diluted in 11.6 ml deionised water were acidified with 0.4 ml 67% v/v HNO3 prior to analysis using Perkin Elmer Optima 2100 ICP-OES to determine the Fe, Zn and Mg content.

2.2.4. Crude bio-oil analysis

To separate the remaining bio-oil and solid residue phase, the reactor was washed repeatedly using chloroform until the solvent was clear, the solution was filtered, and any residual bio-oil washed off the filter paper. The solvent was evaporated using a rotary evaporator set to 40 °C. The ash content of the original strains was not subtracted prior to calculating the mass balance. To determine the higher heating value (HHV), approximately 200 mg bio-oil was weighed into a steel crucible, with ultra-pure water. Filtered digestates were analysed using an Agilent 7700 Series ICP-MS to determine P, Pb, Sn, Mg, Zn and Fe content. Alternative elements, mainly O, were calculated by subtraction in the reactor/mg l−1. The starting pH of the cultures was reduced to pH3, the approximate optimal pH for cultivation of the AMD-1 algae. The pH remained stable throughout the experiment.

2.2.5. Solid residue analysis

The solid residue yield was calculated from the mass of the retentate collected on the filter paper after drying for 12 h in an oven at 60 °C. The filter paper was weighed immediately on removing from the oven, both before and after use, to minimise errors associated with absorption of atmospheric moisture. Solid residue samples were digested in aqua regia. Briefly, 6 ml of HCl (37%; Fisher Tracemetal grade) was added to approximately 100 mg residue. After any initial reaction had subsided, 2 ml concentrated HNO3 (Fisher Trace metal grade) was added and the digest covered and left at room temperature for 15 min. The digest was then heated to 95 °C for 60 min, cooled and made up to 50 ml with ultra-pure water. Filtered digestates were analysed using an Agilent 7700 Series ICP-MS to determine P, Pb, Sn, Mg, Zn and Fe content. Alternative elements, mainly O, were calculated by subtraction and were grouped as ‘other’. SEM analysis of the solid residue was carried out using a JEOL JSM-6480LV system. Elemental composition analysis was carried out using INCA software. Samples were analysed on a Carlo Erba Flash 2000 Elemental Analyser to determine CHN content. Elemental analyses were carried out in duplicate for each sample and average values are reported.

2.2.6. Culturing of AMD-1 and AMD-2 cultures

A mixed community of microalgae (predominantly Euglena- and Chlamydomonas-like morphologies) isolated from the mine drainage of a former tin mine in the UK were grown in AMD supplemented with nitrates and phosphates (see supporting information). Following scale up in conical flasks (3 l), the biomass for HTL was generated in a bubble column with artificial illumination. Bubble columns were constructed using PVC components; 110 mm clear polycarbonate tubing, with a working culture volume of 10 l. Light was supplied via 36 W Grolux fluorescent tube and 36 W 865 daylight fluorescent tube providing 80 μmol photons m−2 s−1. Cultures were aerated by constant bubbling at 3 l min−1. Cultures were grown at 20 °C in cycles of 16 h of light: 8 h dark photoperiods (16:8 h).

The AMD-1 culture was grown on a synthetic acid mine drainage medium (sAMD) supplemented with both phosphate and nitrate salts (a full description is given in the supporting information). The AMD-2 culture was grown on AMD supplemented with phosphate and nitrate salts (see supporting information). Cells counts were conducted via flow cytometry daily and stationary growth phase biomass was harvested by centrifugation.

2.2.7. Culturing the AMD-1 algae with HTL aqueous phase

The bubble column photobioreactors were held at room temperature, which fluctuated between 16 °C to 22 °C (see supporting information for the full temperature profile), air, with no additional CO2, was bubbled through at 5 l min−1 and the bioreactors were inoculated with 1 l of the AMD algae cultured in a SAMD medium (see supporting information), with no additional nitrates or phosphates but with the addition of the aqueous phase from the hydrothermal processing of Spirulina at 350 °C, (diluted 1:100 v/v with deionised water). The starting inoculum was ~105 cells ml−1. The starting pH of the cultures was reduced to pH3, the approximate optimal pH for cultivation of the AMD-1 algae. The pH remained stable throughout the experiment.

3. Results and discussion

3.1. HTL of Spirulina and metal sulphates

Initially, the effect of additional metals present with the algal biomass on the HTL process was examined using the commercially available cyanobacterium Spirulina (A. platensis) with the addition of a range of metal sulphates under batch HTL process conditions. The metal concentrations used (described in Table 1) were based on data collected from AMD from a former tin mine between January and March 2014. The main metal contaminants found in this mine water were Fe, Zn and Mg, with lower amounts of Sn and Pb also being present. To examine the effect of the metals on the HTL process, the five separate metal sulphates were added to the Spirulina biomass at concentrations found in the AMD. Two distinct scenarios were investigated, the first with the main metals Fe, Zn and Mg being present at a low concentration in order to assess the effect of a minimum uptake scenario, where the algae display minimal adsorption of the metals present in dilute AMD streams. In the second scenario the concentration of metals was higher, mimicking a situation where the algae had successfully

<table>
<thead>
<tr>
<th>Metal/scenario</th>
<th>Representative salt(s)</th>
<th>Metal concentration in the reactor/mg l−1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb</td>
<td>PbSO4</td>
<td>10</td>
</tr>
<tr>
<td>Sn</td>
<td>SnSO4</td>
<td>210</td>
</tr>
<tr>
<td>Low uptake (Mg/Zn/Fe)</td>
<td>MgSO4, FeSO4, ZnSO4</td>
<td>17, 43, 90</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>150</td>
</tr>
<tr>
<td>High uptake (Mg/Zn/Fe)</td>
<td>MgSO4, FeSO4, ZnSO4</td>
<td>2665, 5820</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>22,855</td>
</tr>
<tr>
<td>Mg</td>
<td>MgSO4</td>
<td>2665</td>
</tr>
<tr>
<td>Zn</td>
<td>ZnSO4</td>
<td>5820</td>
</tr>
<tr>
<td>Fe</td>
<td>FeSO4</td>
<td>14,396</td>
</tr>
</tbody>
</table>

Table 1: The metal salts used in conjunction with Spirulina to mimic metal contaminated algae used in remediation of AMD. The low uptake scenario mimics a situation where there is poor metal uptake from the AMD, the high uptake scenario mimics the best case, where all the metal present in the AMD has been absorbed.
been used to remediate concentrated AMD metal effluent streams (high uptake scenario).

3.1.1. Effect of additional metal sulphates on the bio-oil yield and HHV of Spirulina

The Spirulina/metal mixtures were processed in batch reactions at either 310 °C or 350 °C. The resulting oil was extracted, weighed and the N content and HHV assessed (Fig. 2). In the majority of cases lower processing temperatures generated higher bio-oil yields than those obtained at ca. 350 °C. This corresponds directly with previous literature findings [25–27]. There was also a positive correlation between bio-oil yields and increasing total metal content (Fig. 2a), and is potentially due to the catalytic effect of the metals present. FeSO₄ is commonly employed as a catalyst in coal liquefaction, for example [28], and significant improvements in oil yield when liquefying pine wood in supercritical ethanol using 5% w/w FeSO₄ have also been reported [29]. Similarly, the addition of both MgSO₄ and ZnSO₄ also increased oil yields, though, to our knowledge, neither metal has previously been investigated as a potential HTL catalyst.

N content of the bio-oil was reduced from 9.7% in the Spirulina feed to 7% in the bio-oil produced by HTL without additional metals. All samples displayed lower bio-oil N levels at higher processing temperatures. The metal concentration had little effect on the N content of the bio-oil, though slightly elevated levels were observed in the process when Sn and Mg were present. Despite the N content of the bio-oil being lower than in unprocessed Spirulina, the presence of metals did not reduce the N content to a suitable level for combustion and a further hydrogation upgrading stage would be necessary to produce hydrocarbon fuels from this process.

Energy recovery = \( \frac{\text{Bio-oil HHV} \times \text{Oil yield}}{\text{HHV of the feedstock}} \) (1)

Higher HHV were observed at the higher processing temperatures, consistent with findings in the literature [15]. For example, *Nannochloropsis* sp. processed at 350 °C yielded 34% oil with a HHV of 38.1 MJ kg⁻¹, while 46% oil was obtained at a processing temperature of 310 °C, though with a much lower HHV of 27.7 MJ kg⁻¹ [13]. Similarly Spirulina yielded higher bio-oil yields at both 220 °C and 350 °C, than at 310 °C, though the oil HHV was lower [30]. Interestingly, for the *Nannochloropsis* the energy recovery (Eq. 1) were remarkably similar at both temperatures, being 50% and 51% respectively, though for the Spirulina the 350 °C reaction gave a far higher energy recovery that the lower processing temperatures (64% opposed to 52%).

Increased concentrations of Mg, Zn and Fe caused a decrease in bio-oil HHV (Fig. 2(c)). This effect was more pronounced at 310 °C than at 350 °C. While the C:H ratio does not change significantly, a higher level of alternative elements (mainly oxygen) are observed in the oil in comparison to the sample with no additional metals (Table 2). It

![Fig. 2. Bio-oil produced by hydrothermal liquefaction of Spirulina with additional metal sulfates, where a) total bio-oil yields calculated as % of the original starting biomass (excluding additional metals), b) total N content in the bio oil and c) The energy content of the bio-oil d) The energy recovery of the bio-oil, calculated according to equation 1.](image-url)
seems plausible that the reduction in the HHV observed in this study is similarly due to an increase in the heteroatom content in the bio-oil and not an increase in aromatisation. The energy recovery for the system was found to be similar for the Spirulina biomass and the samples with low metal input. However, when high levels of metals are present the energy recovery in the bio-oil reaches 62%. This demonstrates that while the energy content is slightly lower, the metals present in the AMD have an overall positive effect on the production of bio-oil.

3.1.2. Assessment of the aqueous phase from the HTL of Spirulina and metal sulphiates

On processing of the algal biomass, the light organic compounds and some alternative inorganic elements are deposited in the aqueous phase. To assess this residue, the aqueous phase was dried in an oven at 60 °C for 12 h and the residue weighed (Fig. 3a). The organic residue in the aqueous phase was consistently reduced at higher processing temperatures: this organic material was presumably partitioning into the bio-oil phase and correlates with the increasing bio-oil yields. Recycling of algal nutrients (such as N, P, K, Fe) would be required for an economically viable algal bio refinery. While the AMD provides most metallic species in abundance, AMD is generally phosphate and nitrogen limited. The total N, NH4+ and phosphate content of the aqueous phase samples were therefore examined (Fig. 3b). Nutrient partitioning into the aqueous phase was influenced both by the reaction conditions and the levels of metal in the sample. The total nitrogen content in the aqueous phase was similar irrespective of temperature, though higher metal loading and could be used as a useful source of NH4+ for nitrogen limited AMD.

Reasonable partitioning of phosphorus into the aqueous phase also occurred and was stable irrespective of the temperature employed. However, while up to 1920 mg l−1 of P was observed at the low metal loadings, on addition of high levels of metals to the system the phosphate levels detected in the aqueous phase dropped dramatically. At the highest metal loading, 22,855 mg l−1 of total metals, phosphate levels in the aqueous phase dropped to 13.9 mg l−1, from 1860 mg l−1 in the aqueous phase from processing of pure Spirulina.

The limitation of aqueous P concentrations is potentially due to the formation of insoluble metal phosphates (particularly iron and zinc phosphates) and their partitioning into the solid residue. Despite additional metals being in excess, small levels of phosphate (up to 15 mg l−1) were still detected in the aqueous residue. Zinc, however, was a notable exception to this. The aqueous phase from a sample processed with 5820 mg l−1 Zn still contained 460 mg l−1 of phosphate when processed at 310 °C, and 720 mg l−1 when processed at 350 °C.

Only trace levels of Sn and Pb were detected in the aqueous phase of the water from the HTL processing of pure Spirulina, and did not increase with the addition of extra Sn or Pb sulphate to the system. At the levels examined Sn and Pb partition almost entirely out of the aqueous phase. At the water from the HTL processing of pure Spirulina, and did not in-
The solid residue was also examined by SEM (see supporting infor-
mation). On addition of metals, the physical appearance of the solid res-
idue altered signifi-
cantly, and the metals were detected in solid residue
from metal-containing reactions at levels not observed in the solid res-
idue from Spirulina liquefaction alone. The results are in good agree-
ment with data obtained through ICP-MS, suggesting that the metals
in the solid residue phase are predominantly oxides. The low magne-
sium content of the mixed-metal additive sample is in agreement
with the high magnesium levels detected in the aqueous phase.

3.2. HTL of algal cultures used in the remediation of acid mine drainage

Following the positive assessment of the HTL for the model com-
ounds, the HTL process was applied to a natural algal community se-
lected for cultivation on AMD. Two scenarios were examined. In both
cases, the same starting algal community was used. The
first scenario,
AMD-1, the algae were cultured in a low pH, synthetic AMD broth. In
AMD-2, the algal culture was grown in real AMD taken from a former
tin mine. 310 °C was selected as the temperature for processing as this
was shown to give optimised bio-oil yields. The initial elemental com-
position of the AMD-1 algae was similar to the Spirulina, though due
to the increased metal uptake, the AMD-2 biomass was notably different
(Table 4). Overall, the mass fractions of the four main product compo-
nents from liquefaction were similar for AMD-1 algae and Spirulina,
though the AMD-2 algae produced a far higher amount of solid residue
on liquefaction (Fig. 6).

Bio-oil yields (calculated as a percentage of the original dry algal bio-
mass) were higher for the AMD-1 algae than for Spirulina with no

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**Table 3**

Analysis of the gas fraction produced from the hydrothermal liquefaction of Spirulina with additional metal sulphates. Gas yield is given as a percentage of the original biomass, on the assumption that the volume is entirely composed of CO₂.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Gas yield (%)</th>
<th>CO₂ (%)</th>
<th>Volatile organic carbon (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>310 °C</td>
<td>350 °C</td>
<td>310 °C</td>
</tr>
<tr>
<td>None</td>
<td>9.8</td>
<td>13.4</td>
<td>98.7</td>
</tr>
<tr>
<td>Pb</td>
<td>8.6</td>
<td>9.5</td>
<td>98.4</td>
</tr>
<tr>
<td>Sn</td>
<td>9.1</td>
<td>14.6</td>
<td>98.5</td>
</tr>
<tr>
<td>Mg Zn Fe (low uptake)</td>
<td>9.8</td>
<td>7.3</td>
<td>99.0</td>
</tr>
<tr>
<td>Mg Zn Fe (high uptake)</td>
<td>15.4</td>
<td>15.6</td>
<td>99.4</td>
</tr>
<tr>
<td>Mg</td>
<td>13.1</td>
<td>10.8</td>
<td>99.3</td>
</tr>
<tr>
<td>Zn</td>
<td>10.7</td>
<td>11.8</td>
<td>97.8</td>
</tr>
<tr>
<td>Fe</td>
<td>13.8</td>
<td>16.2</td>
<td>99.0</td>
</tr>
</tbody>
</table>

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Fig. 3. Analysis of the aqueous phase produced by hydrothermal liquefaction of Spirulina with additional metal sulphates where a) is the yield of residue on removal of the water, b) is the total N content in the water phase, the grey shaded area represents the amount of nitrogen present as NH₄⁺, c) is the total phosphate content in the aqueous phase and d) gives the level of metals present in the aqueous phase on HTL of the combined Mg, Zn, Fe (high uptake) samples (total metal content 22,855 mg l⁻¹).
additives, comparable to yields obtained with additives at the highest metal loading. This increase is likely to be due partly to the comparatively higher C and H content of the biomass, and partly due to catalytic effects from absorbed metals in the biomass. The AMD-2 biomass, with a far lower carbon content, gave a correspondingly lower yield of 8.8% bio-oil, with the solid residue making up 55.3% of the product by weight. Interestingly, although the yield was extremely low, the composition and HHV of the bio-oil were similar to that of the AMD-1 biomass (Table 5). This suggests that the volume of remediated metals will not have a large effect on the quality of the bio-oil, and the quality of the bio-oil should be relatively uniform irrespective of any seasonal or geographical changes in the metal content being remediated.

AMD-1 has a significantly higher level of carbon partitioning into the oil (84%) than either Spirulina or AMD-2 (73% and 42%, respectively) at the same processing conditions, demonstrating that initial biomass concentration cannot be the only factor affecting bio-oil yields. The AMD-1 algae contained 0.46 wt.% Fe and 0.27 wt.% Zn, in addition to 0.32% Mg, which is also present in Spirulina at similar levels.

For the AMD-2, the decrease in bio-oil yield was matched by a large increase in the solid residue yield, with solid residue comprising 55% of the product by weight, compared with ca. 10% for pure Spirulina and AMD-1 (Table 6). Elemental analysis of the solid residue reveals that the CHN ratios are similar to those detected in the solid residue from HTL of both Spirulina with and without metal additives, but distinct from those observed for AMD-1 algal culture. Both metals and phosphorus are present at higher mass fractions in the solid residue phase than in the starting biomass. The AMD-1 solid residue contained moderate levels of Zn and Mg (3% and 3.75%, respectively), and somewhat less Fe (7%) than expected from the model. Sn and Pb were also detected at low levels. The AMD-2 algal solid residue contained predominantly Fe (50%) with little contribution from other metals (0.11% Zn and 0.14% Mg).

In the aqueous phase, the total N is lower for AMD-1 algae than Spirulina, and lower again for the AMD-2 algae, likely to be caused by the comparatively lowered N content of the starting biomass (Fig. 7). A similar pattern was also seen for NH₄⁺. Phosphate analysis of the aqueous phase revealed that the phosphate content of the process water from liquefaction of AMD-1 biomass was similar to that of pure Spirulina, whereas the process water from AMD-2 contained extremely low phosphate levels, presumably a combination of the comparatively

### Table 4

Main elemental composition of the algae (w/w % dry mass) demonstrating low metal uptake (AMD1) and the algal community demonstrating high metal content (AMD2) compared to the Spirulina sample with no additional metal sulphates.

<table>
<thead>
<tr>
<th></th>
<th>C%</th>
<th>H%</th>
<th>N%</th>
<th>P%</th>
<th>Mg%</th>
<th>Zn%</th>
<th>Fe%</th>
<th>Sn%</th>
<th>Pb%</th>
<th>Other%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spirulina</td>
<td>43.47</td>
<td>5.76</td>
<td>9.74</td>
<td>2.06</td>
<td>0.39</td>
<td>0.00</td>
<td>0.13</td>
<td>0.0001</td>
<td>0.003</td>
<td>38.46</td>
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<tr>
<td>AMD-1</td>
<td>47.58</td>
<td>6.95</td>
<td>8.95</td>
<td>2.25</td>
<td>0.32</td>
<td>0.27</td>
<td>0.46</td>
<td>0.0014</td>
<td>0.0117</td>
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<td>AMD-2</td>
<td>17.56</td>
<td>3.37</td>
<td>2.89</td>
<td>2.42</td>
<td>0.10</td>
<td>0.04</td>
<td>23.44</td>
<td>0.0003</td>
<td>0.0015</td>
<td>50.18</td>
</tr>
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</table>

Fig. 4. a) Total solid residue yield for Spirulina processed with additional metal sulphates b) the % of carbon from the original biomass that partitioned into the solid residue phase.

Fig. 5. Elemental composition of the solid residue produced from the hydrothermal liquefaction of Spirulina, showing the high uptake scenario and the individual Mg, Zn and Fe sulphates. The full dataset is given in the supporting information.
lower starting concentration of biomass and the high levels of iron and magnesium in the original starting material leading to precipitation of Zn and Fe phosphates.

Elevated levels of iron were present in the process water produced from the HTL of the AMD-2 algae. Little Fe partitioning to the aqueous phase is observed in the Spirulina model even at relatively high loadings, but the AMD-2 algae material seemingly contained a sufficiently high initial Fe concentration for this to occur. Magnesium recovery in the aqueous phase was also high, although no zinc was recovered. This is in agreement with the Spirulina model: even at high loadings, little zinc recovery in the aqueous phase is observed, and Mg partitions to the aqueous phase relatively readily.

As the acid mine drainage only contains trace levels of phosphate and ammonium, it must be supplemented by these elements to ensure algal growth. The effectiveness of the HTL output streams to supplement microalgal culture was established by combining the aqueous phase produced from the HTL of Spirulina with the sAMD media (Fig. 8). Previous work demonstrated that a dilution factor 1:100 for HTL water was a general good compromise for a reasonable biomass concentration, high growth rate and low toxicity. Chemical analysis showed that the diluted HTL aqueous phase had a total nitrogen and phosphate concentration of 200 mg l\(^{-1}\) and 18 mg l\(^{-1}\) respectively, which should be amenable for use to further stimulate algal growth. The main VOC components of the gaseous products from AMD-1 were similar to the gas products identified from Spirulina liquefied at 310 °C, though AMD-2 only produced propene and isobutylene in significant quantities (see supporting information).

4. Conclusions

HTL is a suitable process to convert algae used in the remediation of acid mine drainage. On liquefaction of Spirulina, bio-oil yields were increased slightly on addition of the metals, and this did improve the carbon recovery in the oil phase, though the HHV was reduced. A corresponding decrease in the total organic content of the aqueous phase was identified. The oil produced from both low metal and high metal systems, were remarkably similar irrespective of the lack of biomass in the AMD-2 sample.

One of the key parts of the proposed biorefinery is the ability to salvage metals captured from AMD. In this work, metals were found to partition almost exclusively into the solid residue phase. This occurred with the notable exception of magnesium, which was recovered at roughly 30–50% in the aqueous phase. However, elevated levels of magnesium in the aqueous phase should also be beneficial for process water recycling. In the AMD algal samples, high metal partitioning in the solid residue phase was also observed, allowing for either effective disposal or recovery.

Another important factor is nutrient recycling for algal cultivation. Unfavourably, high metal loadings drove partitioning of phosphates from the aqueous phase into the solid phase, presenting a potential setback to the recycling of process water as a growth supplement for further algal cultivation. It seems likely that the phosphate would need to be recovered from the solid residue phase through further chemical

---

**Table 5**

<table>
<thead>
<tr>
<th>Bio-oil yield / % of total dry mass</th>
<th>% C</th>
<th>% H</th>
<th>% N</th>
<th>% Other</th>
<th>HHV/MJ kg(^{-1})</th>
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<tbody>
<tr>
<td>Spirulina</td>
<td>35.9</td>
<td>70.8</td>
<td>9.1</td>
<td>7.9</td>
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<td>AMD-1</td>
<td>45.7</td>
<td>68.8</td>
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<td>7.1</td>
<td>16.2</td>
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<tr>
<td>AMD-2</td>
<td>8.9</td>
<td>68.6</td>
<td>7.2</td>
<td>7.5</td>
<td>16.5</td>
</tr>
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</table>

---

**Fig. 6.** Mass balance for the algae used in the remediation of acid mine drainage on being processed by HTL. The percentages given were calculated from the weight of the original starting material on drying (biomass and any metals remediated from the AMD).

**Fig. 7.** Nutrient content of the aqueous phase on the hydrothermal processing of algal cultures used in the remediation of acid mine drainage.

**Fig. 8.** Cell count and phosphate use of AMD-1 cultured in a 10 l bubble column bioreactor on sAMD with the supplementary aqueous phase produced from the HTL of the Spirulina biomass. The bioreactor was run in duplicate.
processing. Nevertheless, the phosphate that did partition into the aqueous phase could be metabolised by the algae effectively.

Processing at lower temperatures yielded a gas phase composed of higher purity CO₂ with lower VOC levels, which is favourable for partial gas phase recycling into algal cultivation. On liquefaction of the algal communities used to remEDIATE AMD, the CO₂ produced was around 99% pure.

In general, processing temperatures were found to have a more substantial influence than metal loadings over product yields and properties. HTL processing was found to be a suitable method of processing algae used in the remediaTion of acid mine drainage. The oil is of a high enough quality for further hydrogenation into fuels, whereas the majority of the metals are almost exclusively recovered in the solid residue phase. There is also scope for the recovery of NH₄⁺ and limited P from the aqueous phase to supplement algal growth and aid in the further remediaTion of metal rich effluent.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fuproc.2015.10.017.

References


Table 6

Elemental composition of the solid residue produced by the hydrothermal liquefaction of algae with a low metal uptake (AMD1), and a high metal uptake (AMD2), used in the remediation of acid mine drainage. The solid residue is given as a percentage of the original total dry algal biomass.

<table>
<thead>
<tr>
<th>Feedstock</th>
<th>Solid residue yield/ %</th>
<th>C %</th>
<th>H %</th>
<th>N %</th>
<th>P %</th>
<th>Mg %</th>
<th>Zn %</th>
<th>Fe %</th>
<th>Sn %</th>
<th>Pb %</th>
<th>Other %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spirulina</td>
<td>10.31 %</td>
<td>9.34</td>
<td>1.12</td>
<td>1.78</td>
<td>17.65</td>
<td>6.28</td>
<td>0.03</td>
<td>1.59</td>
<td>0.0015</td>
<td>0.0047</td>
<td>62.21</td>
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<tr>
<td>AMD-1</td>
<td>10.85</td>
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<td>2.86</td>
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<td>3.75</td>
<td>3.02</td>
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<td>0.01</td>
<td>0.12</td>
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<td>AMD-2</td>
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<td>1.46</td>
<td>1.68</td>
<td>4.48</td>
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<td>0.11</td>
<td>50.1</td>
<td>0.00</td>
<td>0.01</td>
<td>31.54</td>
</tr>
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