PHD

Modelling of fermentation kinetic processes by non-linear optimisation techniques.

Gutierrez, Salvador Lopez

Award date:
1980

Awarding institution:
University of Bath

Link to publication

Alternative formats
If you require this document in an alternative format, please contact:
openaccess@bath.ac.uk

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Download date: 03. Jan. 2021
MODELLING OF FERMENTATION KINETIC PROCESSES
BY NON-LINEAR OPTIMISATION TECHNIQUES

Submitted by

SALVADOR LOPEZ GUTIERREZ

for the degree of Ph.D. of the University of Bath

1980

Copyright.

Attention is drawn to the fact that the copyright of this thesis rests with its author. This copy of the thesis has been supplied on condition that anyone who consulted it is understood to recognise that its copyright rests with its author and that no quotation from the thesis and no information derived from it may be published without the prior written consent of the author.

This thesis may be made available for consultation within the University Library and may be photocopied or lent to other libraries for the purpose of consultation.

[Signature]
ACKNOWLEDGMENTS

The author is indebted to the following for their help in the development of this thesis.

- Dr. John E. L. Bowcott for his continuous advice, encouragement and infinite patience.
- Concejo Nacional de Ciencia y Tecnologia (CONACYT, Mexico) for its financial support.
- Academic Staff of the University of Bath.
- Technical Staff of the University of Bath, particularly to Mr. T. B. Walton for his help and advice.
- Postgraduate students: Mrs. M. Agudelo, Miss C. Bazilio and Mr. J. Hernandez for their continuous help, advice and encouragement.
MEMORANDUM

The work described in this thesis was conducted in the Department of Chemical Engineering of the University of Bath during the period October 1977 to April 1980 and has not been submitted for any other degree. All the work described is the original work of the author except where specially acknowledged.
SUMMARY

A non-linear optimisation procedure is proposed to model fermentation kinetic processes. The fundamentals of the method are based on the minimisation of an objective function which does not require the solution of the model equations. The enzyme kinetics expressions used to model pH effects on growth rate kinetic constants are analysed. A model for the pH effect (PHEM model) on growth rate associated and cell concentration associated reaction parameters is proposed.

Model identification and parameter evaluation for the batch and continuous culture of Candida lipolytica in a glucose-ammonium medium at 30 °C and several pH levels (4 to 6 in continuous culture and 3 to 7 in batch culture) is carried out in order to examine the optimisation procedure proposed here. The optimisation approach was found to be highly efficient for the analysis of data from the continuous culture, but failed in the analysis of data from the batch culture.

The continuous culture was found to be described adequately by a double kinetic Contois-Contois interactive model with glucose and ammonium as limiting nutrients. The batch culture was found to be described adequately by a single kinetics Monod model with glucose as the rate limiting nutrient and endogeneous respiration repressed by the carbon source.

The pH affected both continuous and batch cultures, significantly. The pH effect is examined on the basis of the response of the model parameters to hydrogen ion concentration. pH effects on growth rate constants, stoichiometric coefficient of carbon and nitrogen sources, endogeneous respiration coefficients and inhibition constant of glucose (for endogeneous respiration in the batch culture) are discussed. Some examples of the PHEM model are given, from data found in the literature and from results found in this work.
CONTENTS

SUMMARY. iv
NOMENCLATURE. xiii
INTRODUCTION. 1

CHAPTER 1. BACKGROUND.

1.1 Generalities. 3
  1.1.1 Models for transformation processes in microbial systems. 4
  1.1.2 Non-structured model approach for the modelling of microbial transformation processes. 7
    1.1.2.1 Kinetics of growth. 7
    1.1.2.2 Substrate consumption and product formation kinetics. 9
    1.1.2.3 Other effects in transformation process kinetics. 11
  1.1.3 Transfer processes in fermentation systems. 14
    1.1.3.1 Transfer processes around the biological material. 15
    1.1.3.2 Transfer processes at non-biological interphases. 20
  1.2 Double substrate limited growth kinetics. 26
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2.1 Double substrate kinetics interactive models.</td>
<td>27</td>
</tr>
<tr>
<td>1.2.2 Double substrate kinetics non-interactive models.</td>
<td>28</td>
</tr>
<tr>
<td>1.2.3 Double substrate enzyme models</td>
<td>31</td>
</tr>
<tr>
<td>1.3 Non-structured models for the pH effect on fermentation processes.</td>
<td>35</td>
</tr>
<tr>
<td>1.3.1 Effects of pH on the morphological and physiological characteristics of microorganisms.</td>
<td>35</td>
</tr>
<tr>
<td>1.3.2 Non-structured models with pH dependant functions.</td>
<td>41</td>
</tr>
<tr>
<td>1.4 Non-linear parameter estimation in process models.</td>
<td>52</td>
</tr>
<tr>
<td>1.4.1 Objective functions in optimisation techniques.</td>
<td>54</td>
</tr>
<tr>
<td>1.4.2 The solution of the optimisation problem.</td>
<td>57</td>
</tr>
<tr>
<td>1.5 Objectives of this work.</td>
<td>60</td>
</tr>
</tbody>
</table>

CHAPTER 2. THEORY.

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1 Optimisation approach for modelling multiparameter kinetic processes in fermentation systems.</td>
<td>63</td>
</tr>
<tr>
<td>2.1.1 Objective functions for one-response system.</td>
<td>64</td>
</tr>
<tr>
<td>2.1.2 Objective functions with model structure equations only.</td>
<td>65</td>
</tr>
</tbody>
</table>
2.1.3 The solution of the optimisation problem.
   2.1.3.1 Steepest descent algorithm.
   2.1.3.2 Newton algorithm.

2.1.4 Multiresponse objective functions with model structure equations only.

2.2 Kinetic model for growth.
   2.2.1 Model structure equations.
   2.2.1.1 Model structure equations for continuous culture.
   2.2.1.2 Model structure equations for batch culture.

2.3 Analysis of enzyme kinetics models for pH effects

2.4 Model for the pH effect on stoichiometric coefficients and rate constants for substrate consumption, product formation and endogeneous respiration, (PHEM model).
   2.4.1 A model for the pH effects on stoichiometric coefficients for growth associated reactions.
   2.4.2 A model for the pH effects on rate constants of cell concentration associated reactions.
CHAPTER 3. EXPERIMENTAL.

3.1 Materials and equipment. 102
3.2 Analytical techniques. 108
3.3 Operation. 108
   3.3.1 Batch cultures. 108
   3.3.1 Continuous cultures. 109
3.4 Results. 110

CHAPTER 4. ESTIMATION OF PARAMETERS.

4.1 Estimation of parameters of the continuous culture model. 115
4.2 Estimation of parameters of the batch culture model. 125
4.3 A test of the insignificance of mass transfer effects. 134
   4.3.1 Mass transfer in continuous culture. 134
   4.3.1.1 Transfer of oxygen in the continuous culture. Non-biological interphase. 135
   4.3.1.2 Transfer of oxygen and glucose from the culture fluid to the cellular material. 138
4.3.2 Mass transfer in the batch culture. 141
CHAPTER 5. DISCUSSION.

5.1 The optimisation technique and the kinetic model for the continuous culture.  
5.1.1 Model discrimination.  
5.1.2 Comparison of the model parameters for continuous culture with other values reported in the literature.  
5.1.3 The optimisation procedure.  

5.2 The kinetic model for the batch culture.  
5.2.1 Model response for the batch culture.  
5.2.2 An alternative model for the batch culture.  

5.3 The pH effect on the fermentation process.  
5.3.1 pH effect on growth rate constants.  
5.3.2 pH effect on stoichiometric coefficients.  
5.3.3 pH effect on endogeneous respiration coefficients.  
5.3.4 pH effect on glucose inhibition constant for endogeneous respiration of the batch culture.  

5.4 Some examples of the PHEM model.  

CONCLUSIONS.
APPENDICES.

APPENDIX

A. Some enzyme kinetics mechanisms. 184

B. Mathematical background. 191

B1. Extreme values of a function of several variables. 191

B2. Variance-covariance matrix of $X$. 193

B3. First and second partial derivatives of model structure equations of the continuous culture Contois-Contois kinetics. Mode 4. 194

B4. Algebraic solution of the model structure equations of the continuous culture at steady state. Contois-Contois kinetics. 197

B5. Computer programme flow chart of the optimisation procedure. 198

B6. Modes of the model structure equations of the Monod-Monod model for the batch culture. 222

B7. Description of the subroutine E02CAF for the polynomial fit of the model parameters as functions of pH. 224

C. Analytical techniques. 225

C1. Glucose determination. 225

C2. Ammonium ion determination. 227

D. Parameter evaluation of the batch culture model. 229

D1. Plots for the evaluation of stoichiometric and endogeneous respiration coefficients of the batch culture model. 229
D2. Plots for the evaluation of kinetic parameters of the batch culture model. 229
D3. Evaluation of kinetic parameters of the alternative model for the batch culture. 229

E. Estimation of mass transfer parameters. 242
E1. Estimation of $k^{a}$ for oxygen transfer from gas phase to culture fluid in the continuous culture. 242
E2. Estimation of $(k_{1}a)_{1g}$ and $(k_{1}a)_{0,2}$ for the transfer of dissolved species from the culture fluid to the biological transport zone. 246
E3. Estimation of the parameter $k'_{2}$ of the Powell's model for the batch culture. 248

F. Coefficients and plots of the polynomial fit of the model parameters as function of pH. 250
F1. Coefficients and plots of the polynomial fit of the Contois-Contois kinetic model parameters of the continuous culture. 250
F2. Coefficients and plots of the polynomial fit of the parameters of the alternative model for the batch culture. 253

G. Model response plots for batch and continuous cultures. 255
G1. Model response for the continuous culture at pH 5 and 5.5 255
<table>
<thead>
<tr>
<th>APPENDIX</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>G2. Model response for the batch culture.</td>
<td>255</td>
</tr>
<tr>
<td>Alternative model for the batch culture at pH 3, 4 and 5.5</td>
<td></td>
</tr>
</tbody>
</table>

REFERENCES. 263
NOMENCLATURE

\( a \) : volumetric surface area of transfer.

\( a_i \) : internal surface area of diffusion shell in a spherical microorganism.

\( a_m \) : external surface area of diffusion shell in a spherical microorganism.

\( B \) : Contois constant defined in Table 1.1

\( B_g \) : Contois constant for glucose. (kg glucose/kg dry biomass)

\( B_n \) : Contois constant for ammonium. (kg ammonium/kg dry biomass)

\( C \) : concentration.

\( \mathbf{C} \) : model structure equation vector.

\( C_e \) : concentration of enzyme E.

\( C_{e_0} \) : total enzyme concentration.

\( C_{es} \) : enzyme-substrate (ES) complex concentration.

\( C_{e_i} \) : concentration of enhancing growth nutrients. Equation (1.33)

\( C_g \) : glucose concentration. (kg/m\(^3\))

: gas bulk concentration.

\( C_{gi} \) : concentration at the interphase, gas side.

\( C_H \) : H ion concentration.

\( C_j \) : components of the model structure equation vector \( \mathbf{C} \).

\( C_l \) : liquid bulk concentration.

: substrate concentration at internal shell of diffusion zone of a spherical microorganism.

\( C_{li} \) : concentration at the interphase, liquid side.
\( C_n \): ammonium ion concentration. \((\text{kg/m}^3)\)

\( C_{nF} \): ammonium ion feed concentration in continuous culture.

\( C_{gF} \): glucose feed concentration in continuous culture.

\( C_{O2} \): oxygen concentration.

\( C_{OH} \): OH ion concentration.

\( C_p \): product P concentration.

\( C_s \): substrate S concentration.

\( C_{sj} \): concentration of essential nutrient for growth S_j.

Equation (1.33)

\( C_{SH} \): substrate - H ion complex concentration (SH).

\( C_{SOH} \): substrate - OH ion complex concentration (SOH).

\( C_{s1} \): concentration of limiting substrate S_1.

\( C_{s2} \): concentration of limiting substrate S_2.

\( C_x \): cell concentration as dry biomass. \((\text{kg dry biomass/m}^3)\)

\( C^* \): concentration of substrate at external shell of diffusion zone (bulk fluid) of a spherical microorganism.

\( C_{1}^* \): liquid phase concentration which is in equilibrium with gas bulk concentration \( C_g^* \).

d : diameter of a spherical microorganism.

\( D \): dilution rate (1/hr)

\( D_{av} \): average diffusivity for transport through a membrane pore. Equation (1.66).

\( D_g \): diffusivity coefficient of gaseous species.

\( D_s \): diffusivity coefficient of glucose.

\( D_m \): diffusivity coefficient defined in Equation (1.14)

\( D_o \): constant defined in Equation (1.66).
\( e_i \): output error at the \( i \)th experimental observation. Equation (1.89).

\( e_1 \): model structure equation error defined by Equation (1.90).

\( e_2 \): output error defined by Equation (1.90).

\( E \): enzyme.

\( EI \): enzyme-inhibitor complex

\( ES \): enzyme-substrate complex.

\( f \): function.

\( F \): function.

\( F_i \): implicit function vector for the model structure equations defined by Equation (1.79).

\( F \): components of \( F_i \).

\( g \): refers to gaseous component.

\( I \): refers to glucose.

\( H \): Henry's law constant.

\( H \): Hessian matrix of objective function \( J(\chi) \).

\( I \): inhibitor.

\( I \): identity matrix.

\( J(\chi) \): objective function defined by Equation (1.82).
\[ \begin{align*}
k & : \text{rate constant} \\
k_d & : \text{rate of cell deactivation constant.} \\
: & \text{rate of natural death of cell constant.} \\
k_e & : \text{endogeneous respiration coefficient. (1/hr).} \\
k_g & : \text{mass transfer coefficient in gas film.} \\
k_l & : \text{mass transfer coefficient in liquid film.} \\
k_a & : \text{volumetric mass transfer coefficient.} \\
k_2' & : \text{constant defined by Equation (1.21).} \\
k_3 & : \text{constant defined by Equation (1.22).} \\
: & \text{rate constant defined by Equation (1.40).} \\
K & : \text{equilibrium constant.} \\
K_g & : \text{saturation constant for glucose. (kg glucose/m}^3) \\
K_{g_i} & : \text{inhibition constant for glucose (endogeneous respiration repression) (kg/m}^3). \\
K_H & : \text{inhibition by H ions constant.} \\
K_I & : \text{inhibition by I constant.} \\
K_l & : \text{overall liquid-side mass transfer coefficient.} \\
K_m & : \text{Michaelis-Menten constant.} \\
K_n & : \text{saturation constant for ammonium ion.} \\
K_{OH} & : \text{inhibition constant for OH ions.} \\
K_p & : \text{inhibition constant for product P.} \\
K_S & : \text{saturation constant for substrate S.} \\
K_{S_i} & : \text{apparent value of saturation constant (at constant pH)} \\
K_{S_{i1}} & : \text{saturation constant for substrate } S_{i1}. \\
K_v & : \text{volumetric absorption coefficient defined in Table 1.3 (II).}
\end{align*} \]
m : stoichiometric coefficient.

m_x : stoichiometric coefficient for cell material synthesis.

m_g : stoichiometric coefficient of glucose. (kg glucose consumed per kg biomass produced)

m_n : stoichiometric coefficient for ammonium (kg ammonium consumed per kg of biomass produced).

MCE : model constitutive equations.

MSE : model structure equations.

n : refers to ammonium ion.

nc : number of constraints of the MSE of a process model.

nep : number of experimental points (observations) in a process model.

nev : number of dependent (experimental) variables of a process model.

niv : number of independent variables of a process model.

np : number of parameters of a process model.

npc : number of parameter's constraints of a process model.

p : refers to product P.

P : metabolic product.

P_x : product from cell lysis, death or respiratory activity.

q : volumetric flow rate of component C.

Q : flux of component C.
r : radial coordinate.
      : reaction rate.

re : endogeneous respiration rate.

rg : rate of glucose consumption. (kg glucose/m^3/hr)

ri : radius of internal shell of diffusion zone of a spherical microorganism.

rm : radius of external shell of diffusion zone of a spherical microorganism.

rn : rate of ammonium ion consumption. (kg ammonium/m^3/hr).

rp : product formation rate.

rs : substrate consumption rate.

rx : cell growth rate. (kg dry biomass/m^3/hr).

r'x : observed specific growth rate.

R : gases constant.
      : rate of substrate uptake. Equation (1.22)
      : weighing matrix, Equation (1.85)

Rb : substrate uptake rate.

R'_b : rate of substrate transport in biological transport zone.

R''_b : rate of substrate consumption in metabolic zone.

R'_\text{max} : maximum specific rate of substrate removal.

Rx : specific growth rate. (kg dry biomass produced/hr/kg of dry biomass)

R'x : observed specific growth rate (r'x/C_x)

s : refers to substrate S.

S : substrate.
\( t \) : time

\( \text{independent variable of a process model} \)

\( t_i \) : value of independent variable of a process model at the

\( i \text{th experimental point} \)

\( t \) : independent variable vector of a process model.

\( t_i \) : independent variable vector corresponding at the \( i \text{th}

\( \text{experimental observation} \)

\( T \) : refers to the transpose of a vector or matrix, i. e. :

\[ A^T = \text{transpose of } A \]

\( V_m \) : maximum specific rate constant of an enzyme-substrate

\( w \) : weighing factor.

\( w_i \) : weighing factor corresponding to the \( i \text{th experimental}

\( x \) : refers to cells \( X \).

\( x_i \) : components of estimated parameter vector \( X \).

\( X \) : cell or single cell.

\( X \) : estimated parameter vector of a process model.

\( Y \) : dependent variable of a process model.

\( \gamma \) : yield constant.

\( Y \) : dependent variable vector of a process model.
$Y_e$: experimental value of dependent variable of one-response process model.

$Y^e$: experimental value of dependent variable vector of a multiresponse process model.

$Y_g$: yield constant for glucose. (kg of dry biomass produced per kg of glucose consumed).

$Y_n$: yield constant for ammonium. (kg of dry biomass produced per kg of ammonium consumed)

$Y_s$: yield constant for substrate S.

$Y_t$: theoretical values of dependent variable of a process model.

$Y^t$: theoretical value of dependent variable vector of a multiresponse process model.

$Z$: implicit function which defines the model constitutive equations of a kinetic process. Equation (2.2).

$\alpha$: kinetic constant defined in Equation (1.18).

$\alpha$: stoichiometric constant for product formation.

$\alpha_i$: true value of parameter vector

$\alpha_i$: components of true value of parameter vector.

$\alpha_x$: proportionality constant defined in Equation (1.5).

$\beta$: rate constant for product formation.

$\beta$: kinetic constant defined by Equation (1.18).

$\delta$: rate of enzyme decay constant. Equation (1.7a).

$\delta$: rate of natural death of cell constant.
δ : cell surface charge.

λ : thickness of diffusion layer.

\[ \mu_m \] : maximum specific growth rate (1/hr).

\[ \mu'_m \] : apparent value of maximum specific growth rate at constant pH.

\( \phi \) : geometric factor defined by Equation (1.20).

\( \phi \) : electrical potential in membrane pore.
INTRODUCTION

Biochemical engineering has experienced a great development in the last three decades since Monod's classic work in 1949 in which was proposed a model to describe a fermentation kinetic process, up to the most recent works in which the potential of a wide variety of resources to control prime importance variables in biological reactors have been described. Despite the breakthrough of research in the area, the nature of the biological processes themselves is at present in a state of incomplete understanding of their physiological and biochemical basis which has proved to be a complex matter.

The needs of the present world cannot wait until the biological sciences have an almost perfect view of the situation, so, the present "state of the art" must be considered the best to satisfy the necessities of everyday life. In no few cases, the biological foundations of a phenomenon are neglected to a great extent when designing practical biochemical processes. Process design demands expressions in the form of formulae and equations, as well as figures within a reasonable range of error for parameters. Such expressions and figures are derived from experimental research in which the concepts of mathematical modelling have a central role. It is in this particular aspect of mathematical modelling that chemical engineering has played its part in the connection between the phenomenological science and the real world, justifying by this sole fact its existence.

Mathematical models and parameter values are essential parts in industrial biochemical process development, this have been demonstrated by the large number of reports published in recent years. Most mathematical models in current use to describe biological phenomena are crude approximations to reality, yet they
have survived the scientific scrutiny due to their applicability for practical purposes and it seems they will stay on as long as no new theories of biological mechanisms suitable for mathematical modelling emerge.

The present work intends to contribute to the modelling of biochemical kinetic processes and to the parameter evaluation techniques in current use. The specific objectives of this work are listed at the end of Chapter one (Section 1.5), after the survey of reports relating to it have been presented and only a list of key concepts within the scope of this report follows below:

- Non-linear optimisation.
- Parameter evaluation by non-linear optimisation procedures.
- Double substrate kinetics.
- pH effect on kinetic parameters.
- Modelling of pH effect on fermentation kinetic processes.

The survey of reports in the literature included the latest reports published up until November 1979 when available in English or translation. Some papers not available in English translation were consulted with personal help from the translation staff of the Scientific Division of the British Library but it was not possible to cover all recently published papers which are originally written in Slavic and Oriental languages for which English translations were not available in the U. K. To those works not mentioned here by involuntary causes, acknowledgment for their effort and contribution is due.
CHAPTER 1

BACKGROUND

1.1 Generalities.

Fermentation processes consist of the transformation of chemicals carried out by microbial systems. Microorganisms are highly complex systems that use advanced biochemical processes which are still far from being completely understood. These biochemical systems make use of raw materials to generate a sophisticated production line of chemicals involved in the process of life.

Two types of processes can be distinguished in a fermentation system: transformation processes and transfer processes. Transformation processes are those in which materials experience the actual chemical change and are carried out by networks of biochemical reactions in specific sites of the inner structure of the cell. Transfer processes deal with the phenomena occurring from where the materials enter the system until they reach the actual reaction site. Transfer processes can be considered as pure physical phenomena and the general principles of transport theory is applied to describe them; transformation processes fall in the domain of chemical kinetics and are so treated. A brief review of the modelling of the two types of processes follows below.
Models for the transformation processes in microbial systems.

The occurrence of transformation processes in fermentation is determined by several factors existing in the system such as population number, availability of nutrients, environmental conditions such as pH, temperature, distribution of cell's size and cell's age, etc. All these factors have a certain influence in the overall rate of transformation of chemicals. A mathematical model intending to consider all possible aspects influencing the transformation processes would be ridiculously complicated, hence, oversimplification becomes a real need. This has given rise to the development of several classes of models in which some of the factors mentioned above are considered to a certain extent.

A classification of models in current use may seem superfluous and a list of them due to Roels and Kossen (1978) is presented as a quick reference. The characteristics and the basis of the classification are discussed elsewhere (Fredrickson et al., 1970; Kossen, 1978; Roels and Kossen, 1978).

<table>
<thead>
<tr>
<th>Class of Model</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verbal</td>
<td>Qualitative description of biological processes. Communication among scientists of related disciplines.</td>
</tr>
<tr>
<td>Descriptive</td>
<td>Curve-fitting. Interpolation of data; can be used only within the region where the model was tested experimentally.</td>
</tr>
<tr>
<td>Predictive (explanatory)</td>
<td>Extrapolation of data. Can, with care, be used outside the region where the model was tested experimentally.</td>
</tr>
<tr>
<td>Class of Model</td>
<td>Application</td>
</tr>
<tr>
<td>---------------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Mathematical</td>
<td>Quantitative description of biological processes.</td>
</tr>
<tr>
<td>Deterministic</td>
<td>Systems with many microorganisms, (( &gt; 10^4 )).</td>
</tr>
<tr>
<td>Stochastic</td>
<td>Systems with few microorganisms, (( &lt; 10^4 )).</td>
</tr>
<tr>
<td>Discrete time</td>
<td>Systems where events occur at a limited number of times (e.g., synchronous cultures).</td>
</tr>
<tr>
<td>Continuous time</td>
<td>Systems where events (e.g., division of microorganisms) are evenly distributed in time.</td>
</tr>
<tr>
<td>Distributed</td>
<td>Systems where the biomass and other quantities are considered to be evenly distributed throughout the culture fluid.</td>
</tr>
<tr>
<td>Segregated</td>
<td>Systems where the cellular nature of the microbial life is taken into account.</td>
</tr>
<tr>
<td>Structured</td>
<td>Systems where the internal structure of the cells is taken into account.</td>
</tr>
<tr>
<td>Non-structured</td>
<td>Systems where the internal structure of the cells is not taken into account.</td>
</tr>
</tbody>
</table>

A given model may have several classifications at the same time; the models concerning this work are Descriptive-mathematical-deterministic-distributed-non-structured models and they will be here referred to as non-structured models only.
The scheme for non-structured models is simple. The cells are considered a chemical species \( X \), interacting with one or more substrates \( S_1, S_2, \ldots \), and producing either more cells or product \( P \), or both in agreement with the following chemical equation:

\[
\begin{align*}
\frac{(m_x + m_p)S_1 + (m_{2x} + m_{2p})S_2 + \ldots}{X} & \rightarrow X + P
\end{align*}
\]

(1.1)

where: 
- \( m_x \) : stoichiometric coefficients for cells material synthesis.
- \( m_p \) : stoichiometric coefficients for products synthesis.

In addition to the basic chemical equation (1.1) some other effects can be introduced, e.g.

(a) Cells producing respiratory, death or lysis products:

\[
\frac{X}{m_x X} \rightarrow P_x
\]

(1.2)

(b) Metabolic products reconsumed by cells:

\[
\frac{X}{m_P X} \rightarrow X
\]

(1.3)

Kinetic studies demand expressions for the rate of production or consumption of chemical species. It is a subject to which microbiologists, biochemists, biochemical engineers and scientists working in areas related to the fermentation field have devoted a great deal of time and thought in the last fifty years or so. Non-structured models have played an important role in modelling of the microbial metabolism and they are discussed in the following Section.
1.1.2 Non-structured model approach for the modelling of microbial transformation processes.

The three aspects that have been mainly considered by non-structured models are: cell growth rate, substrate consumption and product formation. Around these three concepts some other features of the microbial life such as accelerated growth, endogeneous respiration, viability and deactivation of cells, etc., have been incorporated. A summary of these aspects is presented in the following Sections.

1.1.2.1 Kinetics of growth.

Although the growth of cells during fermentation has been modelled since early this century (Bader, 1978), Monod's model introduced in 1949 is the most widely used up to date. It rests on a very large quantity of experimental research in which the following assumptions have been made:

- The growth rate of cells is limited by only one substrate $S$, present in the medium.

- The growth rate ($r_x$) of the microorganisms is proportional to their concentration ($C_x$) and proportional to some function of the limiting substrate concentration ($C_s$).

- The functional form for the substrate effect can be taken as: $C_s/(K_s + C_s)$.

Therefore, the expression for the growth rate is given by:

$$ r_x = \mu m C_x \frac{C_s}{K_s + C_s} \quad (1.4) $$
where: $r_x$ : growth rate of cells,

$C_x$ : cell concentration.

$C_S$ : limiting substrate concentration.

$\mu_m$ : proportionality constant termed maximum specific growth rate.

$K_S$ : constant termed saturation constant or half-maximum growth rate constant.

Due to the mathematical properties of the function $C_S/(K_S + C_S)$ which predict that for $C_S \gg K_S$, the growth rate is independent of $C_S$, the Monod model and others that show the same characteristic are referred to as "saturation kinetics" models.

It must be pointed out that the Monod model does not intend a mechanistic interpretation of the microbial growth and it was stated on an empirical basis with no theoretical considerations. Most of the non-structured models have been developed along the same line of thought and the functional differences of the growth rate expressions obey more a better-fit-of-data criterion than to the biological basis of cellular growth. Some of the models proposed in this sense over this century are listed in Table 1.1.

The expressions listed in Table 1.1 consider only the kinetic expression for chemical transformation and any consideration of transfer phenomena must be properly related to a given kinetic equation to give the expression for the overall observed rate of growth. Some of the expressions derived from kinetic and mass transfer analysis are listed in Table 1.2 (Section 1.1.3).

A general characteristic of the models previously mentioned is that only one substrate is considered as growth limiting, for this reason, they are termed single substrate kinetic models. More than one substrate limited growth is examined in Section 1.2 with emphasis on two substrates or as it is known,
TABLE 1.1 Non-structured models expressions for microbial growth rate. From Roels and Kossen (1978).

<table>
<thead>
<tr>
<th>Growth rate expression</th>
<th>Blackman (1905)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( r_x = C_x \frac{(C_s/A)}{if \ C_s &lt; \mu m} )</td>
<td>A, ( \mu m ), constants.</td>
</tr>
<tr>
<td>( r_x = \mu m ) if ( C_s \geq \mu m )</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tessier (1936)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( r_x = \mu m C_x (1 - \exp(-kC_s)) )</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Monod (1949)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( r_x = \mu m \frac{C_x C_s}{K_s + C_s} )</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Moser (1958)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( r_x = \mu m \frac{C_x C_s b}{K_s + C_s b} )</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Contois (1959)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( r_x = \mu m \frac{C_x C_s}{BC_x + C_s} )</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Konak (1974)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \frac{\partial r_x}{\partial C_s} = k (\mu m - r_x)^p )</td>
</tr>
</tbody>
</table>

Double substrate kinetics. Before carrying on, it must be pointed out that none of the models mentioned before has proved universal validity and their applicability is restricted to the particular systems on which they have been tested.

1.1.2.2 Substrate consumption and product formation kinetics.

Substrate consumption is directly linked to cell's growth. Monod (1949) proposed that the rate of consumption of the limiting growth substrate is growth associated, i.e., it can be expressed as a linear function of the cell's growth rate:
Equation (1.5) is frequently written as:

\[ r_s = -\alpha_x r_x \]  

Therefore \( \alpha_x = \frac{1}{Y_s} \).

The quantity \( Y_s \) is termed "yield coefficient" or "yield factor" (unit mass of cells produced by unit mass of substrate consumed).

No modification of importance has been proposed for the substrate consumption rate expression. This, due to the general agreement found between Equation (1.5) and experimental results.

In the case of fermentation products, it has been found that they follow two main formation patterns: growth associated and concentration of cell associated. These concepts were first proposed by Luedking and Piret (1959) in their now classic study on lactic acid fermentation. They found that the rate of lactic acid production by Lactobacillus delbrueckii from glucose, could be correlated to the cell's growth rate and cell concentration by the following expression:

\[ r_p = \alpha r_x + \beta C_x \]  

where: \( r_p \) : rate of product formation.

\( \alpha \) and \( \beta \) : constants.

Equation (1.7) contains two terms which have been identified with two different biochemical processes (Luedking and
Piret, 1959). The term $\alpha r_x$ represents the rate of product formation linked to the metabolic pathways for synthesis of cellular material. If the cell material synthesis stops, i.e., growth ceases, the product formation by this mechanism stops.

The term $\beta C_x$ has been called enzyme-like product formation rate, due to the fact that even if there is no growth, the model still predicts product formation. This is a well known phenomenon in fermentation which consists of the diversion of the metabolic activity from cellular growth to product formation which occurs when the fermentation culture is exhausted of certain nutrients indispensable for growth. (Mazza and Ertola, 1970; Sonnleitner et al., 1979; Kristiansen and Sinclair, 1979).

The two processes for product formation may be present during the whole course of the fermentation (Luedking and Piret, 1959; Aiyar and Luedking, 1966) or only one of them may be significant (Yadav and Gupta, 1976; Kristiansen and Sinclair, 1979).

In a recent study, Kristiansen and Sinclair (1979) proposed that for the citric acid production by A. foetidus from glucose, the rate of product formation can be expressed as:

$$r_p = \alpha r_x - \gamma C_p$$

(1.7a)

where: $C_p$: product concentration.

$\gamma$: constant.

the term $\gamma C_p$ being representative of "enzyme decay" due to repression effect by product accumulation.

1.1.2.3 Other effects in transformation process kinetics.

Some concepts derived from kinetic studies on fermentation that have been taken into account to improve the performance of non-structured models are summarised below.
A. Endogeneous respiration.

Herbert (1958) introduced the concept of endogeneous respiration to account for the fall of cell concentration observed at long residence times in continuous cultures. When the supply of nutrients from the environment is scarce, cells use some of their own material as a source of energy to survive. The cellular material is degraded to oxidation products causing a fall in the cellular mass concentration. The rate of this cell consumption by endogeneous respiration can be expressed as:

\[ r_e = k_e C_e \]  \hspace{1cm} (1.8)

where:

- \( r_e \): rate of cellular material consumption by endogeneous respiration,
- \( k_e \): endogeneous respiration coefficient.

B. Viability of biomass.

Posgate and Hunter (1962) performed an extensive study on cellular viability in starving bacterial populations in batch and continuous cultures and showed that the total biomass was composed of a fraction of viable cells and a fraction of dead cells, which proportion (dead/viable) increased under starving conditions, i.e., end of growth phase in batch cultures or very low dilution rate in continuous cultures. There is experimental evidence (Lockhart and Powelson, 1954; Roman, 1957; Mitchison, 1958) that the cells have a limited life-span and that they become deactivated for growth after a certain number of reproduction cycles which causes some fraction of the population of the culture to be observed as dead cells. This subject has been treated in relation to activity of product formation by Shu (1961) in a model that considers distribution of states (cell age). Sinclair and Topiwala (1970) introduced the rate of natural death of cells in a model to predict viability conditions in a continuous culture in agreement with:
\[ r_{nd} = \gamma C'_x = \gamma v C_x \] (1.9)

where:
- \( r_{nd} \): rate of natural death of cells.
- \( \gamma \): proportionality constant.
- \( v \): viability coefficient defined as the ratio of viable cells over total cells \( (C'_x/C_x) \).
- \( C'_x \): viable cell concentration.

C. Growth inhibition.

The inhibition effects of several chemicals in microbial activity is a very well known subject. The basis of inhibition mechanisms are found in the enzymic processes inside the cell and in the alterations caused to cellular structure by chemical agents. Non-structured models have incorporated some expressions rationalised from enzyme kinetics into the models for fermentation processes. Some examples are given below.

(a) Growth inhibition by substrate. Andrews (1968)

\[ r_x = \mu m C_x \frac{C_s}{K_s + C_s + C_s^2/K_i} \] (1.10)

- \( K_i \): constant.

(b) Growth inhibition by product. Aiba et. al. (1968).

\[ r_x = \mu m C_x \frac{C_s}{K_s + C_s} \frac{K_p}{K_p + C_p} \] (1.11)
(c) Growth inhibition by H ions, substrate in excess. Ibragimova et. al., (1969).

\[ r_x = \mu m_c x \frac{K_H}{K_H + C_H} \] (1.12)

(d) Growth inhibition by substrate. Wayman and Tseng (1976).

\[ r_x = \mu m_c x \frac{C_s}{K_s + C_s} \quad \text{for } C_s < C_{sc} \] (1.13)

\[ r_x = \mu m_c x \frac{C_s}{K_s + C_s} - k_i (C_s - C_{sc}) \quad \text{for } C_s \geq C_{sc} \]

where: 
- \( k_i \) : constant
- \( C_{sc} \) : critical concentration of substrate below which growth is not inhibited by the substrate concentration. Above \( C_{sc} \) inhibition is linear with respect to substrate concentration.

1.1.3 Transfer processes in fermentation systems.

In fermentation reactors it is possible to distinguish two types of processes in which transfer phenomena are important and that, under certain conditions, can control the whole process. Those two processes are:

(a) Transport of gaseous species from gas phase to liquid phase through the interphase film (or a different interphase, as oil-water).

(b) Transport of dissolved chemicals from liquid phase to the biological zone in which the transformation processes occur.
The transport phenomena of type (a) are of the same nature as those occurring in two-phase non-biological reactions, in which a great deal of research has been carried out. The general results for non-biological systems can be applied to fermentation processes when reasonable hydrodynamic and physico-chemical similarities exist. The transport process of type (b) are to a certain extent related to the external structure of the biological materials in which the chemicals "sink" to be transformed. Some general principles and ideas from chemical reactions involving particles and catalyst pellets have been applied to biochemical reactors. A brief description of the equations and correlations that have been proposed to deal with the above mentioned problems are mentioned below.

1.1.3.1 Transfer processes around the biological material.

The basic principles of the theory can be exemplified with the model for mass transport around a single cell. A current model (Powell, 1967) assumes that the cell has spherical geometry and it is surrounded by a film which presents resistance to mass flow. Figure 1.1 depicts the regions of a spherical cell (Atkinson, 1974).

The transport mechanisms in the external shell follows the concept of passive transport, i.e., the mass transfer is driven by a concentration gradient and chemicals are transferred from liquid bulk up to the limits of the biological transport zone. In the intermediate shell, the permease model (Cohen and Monod, 1957; Cirillo, 1961) states that the material is transported by an enzymic mechanism mode by carriers termed "permeases" and this type of transport is not subjected to diffusion laws; in view of which it is possible to transport materials against their concentration gradient. This type of transport has been termed "active transport" and, in agreement with the permease model, the expression for the transfer rate is equivalent to the Michaelis-Menten expression (see
FIGURE 1.1 Transport and metabolic zones in a spherical microorganism model.

A: molecular diffusion zone.
B: biological transport zone.
C: metabolic zone.

Section 1.3) for enzymic reactions. The permease model for active transport in the biological transport zone is currently under strong scrutiny (Hamilton, 1975; Simoni and Postma, 1975, Harold, 1977) due to its failure to fulfill energetic considerations involved in the active transport process.

If steady state conditions are assumed to exist for substrate transport through the transport zones and substrate uptake by the metabolic zone, the rate of substrate removal from the bulk fluid is given by:

\[ r_s = a_m \left( \frac{dC_s}{dr} \right) = R' = R'' \]

(1.14)

where:

- \( r_s \): rate of substrate removal.
- \( a_m \): surface area of external shell of molecular diffusion zone.
- \( D_m \): diffusivity coefficient.
r: flow coordinate (radial)  
\( R''_b \): rate of substrate transport in biological transport zone  
\( R''''_b \): rate of substrate consumption in metabolic zone.  
\[ \frac{dC_s}{dr} \text{ at } \text{m surface of diffusional shell.} \]

As pointed out by Atkinson (1974), equation (1.14) can be equalled to \( R''_b \) or to \( R''''_b \) as long as the steady state condition exists, therefore, the rate of substrate removal can be related to either the biological transport rate equation or to the substrate uptake rate expression for the metabolic region. Equation (1.14) can then be written as:

\[ a_m (\frac{dC_s}{dr}) = R_b \]  

(1.15)

where \( R_b \) is the actual kinetic expression for substrate uptake rate.

From the steady state condition in the diffusional shell it can be stated that:

\[ 4\pi r^2 (\frac{dC_s}{dr}) = \text{constant} \]  

(1.16)

assuming the boundary conditions:

at \( r = r_i \) \hspace{1cm} \( C_s = C_1 \)  

(1.17)

at \( r = r_m \) \hspace{1cm} \( C_s = C^* \)

and if \( R_b \) is assumed to follow a Monod kinetics type expression, e.g.:

\[ R_b = \frac{\alpha C_s}{\beta + C_s} \]  

(1.18)
\( \alpha \) and \( \beta \) constants; the following expression can be derived:

\[
C^* = C_i + \left( \frac{\alpha C_i}{\beta + C_i} \right) \left( \frac{r_i^2}{D_m} \right) \left( \frac{1}{r_i} - \frac{1}{r_m} \right)
\]  

(1.19)

where:

- \( C_i \): substrate concentration at the internal surface of diffusional shell.
- \( C^* \): substrate concentration at the external surface of diffusional shell (bulk fluid).
- \( r_i, r_m \): internal and external radii of diffusional shell.

A geometric factor \( \phi \) can be defined as:

\[
\phi = r_i^2 \left( \frac{1}{r_i} - \frac{1}{r_m} \right)
\]  

(1.20)

If \( D_m \rightarrow \infty \) or \( r_m \rightarrow r_i \) (\( \phi \rightarrow 0 \)) the second term of the right-hand side of Equation (1.19) vanishes giving \( C^* = C_i \) and in this case, it can be said that no mass transfer limitation exists and the rate of substrate removal can be expressed by the kinetic expression \( R_b \) in terms of the bulk fluid concentration \( C^* \). Otherwise, the following result arises (solving Equation 1.19 for \( C_i \)):

\[
C_i = \frac{1}{2} \left( \beta + \alpha \frac{\phi}{D_m} - C^* \right) \left[ -1 + \left( 4 \beta C^* (\beta + \alpha \frac{\phi}{D_m} - C^*)^{-2} \right)^{\frac{1}{2}} \right]
\]  

(1.21)

The following quantities are defined (Atkinson, 1974):

\[
R = \left( a_i / \rho v_m \right) \left( \alpha C_i / ( \beta + C_i ) \right)
\]  

(1.22a)

\[
R_{\text{max}} = \frac{\alpha a_i}{(\rho v_m)}
\]  

(1.22b)

\[
k_3 = 1/\beta
\]  

(1.22c)
where: $a_i$ : internal surface area of diffusion shell.
$v_m$ : wet volume of individual cell.
$\rho_0$ : density of cell.
$R$ : rate of substrate uptake.
$R_{\text{max}}$ : maximum specific rate of substrate removal.

hence, Equation (1.21) can be rearranged as follows:

$$R = \frac{R_{\text{max}}}{2(k_2')^2} \left[ (1 + (k_2')^2 + k_3C^*) - \left(1 + (k_2')^2 - k_3C^*\right)^2 + 4k_3C^* \right]^{\frac{1}{2}}$$

(1.23)

Equation (1.23) expresses the rate of substrate removal in terms of the bulk fluid concentration $C^*$, when mass transfer limitation exists. It must be noticed that when $D_m \rightarrow \infty$ or $\phi \rightarrow 0$ (which are the conditions for no mass transfer limitation), $k_2' \rightarrow 0$. Therefore, the value of $k_2'$ provides a criterion to detect possible mass transfer limitations in a single cell culture.

Following the basic assumptions of mass transfer through a diffusional film surrounding biological materials such as microbial flocs and films, the concept of effectiveness factor has been developed in analogy with the effectiveness factor introduced by Thiele (1939) in heterogeneous catalytic reactions. A discussion of the large number of reports available in the literature concerning this subject is outside the scope of this work. Table 1.2 summarises some of the equations derived from mass transfer considerations to express the rate of substrate removal or biomass growth in microbial aggregates.
TABLE 1.2 Some reaction rate equations in microbial aggregates with mass transfer considered. From B. Atkinson (1974).

<table>
<thead>
<tr>
<th>N : Flux of substrate</th>
<th>G : Specific growth rate</th>
<th>R : rate of substrate removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R = R_{\text{max}} (1 - X_c^3)$, $0 \leq X_c \leq 1$</td>
<td></td>
<td>Microbial floc with volume $V_p$ and surface area $A_p$. Diffusion with biochemical reaction. Muller et al. (1966)</td>
</tr>
<tr>
<td>$2k_3C^*/3(k_2V_p/A_p) = 1 - 3X_c^2 + 2X_c^3$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$N = (k_1/k_2)C^*$</td>
<td></td>
<td>Film (low substrate concentration). Diffusion with biochemical reaction. Atkinson et al. (1967)</td>
</tr>
<tr>
<td>$G = \frac{G_{\text{max}}}{2(k_2')^2} \left[ \frac{1 + (k_2')^2 + k_3C^<em>}{(1 + (k_2')^2 - k_3C^</em>)^2 + 4k_3C^*} \right]$</td>
<td></td>
<td>Microbial floc. Diffusion with biochemical reaction. Powell (1967)</td>
</tr>
<tr>
<td>$N = (k_1x/k_3)(k_3C^<em>/(1 + k_3C^</em>))$</td>
<td></td>
<td>Film (empirical) Koneguy and Andrews (1968).</td>
</tr>
<tr>
<td>$x = L$ if $L \leq d$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$x = d$ if $L \geq d$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

L : film thickness; d : maximum thickness of active microbial layer.

1.1.3.2 Transfer processes at non-biological interphases.

Mass transport through non-biological interphases can be treated by the molecular diffusion theory in the same basic aspects as those applied to describe the transport of substrates around a single cell.
However, the theory involving mass transfer coefficients seems to be more adequate to deal with the situations that are found in practice. The model of a series of resistances which appears in an interphase, as say gas-liquid, has been extensively studied and it is mentioned here as a matter of quick reference only, using a gas-liquid interphase as an example.

The transfer of a gaseous component through a gas-liquid interphase occurs in a series of steps, namely:

i) Transport from the gas bulk through the gas-side film to the gas-side of the gas-liquid interphase.

ii) Movement from the gas-side to the liquid-side of the gas-liquid interphase.

iii) Transport from the liquid-side of the gas-liquid interphase to the liquid bulk through the liquid film.

Assuming that the transport occurs under steady state conditions through the films, the net flux of gas through each region can be described as:

\[
\text{gas side } Q = k_g (C_g - C_{gi}) \tag{1.24}
\]

\[
\text{liquid side } Q = k_l (C_{li} - C_l) \tag{1.25}
\]

where:

- \( C_g \): gas bulk concentration.
- \( C_{gi} \): concentration at the interphase, gas side.
- \( C_{li} \): concentration at the interphase, liquid side.
- \( C_l \): liquid bulk concentration.
- \( Q \): flux of component C.
- \( k_g \): gas side mass transfer coefficient.
- \( k_l \): liquid side mass transfer coefficient.

The movement through the interphase is assumed to be instantaneous and in equilibrium, providing that no consumption of
the component occurs at the interphase, in which case the concentrations \( C_{gi} \) and \( C_{li} \) at the interphase are related by a partition type relationship as the Henry's law:

\[
H \ C_{li} = C_{gi}
\]  

(1.26)

\( H \) : Henry's law constant.

Extensive research has shown that only under certain conditions the gas side resistance is significant (Danckwerts, 1970), this fact offers a particular advantage in the study of mass transfer due to the immediate consequences which are as follows: the overall liquid-side mass transfer coefficient is defined as:

\[
\frac{1}{K_l} = \frac{1}{k_1} + \frac{1}{H k_g}
\]  

(1.27)

\( K_l \) : overall liquid-side mass transfer coefficient.

In terms of \( K_l \), the flux of gas is given by:

\[
Q = K_l (C^*_{l} - C_{l})
\]  

(1.28)

\( C_{l} \) being the liquid phase concentration of \( C \) which is in equilibrium with the gas bulk concentration \( C_g \):

\[
H \ C_{l} = C_{g}
\]  

(1.29)

If the gas-side resistance is negligible (\( k_g \) very large), then \( K_l = k_1 \), (from Equation (1.27)). The flux of gas can be expressed as:

\[
Q = k_1 (C_g/H - C_{l})
\]  

(1.30)
Equation (1.30) is an expression in terms of the gas bulk and liquid bulk concentrations which are variables easy to measure.

The flux equation is converted to volumetric rate by multiplying by the specific surface area of transport, i.e.:

\[ q = k_{1a}(C_g/H - C_l) \]  

(1.31)

where:
- \( q \): volumetric rate of gas transfer.
- \( a \): volumetric surface area of transfer.

Equation (1.31) is one of the most common expressions used to describe mass transfer rates in gas-liquid interphases.

The value of \( k_{1a} \) in fermentation systems is estimated by several techniques; among the most popular are:

(a) Measuring the rate of gas consumption in a reaction system of known kinetics, in which the limiting step is the transport of gas into the liquid (Danckwerts, 1970; Bailey and Ollis, 1977).

(b) Measuring the rate of change of dissolved gas concentration through a transient period between two steady state conditions under similar hydraulic regimes.

(c) Measuring gas bulk and liquid bulk concentrations in a steady state reaction system. (Chain et al., 1966; Sinclair and Ryder, 1975).

In addition to the methods mentioned above, there are in the literature some correlations to determine mass transfer coefficients under certain experimental conditions. These are summarised in Table 1.3.
TABLE 1.3 Mass transfer coefficient correlations.

I. Falling or rising bubbles, droplets or solids.

From Bailey and Ollis, 1977.

\[
\text{Gr} = \frac{d^3 \rho_l (\rho_l - \rho_g)}{\mu_c^2} \quad \text{Grashof number.}
\]

\[
\text{Sh} = k_1 \frac{d}{D_g} \quad \text{Sherwood number.}
\]

\[
\text{Sc} = \frac{\mu_c}{(\rho D_g)} \quad \text{Schmidt number.}
\]

\[
\text{Re} = \frac{\rho d u}{\mu_c} \quad \text{Reynolds number.}
\]

\[
\text{Pe} = \frac{u d}{D_g} \quad \text{Peclet number.}
\]

- \( d \): diameter of bubble, droplet or solid body.
- \( \rho_l \): gas (or light phase) density.
- \( \rho_l \): liquid (or heavy phase) density.
- \( \mu_c \): liquid viscosity
- \( D_g \): gas diffusivity.
- \( u \): relative velocity of gas bubble respect to the liquid.
- \( k_1 \): mass transfer coefficient of liquid film.

<table>
<thead>
<tr>
<th>Correlation</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{Sh} = 1.01 \ (Pe)^{1/3} )</td>
<td>Isolated sphere with rigid interphase, e.g., small bubbles in a fermentation broth containing surface active agents.</td>
</tr>
<tr>
<td>( \text{Sh} = 0.39 \ (Gr)^{1/3} (Sc)^{1/3} )</td>
<td>Isolated spheres with small ( \text{Re} ) in which case: [ u = \frac{d^2 (\rho_l - \rho_g) g}{18 \mu_c} ]</td>
</tr>
</tbody>
</table>
\[ \text{Sh} = 2.0 + 0.60(\text{Re})^{1/3}(\text{Sc})^{1/3} \] (Single bubble with large Re in non-circulating laminar flow.)

\[ \text{Sh} = 0.31(\text{Gr})^{1/3}(\text{Sc})^{1/3} \] (Bubble swarm with \( d < 2.5 \) mm gases into liquids that consumes the gas chemically.)

\[ \text{Sh} = 0.42(\text{Gr})^{1/3}(\text{Sc})^{1/3} \] (Same as above but \( d > 2.5 \) mm)

\[ \text{Sh} = 2.0 + 0.31(\frac{\rho_g}{\mu_g}D_g^{1/3}) \] (Small particles as microbial cells, clumps, flocs, gas-oil dispersions, etc.)

---

**II. Bubbled reactors with mechanical agitation. From Aiba et al., (1973).**

- \( P \): Power consumption of liquid agitation in gassed system.
- \( V \): Liquid volume.
- \( u_s \): Nominal (superficial) velocity of gas (or air) based on cross sectional area of vessel.
- \( K_v \): Volumetric absorption coefficient.

\[ K_v = 0.0635(P_g/V)^{0.95}(u_s)^{0.57} \] (Bubble reactor with vaned disk impeller.)

\[ K_v = 0.038(P_g/V)^{0.53}(u_s)^{0.67} \] (Bubble reactor with paddle impeller.)
1.2 Double substrate limited growth kinetics.

The non-structured models described in Section 1.1.2.1 account only for one limiting substrate. The possibility of more than one substrate affecting the growth rate has been considered sporadically.

Expressions of the type:

\[ r_x = \mu_m \prod_i \left( \frac{C_{si}}{K_{si} + C_{si}} \right) \]  

have found some application in media formulation, although they have not been used for kinetic purposes (Votruba et al., 1975) for \( n > 3 \). Tsao and Hanson (1975) proposed a general expression given by:

\[ r_x = C_x \left( 1 + \sum_i \frac{K_i C_{Ei}}{K_{Ei} + C_{Ei}} \right) \left( \prod_j \frac{\mu_{mj} C_{sj}}{K_{sj} + C_{sj}} \right) \]  

in which \( C_{si} \) are the concentrations of essential nutrients; if one of them is absent, the growth rate goes to zero. \( C_{Ei} \) are the concentrations of the growth rate enhancing substrates; their presence is not essential for growth but growth is enhanced when they are supplied.

Different substrates affect the growth rate in different ways when they are supplied in limited amount. Some of these effects have been suggested to be (Acevedo and Cooney, 1975):

Carbon: would restrict the supply of energy and its availability for synthesis.

Nitrogen and Sulphur: would restrict protein synthesis.

Phosphorus, magnesium: would restrict nucleic acid and/or cell and potassium membrane and/or cell wall synthesis.
The modelling of multiple substrate kinetics has been reduced to take into account two substrates only. When two substrates are considered to be affecting the growth rate and are included in the growth rate expression, the model is referred to as a double substrate kinetic model and the process as double substrate limited growth. Double substrate kinetics have only been used to a limiting extent mainly for two reasons: the first is that single substrate models have been able to describe a wide range of fermentations quite adequately and the second is the additional experimental and mathematical difficulties that the modelling involves.

Double substrate kinetics may be worthy of modelling if there is clear evidence that the double limitation exists for a given set of conditions during fermentation. Commonly, one of the limiting substrates is the carbon source and the second limiting substrate may be any other of the major components of cellular material. The cell low-content components are excluded from limitation because it is easy to achieve economical saturation levels during the whole course of the fermentation. The control of this type of component is centred on other aspects such as inhibition or to drive the fermentation through a desired pathway.

Up to the present, two main categories of double substrate kinetic models have been developed, these are: interactive models and non-interactive models. A third possibility has been derived from double substrate enzyme kinetics.

1.2.1 Double substrate kinetics interactive models.

The basic feature of the interactive models is that the growth rate is affected by two substrates simultaneously to a certain extent. This effect has been called 'multiplicative' (Droop, 1974). The effects of the two substrates cannot be separated from each other, although the extent to which the growth
The simplest way to develop a double substrate interactive model is by multiplying their substrate functions \( f(C_{s1}) \) and \( g(C_{s2}) \) directly. The functional form of \( f \) and \( g \) can be any of those mentioned before (Monod, Contois, Tessier, etc.), the combination of such models are known as Monod-Monod, Monod-Contois, etc., e.g.:

\[
rx = \mu_m C_x \frac{C_{s1}}{K_{s1} + C_{s1}} \frac{C_{s2}}{K_{s2} + C_{s2}}
\]

Equation (1.34) describes the Monod-Monod interactive double substrate kinetic model for growth. The values of the saturation constants can be assessed separately in experiments keeping one substrate in saturation conditions and the other as the limiting one, yet the applicability of the double limitation function is restricted to conditions in which both substrates have concentration levels below the saturation value. Some examples of this model are given below:

- Lactilobacillus casei in glucose/riboflavin. Monod-Monod. (Megee et. al., 1972)

1.2.2 Double substrate non-interactive models.

In this case, a single substrate affects the growth rate at any time and the limitation can shift from one substrate to another depending on whichever causes the lowest growth rate, hence,
the non-interactive model can be stated, in the case of saturation kinetics models, as:

\[ r_x = \mu_m C_x f(C_{s1}) \text{ if } f(C_{s1}) < g(C_{s2}) \]

(1.35)

\[ r_x = \mu_m C_x g(C_{s2}) \text{ if } g(C_{s2}) < f(C_{s1}) \]

Bader (1978) stated that the conditions for the shift of limitation should be:

\[ r_x = \mu_m C_x f(C_{s1}) \text{ if } C_{s1}/K_{s1} < C_{s2}/K_{s2} \]

(1.36)

\[ r_x = \mu_m C_x g(C_{s2}) \text{ if } C_{s2}/K_{s2} < C_{s1}/K_{s1} \]

As it was exemplified with a Monod-Monod model, these conditions are perfectly correct, but when using different functional forms for \( f \) and \( g \), the conditions mentioned by Bader do not imply the conditions stated in Equation (1.35). It should be mentioned that the conditions stated in Equation (1.35) are more general.

Sinclair and Ryder (1975) concluded that in their study of \( C. \) utilitis (glycerol/oxygen, Monod-Monod and Monod-Contois) the growth rate can be described by both an interactive as well as a non-interactive model. Droop (1974) studied a non-interactive model parallel to an interactive one (Monocrysis lutheri in Phosphorus/vitamin \( B_{12} \)) and concluded that the experiment was better described by a non-interactive model.
Bader (1978) has stated that for a continuous culture chemostat, the following inequalities must hold for the growth being double limited in either interactive or non-interactive models:

\[
\alpha_1 + \frac{b_{\text{max}}}{K_2} > \beta_f > \frac{b_{\text{min}}}{K_2} + \alpha_1 \quad (1.37)
\]

\[
\alpha_1 + K_1 Y_1/K_2 Y_2 (\alpha_f - \alpha_1) > \beta_f > K_1 Y_1/K_2 Y_2 (\alpha_f - \alpha_2) + \alpha_2 \quad (1.38)
\]

where: (f subscript stands for feed conditions)

- \(K_1, K_2\) : saturation constants
- \(Y_1, Y_2\) : yield coefficients.
- \(\alpha = \frac{C_{s1}}{K_1}, \quad \beta = \frac{C_{s2}}{K_2}\) (steady state values).
- \(\alpha_1\) : values of \(\alpha\) for which \(\alpha = \beta\).
- \(\alpha_2\) : minimum value of a function \(f(\mu/\mu_m)\) close related to the wash-out dilution rate.
- \(\alpha_1\) : maximum value of the function \(f(\mu/\mu_m)\) closely related to the lowest dilution rate at which the maintenance effects become dominant.
- \(\mu\) : specific growth rate \(\left(\frac{r_x}{C_x}\right)\).

The functional form of \(f(\mu/\mu_m)\) depends on the kinetic model adopted.
Such conditions define the operational region of double limited growth which can be overcome by handling (increasing normally) $\beta_f$ out of the region in which double limitation would occur.

1.2.3 Double substrate enzyme models.

A third approach to the modelling of double substrate kinetics has been developed by analogy to enzyme kinetics. Enzyme kinetics is itself a major area of research, therefore, only the fundamentals of the Michaelis-Menten model for enzyme reactions are mentioned.

The Michaelis-Menten model for an enzyme-substrate reaction is outlined in the following scheme ($C_e$ concentration of enzyme E; $C_s$ concentration of substrate S; $C_{es}$ concentration of enzyme-substrate complex ES; $C_e^o$ total enzyme concentration):

$$E + S \xrightleftharpoons[k_2][k_1] (ES) \text{ in equilibrium} \quad (1.39)$$

$$\xrightarrow[k_3]{} (ES) \rightarrow P + E \text{ irreversible} \quad (1.40)$$

plus a balance of total enzyme $C_e^o$ (constant):

$$C_e^o = C_e + C_{es} \quad (1.41)$$

Applying the equilibrium condition of Equation (1.39) and the enzyme balance, assuming that the reaction rate of product formation $r_p$ is first order in $C_{es}$, the following expressions is obtained:

$$r_p = k_3 \frac{C_e^o}{k_2/k_1 + C_s} \quad (1.42)$$
\[ k_c^o \] is termed as the maximum specific rate \( (V_m) \) and 
\[ \frac{k_2}{k_1} \] as the Michaelis-Menten constant for the enzyme-substrate system. The reaction rate is therefore expressed as:

\[
r = \frac{V_m c_s}{K_m + c_s}
\]

(1.42a)

Based on the Michaelis-Menten model, more complex expressions for enzyme kinetics have been stated.

In enzyme reactions mechanisms involving two substrates, a great number of possibilities can be contemplated. A few of them are outlined below. Double arrows indicate equilibrium reactions and dashed double arrows indicate possible equilibrium reactions.

Double route model.

\[
\begin{align*}
(S_1) & \xrightarrow{S_2} (ES_1) \\
E & \xrightarrow{S_2} (ES_1S_2) \quad \xrightarrow{k_1} P + E \\
S_2 & \xrightarrow{S_1} (ES_2) \\
\end{align*}
\]

(1.43)

The formation of the complex enzyme-substrate is independent of the substrate first attached to the enzyme.

Parallel route model.

\[
\begin{align*}
(S_1) & \xrightarrow{S_2} (ES_1) \\
E & \xrightarrow{S_2} (ES_1S_2) \\
& \xrightarrow{k_1} P + E \\
S_2 & \xrightarrow{S_1} (ES_2) \\
\end{align*}
\]

(1.44)
There is a distinction between the complexes $(ES^1 S_2)$ and $(ES^2 S_1)$ producing $P$ at different rates.

Single compulsory route model.

One of the complexes is inactive.

Double inhibition by substrate double route model:

Double attachment to the enzyme by the same substrate producing inactive complexes.

* A brief list of mechanisms and names for enzyme kinetics is given in the Appendix A.
Expressions for the reaction rate can be derived from the Michaelis-Menten assumptions and the total enzyme balance. For a single mechanism such as the double route model, the following expression can be derived (route 1 or route 2 only, Bader, 1978):

\[
\frac{r_p}{K_1 C^0} = \frac{C_{s1} C_{s2}}{K_2 + K_3 C_{s1} + K_4 C_{s2} + C_{s1} C_{s2}}
\]

(1.47)

For a double inhibition by substrate, double route model, the reaction rate expression is (Tarasova et al., 1977):

\[
\frac{r_p}{K_1 C^0} = \frac{C_{s1} C_{s2}}{K_2 + K_3 C_{s1} + K_4 C_{s2} + K_5 C_{s1}^2 + K_6 C_{s2}^2 + C_{s1} C_{s2}}
\]

(1.48)

In equations (1.47) and (1.48), \( K_i (i = 1,6) \) are the algebraic combinations of the rate and equilibrium constants involved in the network.

An expression of the type: \( \frac{C_{1} C_{2}}{a + b C_{1} + c C_{2} + C_{1} C_{2}} \)

can be given as: \( \frac{C_{1} C_{2}}{(K_1 + C_{1})(K_2 + C_{2})} \) where:

\[a = K_1 K_2, \quad b = K_2 \quad \text{and} \quad c = K_1 \]

Therefore, a single route possibility of the double route model, will lead to an expression that mathematically has the same form of the double kinetic Monod-Monod interactive model. Hence, by analogy, the expressions derived from enzyme kinetics could be used as the growth rate expressions in fermentation.

An example of an enzyme kinetic model for double substrate limited growth can be found in the work of Tarasova et al. (1976) (Act. aureofaciens in ammonium/carbohydrate).

Enzyme kinetic double substrate models are essentially
interactive models. Although derived from theoretical considerations, they could be applied to a wide range of practical situations, but have the disadvantage of involving many parameters, and their assessment becomes uncertain or complicated.

1.3 Non-structured model for the pH effects on fermentation processes.

Before describing the current models for pH effects on fermentation, a brief review of the pH effects on the microbial activity is presented below.

1.3.1 Effect of pH on the morphological and physiological characteristics of microorganisms.

Microorganisms growth shows a wide range of responses to the environmental pH (Thimann, 1963). Bacteria usually grow in a range of pH between 4 and 8, the optimum being around 7, with some interesting exceptions such as the Acetobacter (Zymosarcina ventriculi) and the sulphur oxidising bacteria (Thiobacillus thio-oxidans) which can grow at pH lower than 4. Yeast and fungi generally grow better at acidic pH levels, some of them even at pH's lower than 2. Animal pathogens grow in a narrow range around neutral values, the optima being about 7.2 - 7.4. Actinomycetes are somewhat acid tolerant and some have their optimum for growth at about pH 8.5.

When the environmental pH of microorganisms runs to the extremes of their range for growth, the effects can be dramatic. Filamentous mould Penicillium chrysogenum (Pirt and Callow, 1959) growing normally at pH 6, shows a dramatic change in its morphology when the pH is varied in the range 6 - 7.4; the filaments become multi-hyphae and shorter, the filamentous pattern changes to a pellet form followed by precipitation and a deep change in the rheological properties of the broth is observed.
Yeast, Candida utilis (Lirova et al., 1978b) growing at extreme pH values (2.3 and 7.8) show a severe disruption of their morphology: thickening of cell walls, enlargement of vacuoles, deformation of mitochondria membranes, etc. These changes are linked to modifications of the physiological activity. The effects are diverse and complicated. Some examples of the measurable changes in the cell physiology are summarised in Figures 1.2 to 1.7 (the curves show only qualitative information, the original papers must be consulted to obtain figures).
FIGURE 1.3  Effect of pH on growth rate of microorganisms.

(1) Andreeva e. al. (1969).
(2) Martin and Hempfling (1976).
(3) Ibragimova et. al. (1969)
(4) Stouthamer and Bettenhausen. (1976)
(5) Abbot et. al., (1973)
FIGURE 1.4 Effect of pH on nutrients uptake of microorganisms.

(1) economic coefficient, (2) nitrogen consumption, (3) phosphorus consumption. Lirova et al. (1978b)

(4) economic coefficient, (5) nitrogen consumption, (6) phosphorus consumption. Lirova et al. (1978a)

(8) Biomass yield, Paredes-Lopez et al. (1976)
(10) Biomass yield, Eroshin et al. (1976).
FIGURE 1.5 Effect of pH on metabolites production of microorganisms.

(2) 3-hydroxibutanoate. Vollbrecht and Schlegel (1978).
(5) Tetracycline production. Tarasova et. al. (1976).
(6) Isocitric acid. Marchal et. al. (1977).
(8) Citric acid (concentration). Kristiansen and Sinclair (1979).
FIGURE 1,6  Effect of pH on enzyme synthesis of microorganisms.

(1) Phosphohydrolase activity. Lirova et. al., (1978b)
(2) Acid Protease production. Singh et. al. (1975)

Effect of pH on dissolved oxygen in a chemostat. Brown and Zainudeen (1977)


FIGURE 1.7 Some effects of pH on fermentation processes.
It has been reported that the biological material experiences changes in its biochemical composition (Gaudy, 1975) when the cells undergo shock pH changes. See Figure 1.8

From the above information, the importance given to pH as a prime control variable is obvious. In the following section, some of the model proposed for pH effects are described.

1.3.2 Non-structured models with pH dependant functions.

The first to propose a kinetic model for pH effects on fermentation was Ibragimova et. al. (1969). It was a simple model derived from a non-competitive inhibition enzymic mechanism (see Appendix A) for the observed growth rate of Propionibacterium shermanii in lactate (see Figure 1.3, curve (3)).
It was concluded that the specific growth rate \( R = \frac{r_x}{C_x} \) would be described as:

\[
R_x = \mu_m \frac{K_H}{K_H + C_H}
\]  

or

\[
R_x = \mu_m \frac{K_{OH}}{K_{OH} + C_{OH}}
\]

(substrates were assumed to be at saturation levels), and that a single metabolic reaction (the one affected by H or OH ions) was the rate limiting step for the whole process.

There is sufficient evidence to show that the pH level affects the fermentation process in a complex way. The reduction of the whole process to a single metabolic reaction may seem risky from the biochemical and physiological point of view, but the important fact (for modelling considerations) is that such functions depict the observed results with good agreement. In fact, what is sought when modelling bell-shaped curves is a class of functions which present a maximum (or a minimum) over a given interval of the independent variable and, in addition, some symmetry.

For example, consider the following case for its simplicity. Figure 1.9 presents the data from Vollbrecht and Schlegel (1978). The particular Y-axis coordinate (maximum concentration of metabolite produced in g/l) would be incorporated in the model as a variable derived from the mass balance equations (see reference for particulars, no model was proposed). Note the asymptotic decrease of Y at the extremes of the pH range, the presence of a well defined maximum and the symmetry. The characteristics of the curve are very suggestive and have led some researchers to propose mechanisms which generate curves that behave in such a way.

Andreyeva and Biryukov (1973) listed some of the enzyme kinetic mechanisms in which inhibition by H and OH ions are considered and they discussed their feasibility for modelling pH dependant variables in fermentation. For example, the mechanisms:
Non-competitive inhibition mechanism (I inhibitor, E enzyme, S substrate)

(a larger list is in the Appendix A)

\[
\frac{R_X}{\mu_m} = \frac{C_S}{K_s + C_S} \frac{K_I}{K_I + C_{I}} \quad (1.51)
\]

will lead to an expression of the type:

\[
\frac{R_X}{\mu_m} = \frac{C_S}{K_s + C_S} \frac{K_I}{K_I + C_{I}} \quad (1.52)
\]

if I = H or OH ion, then ((H)(OH)) = 10^{-14}:

\[
\frac{R_X}{\mu_m} = \frac{C_S}{K_s + C_S} \frac{K_H}{K_H + C_H} \quad (1.53)
\]

or

\[
\frac{R_X}{\mu_m} = \frac{C_S}{K_s + C_S} \frac{K_{OH}}{K_{OH} + 1/C_H} \quad (1.54)
\]

As the possibility of inhibition by both H and OH ions exists, it can be found that non-competitive inhibition by H and OH ions mechanism will produce the expression:
What has recommended the use of such approximation in fermentation models are the mathematical properties of the functions \( \frac{K_H}{K_H + C_H} \) and \( \frac{K_{OH}}{K_{OH} + 1/C_H} \), and the fact that the product of these behaves, in a good approximation, as the curve showed in Figure 1.9. Figure 1.10 shows some bell-shaped curves generated by the function:

\[
F(C_H) = \frac{p}{p + C_H} \frac{q}{q + 1/C_H}
\]

in F-pH coordinates. (1.56)
FIGURE 1.10 The function $F(C_H)$ in $F - \text{pH}$ coordinates.

$$F(C_H) = \frac{p}{p + C_H} - \frac{q}{q + 1/C_H}$$

(1) $p = 10^{-2}$, $q = 10^6$

(2) $p = 10^{-8}$, $q = 10^6$

The use of two parameters allows mobility of the absolute value of $F$ and the location of the maximum throughout the operative pH range. The asymptotical behaviour at the extremes is evident and therefore the attraction of such mechanisms (it should be said, the attraction of such expressions) may seem irresistible.
It can be proposed that the variable $Y$ (maximum concentration of metabolite produced) may be expressed as:

$$
Y = \alpha \frac{p}{p + C_H} \frac{q}{q + 1/C_H}
$$

(1.57)

for some suitable values of $\alpha$, $p$ and $q$. But caution should be exercised when stating that this variable is influenced by a non-competitive inhibition by $H$ and OH ions mechanism simply by analogy.

The approach mentioned above, has been successfully used for the modelling of pH dependant variables. Some examples are cited below.


$$
R_x = \mu_m \frac{K_i}{K_i + C_i}
$$

(1.58)

$I = H$ or $OH$.

(b) Inhibition by substrate in high concentration (Haldane type inhibition). Mixed population in a digestive process. Andrews (1969).

$$
R_x = \frac{\mu_m}{1 + \frac{K_i}{C_i} + \frac{C_i}{K_i}}
$$

(1.59)

This model was proposed simultaneously (Feb 1969) with the model of Ibragimova et. al., (Equations 1.49 and 1.50) but no conclusive evidence was presented.

\[
q = \frac{K_1 C_s}{K_2 (1 + K_3/C_H + C_s/K_4) + C_s (1 + K_5/C_H + C_s/K_6)}
\]

\[1.60\]

\(q\) : respiration intensity \((\mu l O_2/ml/hr)\).


\[
R_x = \frac{\mu m C_s}{K_s + C_s + C_s^2/K_1 (1 + K_3/C_H)} \frac{1}{1 + K_2/C_H + C_s/K_1}
\]

\[1.61\]


\[
R_x = \frac{\mu m}{K_{1H} + K_{1OH} + 1/C_H} \frac{K_{1OH}}{K_{1OH} + 1/C_H}
\]

\[1.62\]

\[
q = q_{max} \frac{K_{2H}}{K_{2H} + C_H} \frac{K_{2OH}}{K_{2OH} + 1/C_H}
\]

\[1.62\]

\(q\) : rate of antibiotic production.

In 1973 Muzychenko et al., proposed a different approach for the modelling of pH dependant functions, involving concepts of the transport phenomena through the cell membrane. The model is a combination of passive transport (diffusion described by Fick's law) and resistance due to the energetic barrier of the cell surface caused by ionisation of acidic and basic groups present in the pore structure of the membrane.
The model was stated as follows:

(a) The density of the cell surface charge ($\delta$) is a function of pH, derived from the dissociation constants of basic and acidic groups. This charge density is uniformly distributed along the pores of the membrane.

$$\delta = \delta (pH) \quad (1.64)$$

(b) An electrical potential ($\varphi$), caused by the charges, is present at each point of the pore, being a function of the charge density and geometric characteristics of the pore such as diameter and length.

$$\varphi = \varphi (\delta) = f(pH) \quad (1.65)$$

(c) This electrical potential increases the energetic requirements for transport through the membrane pores, and it can be expressed in the diffusivity coefficient:

$$D_{av} = D_0 \exp (- a \varphi/RT) = D_0 \exp (- b f(pH)/RT) \quad (1.66)$$

$D_{av}$: the average value of diffusivity for the transport in question.

$D_0$, $a$, $b$ : constants.

(d) Finally, applying Fick's law and a mass balance over the cell:

$$\frac{dC_x}{dt} = \mu m C_x C_{sl}/(K_s + C_{sl}) \quad (1.67)$$

$$\frac{dC_{sl}}{dt} = \frac{3}{2} D_{av} C_x (C_s - C_{sl})/(\lambda r) - \alpha \mu m C_x \frac{C_{sl}}{K_s + C_{sl}} \quad (1.68)$$

where:

$C_s$ : substrate concentration in the extracellular medium.

$C_{sl}$ : substrate concentration within the cell.

$C_x$ : cell concentration.
\( K_s \): saturation constant for \( S_1 \) (Monod kinetics)

\( \lambda \): thickness of diffusion layer.

\( r \): cell radius.

\( \alpha \): stoichiometric coefficient.

For no accumulation of substrate in the cell, i.e.,

\[
\frac{dC_{sl}}{dt} = 0:
\]

\[
K_sC_s + \left( C_s - \frac{K_s}{3D_{av}} \right) C_{sl}^2 = 0 \quad (1.69)
\]

As \( D_{av} \) is a function of pH and for constant values of \( C_s, C_{sl}, \lambda, r \) and \( \alpha \), the above expression can be given as:

\[
G(K_s, \mu_m, pH) = 0 \quad (1.70)
\]

or

\[
K_s = g_1(\mu_m, pH) \quad (1.71)
\]

or

\[
\mu_m = g_2(K_s, pH) \quad (1.72)
\]

Graphs for \( K_s \) (at constant \( \mu_m \)) and \( \mu_m \) (at constant \( K_s \)) were produced as function of pH. These are shown in Figure 1.11.

It must be pointed out that the model proposed by Andrews (1969), Equation (1.59), was elucidated from concepts based on membrane permeability to substrates in their ionised and un-ionised forms, from which the analogy with the Haldane function was used.

In addition to the models mentioned above, some empirical relationships have been found to describe the pH effect on the
FIGURE 1.11 Dependence of $\mu_m$ and $K_s$ on the change of pH of the medium, Candida tropicalis, Muzychenko et al. (1973).

fermentation processes. Some examples are given below.

(a) Penicillin production, Andreyeva and Biryukov (1973).

$$q_a = a_0 + a_1(pH) + a_2(pH)^2 \quad (1.73)$$

$q_a$: rate of penicillin production.
(b) Linear relationship between $\mu_m$ and $C_H$. Brown and Halsted (1975).

$$
\mu_m = a - b C_H,
$$

(1.74)
a and b are positive constants.

(c) Saturation constant and endogeneous respiration coefficient for Trichoderma viride in glucose. Brown and Zainudeen (1977).

$$
K_s = a_1 - b_1 C_H,
$$

(1.75)

$$
k_e = a_2 - b_2 C_H,
$$

(1.76)
a_1, a_2, b_1 and b_2 are positive constants.

(d) Rate of cell deactivation constant ($k_d$) and stoichiometric coefficient for citric acid formation. Kristiansen and Sinclair (1979).

$$
k_d = a - b(pH),
$$

(1.77)

$$
\alpha = \exp(c(pH - d)),
$$

(1.78)

$\alpha$: stoichiometric coefficient for citric acid formation.
a, b, c and d are positive constants.

Other model parameters affected by pH have been reported, yet the models do not incorporated pH effects. See Figures 1.12 and 1.13.
FIGURE 1.12 Effect of pH on the stoichiometric coefficient $\alpha$ and rate constant $\beta$, of the product formation model:

$$r_p = \alpha r_x + \beta C_x$$

Graph calculated from the data of Aiyar and Luedking (1966).

FIGURE 1.13 Dependence of $\mu_m$ on pH. Pirt and Callow (1960).
1.4 Non-linear parameter estimation in process models.

The breakthrough of multiparameter models in the fermentation field is long due to the improvement of numerical algorithms and the facilities of current computer systems. Optimisation techniques are becoming a common procedure in parameter evaluation and the current literature is rich in examples developed in the last ten years. Only the necessary background is mentioned here.

Optimisation represents simply a "fit-the-data-" procedure. The fitting equation can be an abstract expression or a specific model which may have a physical meaning. Among the desirable properties of a fitting equation are (Edwards and Wilke, 1968):

(a) Sufficient flexibility to fit many types of data without introducing distortion.
(b) At least some of the fittable parameters have a direct physical meaning.
(c) Continuously differentiable with explicit derivatives with respect to the independent variables.
(d) Parameters easily evaluated.
(e) Easy to use once parameters have been determined.

The optimisation procedure for the estimation of parameters of a process model can be stated as follows:

Let a process model with one dependent variable \( Y \) (measurable), one independent variable \( t \) (usually set to a desired value) and \( np \) (np: number of parameters) parameters \( x_i \), \( i = 1, np \) or \( \mathbf{X} = (x_1, x_2, \ldots, x_np) \) be described by the model structure equation:
\( F(Y,t) = 0 \) \hspace{1cm} (1.79)

with or without the following constraints:

\[ C_j(Y,t_0) = 0, \quad j = 1, nc \] \hspace{1cm} (1.80)

\( nc \) : number of constraints.

and with solution:

\[ Y_t = f(X,t) \] \hspace{1cm} (1.81)

and with \( nep \) (nep: number of experimental points) experimental observations \( Y_{ei} \) \((i = 1, nep)\), corresponding to the values \( t_i \).

The estimation of \( X \) problem or minimisation problem can be stated as follows:

Minimise \( J(X) = J(f(X,t_i), Y_{ei}) \quad i = 1,nep \) \hspace{1cm} (1.82)

subjected to \( npc \) (npc: number of parameter constraints) constraints:

\[ P_{ci}(X) = 0 \quad i = 1, npc \] \hspace{1cm} (1.83)

The value of \( X \) at the minimum of \( J(X) \) is referred to as the best estimated of \( X \) for the observed data. The value of \( J(X) \) at the minimum has been called "goodness of fitting", "performance index", "sum of residuals", among other terms depending on how the function \( J(X) \), which is called "objective function" or "criterion function", is stated.
1.4.1 Objective functions in optimisation techniques.

The optimisation technique in parameter estimation requires the statement of an objective function in terms of the parameters. Some forms of the objective function are summarised below. (From Bekey and Yamashiro, 1976).

1. \[ J(X) = \sum_{i=1}^{np} (x_i - \alpha_i)^2 \] (1.84)

where \( x_i \) are the estimated value of the parameters and \( \alpha_i \) are the true values. Obviously these true values are rarely known.

2. \[ J(X) = \int_{t_0}^{t_r} (Y_t - Y_e)^T R^{-1}(Y_t - Y_e) \, dt \] (1.85)

where:
- \( Y_t(t) \): theoretical values of the dependant variable
- \( Y_e(t) \): experimental values of the dependant variable.
- \( t_0, t_r \): low and upper limits of the dependant variable between which the event occurs.
- \( R^{-1} \): inverse of a weighing matrix \( R \), usually the variance-covariance matrix of observed values.
- \( X \): the parameter vector.
- \( T \): stands for the transpose of a vector or matrix.

3. \[ J(X) = \sum_{i=1}^{nep} (Y_{ti} - Y_{ei})^T R^{-1}(Y_{ti} - Y_{ei}) \] (1.86)

The discrete version of the function described above, with the \( i \) subscript corresponding to the \( i \)th observation.
Equation (1.86) is commonly written as:

4. \[ J(X) = \sum_{i=1}^{nep} w^2_i (Y_{ti} - Y_{ei})^2 \] \hspace{1cm} (1.87)

where \( w^2_i, i = 1, nep \), are the components of the main diagonal of the weighing matrix and the other components are neglected. Equation (1.87) can be further reduced to:

5. \[ J(X) = \sum_{i=1}^{nep} (Y_{ti} - Y_{ei})^2 = \sum_{i=1}^{nep} e_i^T e_i \] \hspace{1cm} (1.89)

where: \( e_i = Y_{ti} - Y_{ei} \), termed output error (difference between model and experimental system responses.

\( e_i^T \): transpose of \( e_i \)

6. A more complex objective function is given by:

\[ J(X) = \int_{t_o}^{t_r} (e_1^2 + w^2 e_2^2)dt \] \hspace{1cm} (1.90)

where: \( e_1 = F(Y_t, \hat{X}, t) - F(Y_t, \alpha, t) \)

\( F \) is the model structure equation defined by Equation (1.79).

\( \hat{X} \): parameter estimation.

\( \alpha \): parameter true values.

\( Y_t \): theoretical value of the dependant variable.

\( t \): dependant variable.
\[ e_2 = Y_t(t) - Y_e(t) \] difference between theoretical and experimental values of the dependant variable.

\[ w^2 : \text{weighing factor.} \]

\[ J(X) = \sum_{i=1}^{nep} (e_{1i}^2 + w_i e_{2i}^2) \quad (1.91) \]

The discrete version of the previous function.

When the process model contains more than one dependant variable (and/or independent variables) the optimisation problem becomes simply the optimisation of \( \text{nep} \) (\( \text{nep} \): number of experimental variables) functions (Marquardt, 1963):

\[ J_i(X), i = 1, \text{nep}. \]

The minimisation procedure can be performed independently for each variable, therefore, the values of \( X \) obtained in each minimisation may differ significantly (Hunter, 1967; Johnson and Berthouex, 1975). To deal with such cases, some objective functions, termed "multiresponse functions" have been proposed, these are:

\[ J(X) = \sum_{i=1}^{\text{nep}} \sum_{j=1}^{\text{nev}} (Y_{tij} - Y_{eij})^2 \quad (1.92) \]

Combined sum of squares:

- \( \text{nev} \): number of dependant variables.
- \( \text{nep} \): number of experimental points.
9. \[ J(X) = \sum_{i=1}^{n_{ep}} \sum_{j=1}^{n_{ev}} w_{ij}^2 (Y_{tij} - Y_{eij})^2 \] (1.93)

Combined sum of squares weighted by response, \( w_{ij}^2 \) are weighing factors.

10. \[ J(X) = \left| \sum_{i=1}^{n_{ep}} (Y_{tij} - Y_{eij})(Y_{tik} - Y_{eik}) \right| \] (1.94)

\( j, k = 1, n_{ev} \) Determinant criterion.

The theoretical basis for the validity of Equations (1.92) and (1.93) were demonstrated by Hunter (1967) and the determinant criterion by Box and Draper (1965).

1.4.2 The solution of the optimisation problem.

Although the general theory for the solution of the linear optimisation problem stated in Equation (1.82) was developed by Gauss in 1809 (Deutsch, 1965), the solution of the non-linear problem constitutes another breakthrough in the field of applied sciences. Some of the algorithms used for the solution of the optimisation problem are described below. (From Bekey and Yamashiro, 1976).

1. Exhaustive enumeration.

Computation of \( J(X) \) for all possible values of \( X \) around the region in which the minimum is suspected to exist and choose
the value of $\mathbf{X}$ which produces the lowest value of $J(\mathbf{X})$. Although impractical for large dimensions of $\mathbf{X}$, the peculiarities of the problem do not cause failure in the algorithm. In addition, it allows the detection of several local minima, if any, around the solution.

2. Random search.

This is a variation of the exhaustive enumeration method in which random values of $\mathbf{X}$ are used to compute $J(\mathbf{X})$; its application is valuable when the bounds of $\mathbf{X}$ are extremely large and would be useful to detect possible starting points for a more complicated algorithm in which the initial estimate of $\mathbf{X}$ is critical.

3. Relaxation method.

This is the general name of various climbing and descending methods whose main feature is the searching for the local minimum (or maximum) along one parameter axis and when located, continuing the search along the second parameter and so forth. The disadvantage of this method consists mainly in the fact that the search will stop if there is a narrow valley in the criterion function surface.


This is a descendant procedure similar to the relaxation method in which each parameter is perturbed from the starting point only by a fixed increment ($\Delta x_i$) and the function $J(\mathbf{X})$ is computed at each increment value. The point at which the minimum is observed is selected as the next starting point. The search will stop if a narrow valley is found.
The following algorithms are generally known as gradient methods due to the use of the information provided by the gradient of the function \( J(X) \), i.e., \( \text{grad}(J(X)) \).

5. Steepest descent.

A classical algorithm where the search of the minimum consists of two steps:

\[
\begin{align*}
\text{(a)} & \quad \Delta X^i = -K \text{grad}(J(X^i)) \quad \text{(1.95)} \\
\text{(b)} & \quad X^{i+1} = X^i + \Delta X^i \quad \text{(1.96)}
\end{align*}
\]

where \( K \) is a constant scalar matrix \( K = kI \), \( k \) is a real positive number and \( I \) is the identity matrix. Implementation of the method consists of the selection of \( k \) and the practical evaluation of \( \text{grad}(J(X)) \).


It is an algorithm derived from an analytical basis in conjunction with the Newton-Raphson algorithm for numerical solution of algebraic equations. It consists of two steps:

\[
\begin{align*}
\text{Solve} & \quad H(X^i)(\Delta X^i) = -\text{grad}(J(X^i)) \quad \text{(1.97)} \\
\text{then} & \quad X^{i+1} = X^i + \Delta X^i \quad \text{(1.98)}
\end{align*}
\]

where \( H(X^i) \) is the Hessian matrix of \( J(X^i) \) (see Appendix B1). When \( J(X) \) is the simple sum of squares (or integral) defined by Equation (1.89), the Newton algorithm is called Newton-Gauss algorithm.

It is the same as the Newton algorithm but the Hessian matrix $H(\mathbf{x}^i)$ is approximated to:

$$
H(\mathbf{x}^i) = \left[ \frac{J(\mathbf{x}^i)}{\text{grad}(J(\mathbf{x}^i))^T \text{grad}(J(\mathbf{x}^i))} \right]^{-1}
$$

(1.98)

Modifications and improvements to the gradient methods can be found in the literature (Marquardt, 1963; Fletcher, 1969).

1.5 Objectives of this work.

The pH is, without doubt, one of the most important variables in a fermentation process. As it was shown in Section 1.3, the effects of pH are extensive and complex and it has been shown (Spitzer, 1976) that the quantitative knowledge of such effects has a great potential in process control and design. Based on the information summarised in this Chapter, the following objectives are intended:

Overall Objective: Development of a method to acquire quantitative information about pH effects in a fermentation system for modelling purposes, which can be used in process control and design in agreement with the following objectives:
1. Development of a method to estimate model structure and parameter values in a multiparameter non-structured kinetic model which presents non-linear characteristics in both variables and parameters, using optimisation techniques. This includes the development of a suitable objective function such that:

(a) the model structure can be determined.
(b) the value of the parameters of the model can be estimated.
(c) the solution of the optimisation problem can be carried out using current algorithms available in standard computer libraries.

2. Test of the method developed in objective 1 by application to the modelling of batch and continuous cultures under double substrate limiting conditions at several constant pH levels.

3. Development of a theoretical discussion of possible enzyme-like kinetic mechanisms to discriminate feasible models in which pH affects the kinetic constants of the growth rate equation.

4. Development of a non-mechanistic model for pH effects on:

(a) growth associated reaction parameters such as stoichiometric coefficients for substrate consumption and product formation,
(b) cell concentration associated reaction parameters such as endogeneous respiration and product formation rate constants.
As it was stated in Section 1.5, the overall objective of this work is the development of a method to obtain information from a fermentation system for modelling purposes using optimisation techniques. The optimisation approach has been previously used in fermentation systems with a relatively low number of parameters (Edwards and Wilke, 1968; Constantinides et al., 1970; Johnson and Berthouex, 1975; Nihtila and Virkkunen, 1977). The assessment of multiparameter models can be carried out within a reasonable time if a suitable technique for parameter evaluation is available without diverting the time saved in experimental work to often tedious or complicated mathematical work. In Section 2.1 the theoretical basis for such a method are discussed.

To examine the scope and limitation of the method mentioned above, a double substrate kinetic non-structured model for continuous and batch cultures is investigated and its fundamentals are stated in Section 2.2.

Application of the mathematical method to the study of the double substrate kinetic model permits the examination of the pH effects on the culture. The pH has been chosen as the variable to study due to its important effects on fermentation and the benefits that can be obtained by a quantitative knowledge of such effects. In Section 2.3 a discussion of the enzyme-like kinetic expressions for the growth rate is developed in order to clarify some bases for modelling of growth rate with pH dependant
functions. In Section 2.4 a non-mechanistic model for pH effects on stoichiometric coefficients and rate constants different from the growth rate constants (such as product formation and endogeneous respiration coefficients) is proposed.

2.1 Optimisation approach for the modelling of multiparameter kinetic processes in fermentation systems.

The optimisation approach for modelling consists of two steps:

(a) Choice a suitable objective function.
(b) Solve the minimisation problem stated by Equation (1.82).

Before carrying on with the statement of the optimisation approach, two terms need to be defined as follows:

1. Model constitutive equations (MCE). These are the mathematical expressions for the conception of the kinetic model. The MCE are independant of the reactor configuration and represent the kinetic phenomenon in terms of the local value of the variables involved.

2. Model structure equations (MSE). These are the expressions derived from application of the conservation principles (mass, energy, momentum, etc.) considering reactor configuration, the MCE and other phenomena that might be involved.

For instance, the mathematical conception of a first order reaction produces the constitutive equation:

\[ r = k C \]  

(2.1)

where: \( r \) : rate of reaction,
\( k \) : constant.
C : concentration of chemical species.

Application of this MCE to a mass balance and/or energy balance to any reactor configuration such as STCR, tubular reactor, etc., produces a set of equations which constitutes the MSE.

2.1.1 Objective function for one-response system.

Consider the kinetic process which can be described by the MCE given by:

\[ Z(\mathbf{x}, Y, t) = 0 \quad (2.2) \]

where:
- \( \mathbf{x} \) : parameter vector \( \text{dim}(\mathbf{x}) = np \)
- \( t \) : independent variable vector \( \text{dim}(t) = niv \)
- \( Y \) : dependent variable or system response \( \text{dim}(Y) = 1 \)

which produces the MSE when a conservation principle is applied to a given reactor configuration:

\[ F(\mathbf{x}, Y, t) = 0 \quad (2.3) \]

For simplicity it is assumed that the MSE can be expressed in terms of the parameter vector \( \mathbf{x} \), system response \( Y \) and independent variable vector \( t \). Solution of Equation (2.3) produces:

\[ Y_t = f(\mathbf{x}, t) \quad (2.4) \]

either analytically or numerically calculated.

Let \( Y_e \) and \( \hat{X} \) be the experimental response of the system and the estimated value of the parameter vector respectively. If the MCE has been proved to apply in the system under study, then
the problem of modelling becomes simply the estimation of the parameter vector, in which case, objective functions of the type defined by Equations (1.86) to (1.89) can be used. Otherwise a better objective function is that given by Equation (1.90), i.e.,

\[ J(X) = \int_{t_0}^{t_r} (e_1^2 + w_2 e_2^2) dt \]  

(2.5)

or its discrete equivalent:

\[ J(X) = \sum_{i=1}^{nep} (e_{1i}^2 + w_2 e_{2i}^2) \]  

(2.6)

where: nep : number of experimental observations.

As defined in Section 1.4.2 (Equation (1.90)), \( e_1 \) describes the deviation from the model structure and \( e_2 \) describes the deviation caused by experimental error:

\[ e_1 = F(X,Y^*,t) - F(\theta,Y^*,t) \]  

(2.7)

\[ e_2 = (Y_t - Y_e) \]  

(2.8)

Equations (2.6) and (2.7) involve the value \( Y_t \) (theoretical value of the system response) which implies the solution of Equation (2.3); the algorithm to solve the minimisation problem will have a degree of complexity depending upon the solution of Equation (2.3) which may be differential or algebraic with an analytical or a numerical solution.

2.1.2 Objective function with model structure equations (MSE) only.

The following objective function is proposed to
solve the optimisation problem of modelling:

\[ J(X) = \sum_{i=1}^{\text{nep}} \left[ F(X, Y_{ei}, t_i) - F(\alpha, Y_{ti}, t_i) \right]^2 \]  

(2.9)

It can be deduced from Equation (2.9) that:

\[ F(\alpha, Y_{ti}, t_i) = 0 \]  

(2.10)

for the true values of parameter vector \( \alpha \), and theoretical solution of the MSE \( Y_{ti} \), therefore, equation (2.9) is further reduced to:

\[ J(X) = \sum_{i=1}^{\text{nep}} \left[ F(X, Y_{ei}, t_i) \right]^2 \]  

(2.11)

The term \( F(X, Y_{ei}, t_i) \) considers the deviation from the model structure as well as the error involved in the experimental values of the dependant variable. The objective function defined by Equation (2.11) has the following advantages over that defined by Equation (2.6):

i) only the functional form of the MSE is required.

ii) does not involve \( Y_{t} \) and therefore the solution of the MSE is not required by the optimisation procedure.

iii) the MSE can be rearranged algebraically to produce a "better behaved" objective function, e.g. to reduce the degree of "non-linearity".

Two algorithms are described in order to compare the degree of difficulty to solve the optimisation problem using the objective functions described by Equations (2.6) and (2.11). For Equation (2.6), numerical solution of the MSE is assumed (a common occurrence in fermentation systems) and the algorithms
considered are the steepest descent (Section 1.4.2, Equations (1.95) and (1.96)) and the Newton algorithm (Section 1.4.2, Equations (1.97) and (1.98)).

2.1.3 The solution of the optimisation problem.

2.1.3.1 Steepest descent algorithm.

The steepest descent algorithm involves the iterative loop:

\[ \Delta \mathbf{x}^j = - K \text{grad}(J(\mathbf{x}^j)) \]
\[ \mathbf{x}^{j+1} = \mathbf{x}^j + \Delta \mathbf{x}^j \] (2.11)

which stops when the difference between two successive values of \[ \mathbf{x}^j \]
is less or equal to a given tolerance criterion. The algorithm calculates \[ \text{grad}(J(\mathbf{x})) \] as many times as the iterative loop is repeated and should have an additional control for the size of \[ K = kI, \] (\[ I \] being the identity matrix) to accelerate the convergence towards a possible solution.

For the objective function given by Equation (2.6), the gradient of \[ J(\mathbf{x}) \] is given by:

\[ \text{grad}(J(\mathbf{x})) = \left( \frac{\partial J}{\partial x_1}, \frac{\partial J}{\partial x_2}, \ldots, \frac{\partial J}{\partial x_{np}} \right) \] (2.12)

where:

\[ \frac{\partial J}{\partial x_j} = \sum_{i=1}^{\text{nep}} 2( e_{i1} \frac{\partial e_{1i}}{\partial x_j} + w_{i21} \frac{\partial e_{2i}}{\partial x_j} ) \] (2.13)
As $e_1$ and $e_2$ are functions of $Y_t$ (see Equations (2.7) and (2.8)), the gradient of $J(X)$ does involve the partial derivatives of $Y_t$ with respect to the parameter vector and in the case in which the MSE does not have analytical solution, a numerical approach must be used to solve it and from there onwards a numerical calculation for the gradient of $J(X)$ must be performed.

In the case of the objective function defined by Equation (2.11), the components of the gradient of $J(X)$ are given by:

$$
\frac{\partial J}{\partial x_j} = \sum_{i=1}^{nep} \left( 2 \prod F(x_i, y_i, t_i) \right) \quad (2.14)
$$

and to calculate such partial derivatives, the solution of the MSE is not required. Figures 2.1 and 2.2 show the flow charts for the steepest descent algorithm for the minimisation of the objective functions given by Equations (2.6) and (2.11).

2.1.3.1 Newton algorithm.

The Newton algorithm consists of the iterative loop:

1. Solve $H(X^j) \Delta X^j = -\text{grad}(J(X^j))$ for $\Delta X^j$

2. $X^{j+1} = X^j + \Delta X^j$

which stops when a given tolerance criterion is satisfied. The algorithm involves the evaluation of the gradient of $J(X)$ and in addition that of the Hessian matrix $H(X)$ which is given by:
FIGURE 2.1 Flow chart for the optimisation of the objective function:

\[ J(X) = \sum_{i=1}^{\text{neq}} (e_{1i}^2 + w_{1i}^2 e_{2i}^2) \] 

by a Steepest descent algorithm.
**FIGURE 2.2** Flow chart for the optimisation of the objective function:

\[ J(X) = \sum_{i=1}^{nep} \left( F(X, Y_{ei}, t_i) \right)^2 \]

by a Steepest descent algorithm.
\[ H(X) = \begin{bmatrix} \frac{\partial^2 J(X)}{\partial x_j \partial x_k} \end{bmatrix} \quad j,k = 1, n_p \]  

(2.15)

np: number of parameters.

For the objective function given by Equation (2.6) the components of the Hessian matrix are given by:

\[
\frac{\partial^2 J(X)}{\partial x_j \partial x_k} = \sum_{i=1}^{\text{nep}} 2 \left[ e_{1i} \frac{\partial^2 e_{1i}}{\partial x_j \partial x_k} + e_{1i} \frac{\partial e_{1i}}{\partial x_j} \frac{\partial e_{1i}}{\partial x_k} + \right. \\
\left. e_{2i} \frac{\partial^2 e_{2i}}{\partial x_j \partial x_k} + e_{2i} \frac{\partial e_{2i}}{\partial x_j} \frac{\partial e_{2i}}{\partial x_k} \right] 
\]

(2.16)

and for the objective function given by equation (2.11), the Hessian matrix components are given by:

\[
\frac{\partial^2 J(X)}{\partial x_j \partial x_k} = \sum_{i=1}^{\text{nep}} 2 \left( F_i \frac{\partial^2 F_i}{\partial x_j \partial x_k} + \frac{\partial F_i}{\partial x_j} \frac{\partial F_i}{\partial x_k} \right) 
\]

(2.17)

where \( F_i = F(X, Y_{e1}, t_i) \)

The evaluation of the Hessian matrix requires a numerical approach in the former case (Equation (2.16)). Figures 2.3 and 2.4 show the flow charts for the optimisation procedure by the Newton algorithm for both objective functions.
Flow chart for the optimisation of the objective function:

\[ J(X) = \sum_{i=1}^{nep} (e_{1i}^2 + w_i e_{2i}^2) \]

by a Newton algorithm.
FIGURE 2.4 Flow chart for the optimisation of the objective function:

\[
J(X) = \sum_{i=1}^{\text{nep}} \left[ F(X, Y_{ei}, t_i) \right]^2
\]

by the Newton algorithm.
2.1.4 Multiresponse objective functions with model structure equations only.

The objective function proposed in the preceding Section was developed for a system with one response only. The multiresponse objective functions have provided (Hunter, 1967; Johnson and Berthouex, 1975) a better criterion in optimisation. The statement of a multiresponse objective function which involves only the model structure equations is as follows:

Let a kinetic process be described by the MSE given by:

\[ F(\alpha, \gamma, t) = 0 \]  \hspace{1cm} (2.17)

where:
- \( \alpha \): parameter vector \hspace{1cm} \text{dim}(\alpha) = np
- \( t \): independent variable vector \hspace{1cm} \text{dim}(t) = niv
- \( \gamma \): dependent variable vector \hspace{1cm} \text{dim}(\gamma) = nev
- \( F \): MSE vector \hspace{1cm} \text{dim}(F) = nev

The difference between the one-response system described by Equation (2.3) and the multiresponse system defined by Equation (2.17) is that the dependent variable vector has dimension higher than one and instead of being described by one equation, it is described by a set of equations represented by the model structure equation vector \( F \).

The multiresponse objective function is given by:

\[ J(X) = \sum_{i=1}^{nep} \sum_{j=1}^{nev} \left[ F_j(X, \gamma_{\text{eq}}, t_i) \right]^2 \]  \hspace{1cm} (2.18)
where: nep : number of experimental points
nev : number of dependant variables (system responses)

The multiresponse objective function given by Equation (2.18) corresponds to a straightforward generalisation of the one-response objective function defined by Equation (2.11). This multiresponse objective function can be compared with those described in Section 1.4.2 (Equations (1.92) and (1.93)).

For an objective function such as that defined by Equation (2.18) in which first and second partial derivatives of \( J(X) \) with respect to the parameters are analytical, a Newton algorithm for the solution of the optimisation problem is recommended. Although a steepest descent algorithm can be used, it must be taken into account that such an algorithm will stop if a narrow valley is found in the objective function values.

2.2 Kinetic model for growth.

A double substrate limited growth model is proposed for the microbial growth, with glucose and ammonium ion as the limiting substrates, i.e.:

\[
x = \int_0^\infty C_x F(C_g)G(C_n) \]

where \( F \) and \( G \) are the functional forms for the substrates glucose \( (C_g) \) and ammonium ion \( (C_n) \) concentration. Monod and Contois kinetics are proposed, i.e.:
for Monod kinetics:

\[ F(C_g) = \frac{C_g}{K + C_g} \]

\[ G(C_n) = \frac{C_n}{K + C_n} \]  

(2.20)

for Contois kinetics:

\[ F(C_g) = \frac{C_g}{B C_g + C_g} \]

\[ G(C_n) = \frac{C_n}{B C_n + C_n} \]  

(2.21)

Endogeneous respiration is taken into account in the form proposed by Herbert(1958):

\[ r_e = k_e C_x \]  

(2.22)

where \( r_e \) is the rate of cell material consumption by endogeneous respiration and \( k_e \) is the endogeneous respiration coefficient.

Growth associated substrate consumption (Monod, 1949) is assumed:

\[ r_g = -m_g r_g \]  

(2.23)

\[ r_n = -m_n r_n \]  

(2.24)

where \( r_g \) and \( r_n \) are the rate of glucose and ammonium consumption and \( m_g \) and \( m_n \) are the respective stoichiometric coefficients.

The parameters involved in the model which are pH dependant are:
\[ m = M_m(pH) \] Maximum specific growth rate.
\[ K_g = K_g(pH) \] Monod saturation constant for glucose.
\[ K_n = K_n(pH) \] Monod saturation constant for ammonium.
\[ B_g = B_g(pH) \] Contois constant for glucose.
\[ B_n = B_n(pH) \] Contois constant for ammonium ion.
\[ k_e = k_e(pH) \] Endogeneous respiration constant.
\[ m_g = m_g(pH) \] Stoichiometric coefficient for glucose.
\[ m_n = m_n(pH) \] Stoichiometric coefficient for ammonium.

At constant pH the parameters have a constant value which can be estimated without consideration of their functional pH dependence.

2.2.1 Model structure equations.

The model structure equations (MSE) are developed here taking into account only the kinetic process. Mass transfer limitation are not considered.

2.2.1.1 Model structure equation for Continuous Culture.

For a continuous culture in an ideal stirred tank fermentor with sterile feed, the mass balance equations are:

For biomass:

\[ \frac{dC_x}{dt} = r_x - k_e C_x - DC_x \] (2.25)
For glucose:
\[
\frac{dC_g}{dt} = D(C_{gF} - C_g) - \frac{m_r}{V} \quad (2.26)
\]

For ammonium ion:
\[
\frac{dC_n}{dt} = D(C_{nF} - C_n) - \frac{m_r}{V} \quad (2.27)
\]

where \( D \) is the dilution rate, \( t \) is time, \( C_{gF} \) and \( C_{nF} \) are the feed concentrations of glucose and ammonium ion respectively.

At steady state:
\[
\begin{align*}
    r_x - (D + k_e)C_x &= 0 \\
    D(C_{gF} - C_g) - \frac{m_r}{V} &= 0 \\
    D(C_{nF} - C_n) - \frac{m_r}{V} &= 0
\end{align*}
\quad (2.28)
\]

The system represented by Equation (2.28) can be expressed as a set of implicit functions \( C_1, C_2 \) and \( C_3 \) given by:
\[
\begin{align*}
    C_1(C_x, C_g, C_n, D, C_{gF}, C_{nF}, X_1, X_2, \mu_m, k_e, m, m) &= 0 \\
    C_2(C_x, C_g, C_n, D, C_{gF}, C_{nF}, X_1, X_2, \mu_m, k_e, m, m) &= 0 \\
    C_3(C_x, C_g, C_n, D, C_{gF}, C_{nF}, X_1, X_2, \mu_m, k_e, m, m) &= 0
\end{align*}
\quad (2.29)
\]

where \( X_1 \) is either \( K_1 \) or \( B_1 \) and \( X_2 \) is either \( K_2 \) or \( B_2 \) depending on the type of function, Monod or Contois, included in the model.
The system (2.29) represents the model structure equations (MSE) for a continuous culture at steady state. Solution of such a system, for \( C_x, C_g \) and \( C_n \), produces expressions of the type (Appendix B4):

\[
\begin{align*}
C_x &= C_x(C_{gF}, C_{nF}, D, X_1, X_2, \mu_m, k_e, m, n) \\
C_g &= C_g(C_{gF}, C_{nF}, D, X_1, X_2, \mu_m, k_e, m, n) \\
C_n &= C_n(C_{gF}, C_{nF}, D, X_1, X_2, \mu_m, k_e, m, n)
\end{align*}
\]  

(2.30)

2.2.1.2 Model structure equations for Batch Culture.

For a batch culture, in an ideal stirred tank fermentor, the mass balance equations are:

For biomass:

\[
\frac{dC_x}{dt} = r_x - k_e x
\]

For glucose:

\[
\frac{dC_g}{dt} = -m_{rx}
\]  

(2.32)

For ammonium:

\[
\frac{dC_n}{dt} = -m_{nx}
\]

with initial conditions: \( C_x(t_o) = C_x^0 \), \( C_g(t_o) = C_g^0 \) and \( C_n(t_o) = C_n^0 \). \( C_x^0, C_g^0 \) and \( C_n^0 \) being the concentrations at time \( t_o \).

The model structure equations for batch culture can then be expressed as:
where \( C_*, C'_t \) and \( C'_n \) stand for the first derivatives of \( C_x, C_g \) and \( C_n \) with respect to time.

The solution of the MSE (if possible) produces expressions of the type:

\[
\begin{align*}
C_x(C'_t, C'_x, C'_g, C'_n, X_1, X_2, \mu, k_e, m_g, m_n) &= 0 \\
C_g(C'_t, C'_x, C'_g, C'_n, X_1, X_2, \mu, k_e, m_g, m_n) &= 0 \\
C_n(C'_t, C'_x, C'_g, C'_n, X_1, X_2, \mu, k_e, m_g, m_n) &= 0
\end{align*}
\]

(2.34)

The evaluation of the parameters of the model and its validity are discussed in Chapters 4 and 5.

2.3 Analysis of enzyme kinetic models for pH effects.

Since the review of Andreyeva and Biryukov (1973) dealing with feasible enzyme kinetic mechanisms for modelling fermentation processes with pH effects, no new model has been proposed with exception of Muzychenko's model (1973) which has provided the best attempt to divert modelling from enzyme kinetics concepts.

So far, only the growth rate of cells has been modelled by analogy with enzyme kinetics and usually the expressions describing growth has been related to other variables, such as utilisation of nutrients and product formation, simply by multiplying the growth rate expression by the appropriate constant.
Such an approach suggests that this form of modelling could well be used to describe many of the observed effects of pH on fermentation.

Despite the relative success of enzyme-like kinetic models in modelling pH effects in fermentation, the following observations must be taken into account. The thickening of cell wall at extreme pH values reported by Lirova et al. (1978b) suggests that the metabolic activity is diverted to cell wall material synthesis to the detriment of some other constituents of the cell. This would cause a change in the chemical composition of the cellular material, as reported by Gaudy (1975) and the requirements for synthesis of proteins, carbohydrate and nucleic acid will change.

Thickening of cell wall increases the physical barrier that the cellular membrane represents to the transport of nutrients and metabolites. This fact also suggests that the intracellular pH must be kept in a certain range out of which disruption of the whole cell organisation occurs. Therefore, in order to keep the pH within the allowed range, the cell must increase its wall thickness to prevent strong ionic exchange.

The above suggestions are in direct opposition to the assumptions of Muzychenko (1973) who suggested that an extreme change of the extracellular pH would affect the intracellular pH to an insignificant extent. The change of the internal cellular structure at extreme pH values reported by Lirova et al (1978b), additionally suggests that not only the transport of nutrients and metabolites through the cell membrane is strongly affected but the whole metabolic network also.

The ionisation of acidic and basic groups mentioned by Muzychenko (1973) contributes to the modification of the fermentation process; whether this phenomenon affects the fermentation in the way suggested by Muzychenko (see Section 1.3.2, Equation 1.64) has
still to be proved, but what is relevant to mention is that
ionisation of such chemical groups modify the metabolic pathways.
The dissociation of chemical groups occurs to a different extent
at different pH levels, either at the extracellular or intracellular
medium (for example, the equilibrium of phosphate ions: \( PO_4^{3-} \),
\( HPO_4^{2-} \) and \( H_2PO_4^{-1} \)) and a simple assessment of their effects on
metabolism is not easy.

In view of the previous observations, the 'bottle-neck'
concept supported by some workers (Ierusalimskii; 1965, Ibragimova,
1969; Andreeva, 1969) in which a single metabolic reaction is
considered to be controlling the whole fermentation process is
unlikely to be realistic. Therefore, modelling of pH effects
using enzyme kinetic mechanisms is valid as long as any of the
expressions derived from such mechanisms describes the experimental
results observed in fermentation, but caution must be exercised in
stating that the mechanism that generates a given expression is the
controlling one for the whole fermentation process.

Bearing in mind the mentioned above, a discussion of
enzyme kinetics models is presented in order to clarify some of
the criteria that could be used as an aid when proposing models
which include pH effects. The number of feasible mechanisms is
immense so only a few of the most representative are examined.
In all the mechanisms examined, Michaelis-Menten kinetics are
assumed and the final expression is intended to be identified as:

\[
R = \frac{\mu_m'(\text{pH})}{K_s'(\text{pH}) + C_s} \quad (2.36)
\]

where the dash denotes apparent values of the constants \( \mu_m \) and \( K_s \)
at a given pH value, \( R \) is the specific growth rate (\( r_x/C_x \)) and
\( C_s \) the substrate concentration.

The constants involved in the model expression are
defined in terms of the equilibrium constants of the equilibrium
reactions (double arrowed lines) in the Appendix A.

In a model derived from a given mechanism, the parameters \( \mu_m \) and \( K'_s \) should show the behaviour with respect to pH derived from the assumptions of the mechanism in question.

(a) Competitive inhibition by H and OH ions.

I = inhibitor.

\[
\begin{align*}
S & \quad \xrightarrow{E} \quad \text{(ES)} \quad \xrightarrow{E + P} \\
I & \quad \xrightarrow{(EI)} \\
S & \quad \xleftarrow{E} \quad \text{(ES)} \quad \xleftarrow{E + P} \\
\end{align*}
\]

if \( I = H \)

\[
R = \frac{\mu_m C_s}{K_s(1 + C_H/a) + C_s} \tag{2.37}
\]

if \( I = OH \)

\[
R = \frac{\mu_m C_s}{K_s(1 + b/C_H) + C_s} \tag{2.38}
\]

\( \mu'_m \) is expected to be independent of pH but \( K'_s \) is a function of pH. (See Figures (2.5) and (2.6).) i.e.: 

\[
\mu'_m = \mu_m \quad \text{constant},
\]

\[
K'_s = K_s(1 + C_H/a) \quad \text{or}
\]

\[
K'_s = K_s(1 + b/C_H)
\]
FIGURE 2.5 $\mu_m'$ and $K_S'$ for competitive inhibition by H ions.

FIGURE 2.6 $\mu_m'$ and $K_S'$ for competitive inhibition by OH ions.
(b) Uncompetitive inhibition by H or OH ion mechanism.

\[ I = \text{inhibitor} \]

\[
\begin{align*}
S & \quad \text{(ES)} \quad \text{E + P} \\
E & \quad \text{I} \\
\text{(EIS)} & \quad \text{I} \\
\end{align*}
\]

\[
\begin{align*}
\text{if } I = H & \quad R = \frac{\mu_m}{1 + C_H/a} \frac{C_s}{K_s/(1 + C_H/a) + C_s} \\
\text{if } I = OH & \quad R = \frac{\mu_m}{1 + b/C_H} \frac{C_s}{K_s/(1 + b/C_H) + C_s}
\end{align*}
\]

Both \( \mu_m \) and \( K_s' \) depend on pH, this case is depicted in Figures 2.7 and 2.8.

(c) Non-competitive inhibition* by H and OH ion mechanism.

\[ I = \text{inhibitor} \]

\[
\begin{align*}
S & \quad \text{(ES)} \quad \text{E + P} \\
E & \quad \text{I} \\
\text{(EI)} & \quad \text{I} \\
\text{(EIS)} & \quad \text{I}
\end{align*}
\]

* The difference between uncompetitive inhibition and non-competitive inhibition lies in the fact that the former mechanism assumes that the inhibitor is not directly attached to the free enzyme, but to the complex enzyme-substrate. In the later case, the inhibitor can be attached to both free enzyme and enzyme-substrate complex.
**FIGURE 2.7** $\mu^*$ and $K_s'$ for uncompetitive inhibition by H ions.

**FIGURE 2.8** $\mu^*$ and $K_s'$ for uncompetitive inhibition by OH ions.
if \( I = H \)

\[
R = \frac{M_0}{1 + \frac{C_H}{a}} \frac{C_s}{K_s + C_s}
\]  

(2.41)

if \( I = OH \)

\[
R = \frac{M_0}{1 + \frac{b}{C_H}} \frac{C_s}{K_s + C_s}
\]  

(2.42)

\( M_0 \) depends on \( pH \) and \( K'_s \) is independent of \( pH \). Figures 2.9 and 2.10

(d) Competitive inhibition by \( H \) and \( OH \) ions.

\[ 
\begin{align*}
\text{H} & \quad \rightarrow \quad \text{(EH)} \\
\text{S} & \quad \rightarrow \quad \text{ES} \\
\text{OH} & \quad \rightarrow \quad \text{(EOH)}
\end{align*}
\]

\[
R = \frac{M_0}{K_s \left(1 + \frac{C_H}{a} + \frac{b}{C_H}\right) + C_s}
\]  

(2.43)

\( M_0 \) is indpendant of \( pH \) and \( K'_s \) depends on \( pH \). Figure 2.11

(e) Substrate ionisation by \( H \) and \( OH \) ions.

\[ 
\begin{align*}
\text{H} & \quad \rightarrow \quad \text{(SH)} \\
\text{S} & \quad \rightarrow \quad \text{ES} \\
\text{OH} & \quad \rightarrow \quad \text{(SOH)}
\end{align*}
\]

\[
\text{E} \quad \rightarrow \quad \text{ES} \\
\text{E} + \text{P}
\]
FIGURE 2.9 $\mu'_m$ and $K'_s$ for non-competitive inhibition by $H$ ions.

FIGURE 2.10 $\mu'_m$ and $K'_s$ for non-competitive inhibition by $OH$ ions.
\[ R = \mu_m \frac{C_{so}}{K_s(1 + C_H/a + b/C_H) + C_{so}} \]  

\[ (2.44) \]

$C_{so}$ is the total substrate concentration present at any time, i.e.:

\[ C_{so} = C_s + C_{SH} + C_{SOH} \gg C_{es} \]

$\mu_m$ is independent of pH, $K'_s$ depends on pH. Figure 2.11

(f) Competitive inhibition and substrate ionisation by H and OH ions.

\[ R = \mu_m \frac{C_{so}}{K_s(1 + C_H/a + b/C_H)(1 + C_H/c + d/C_H) + C_{so}} \]  

\[ (2.45) \]

$C_{so}$ is the total substrate present at any moment.

$\mu_m$ is independent of pH, $K'_s$ depends on pH. Figure 2.12

(g) Competitive inhibition by H and OH ions and ionisation of the substrate-enzyme complex by H and OH ions.
FIGURE 2.11 $\mu_m'$ and $K'_s$ for competitive inhibition by $H$ and $OH$ ions or for substrate ionisation by $H$ and $OH$ ions.

FIGURE 2.12 $\mu'_m$ and $K'_s$ for competitive inhibition and substrate ionisation by $H$ and $OH$ ions.
Both $\mu_m'$ and $K_s'$ depend on pH. Figures 2.13 and 2.14

If $(1 + C_H/c + d/C_H) = (1 + C_H/a + b/C_H)$, then $\mu_m'$ depends on pH and $K_s'$ is independent of pH. Figure 2.15

(h) Non-competitive inhibition by H and OH ions.

The expression for $R$ is exactly the same as that produced by the mechanism (g) and the possibilities are similar. Figures 2.13, 2.14 and 2.15
FIGURE 2.13 $M_m'$ and $K_s'$ for mechanisms (g) and (h) for the case:

\[(1 + \frac{c_H}{c} + \frac{d}{C_H}) > (1 + \frac{c_H}{a} + \frac{b}{C_H})\]

FIGURE 2.14 $M_m'$ and $K_s'$ for mechanisms (g) and (h) for the case:

\[(1 + \frac{c_H}{c} + \frac{d}{C_H}) < (1 + \frac{c_H}{a} + \frac{b}{C_H})\]
It can be seen in the above description that some mechanisms are indistinguishable from each other and the numerical combinations are immense. One the other hand, sometimes extreme values of pH are required to detect the qualitative shape of the curves. This fact could lead to a breakdown of the theory due to the adverse effects of extreme pH values on the microbial physiology.

As it was mentioned before, all the mechanisms examined here, only account for the pH effect on the saturation constant and maximum specific growth rate of a single Monod model and they do not predict any pH effect on other parameters such as stoichiometric coefficients and endogeneous respiration constant.

The following model is proposed for pH effects on the parameters which the previously examined mechanisms did not consider, i.e., stoichiometric and endogeneous respiration coefficients.
Model for the pH effects on stoichiometric coefficients and rate constants for substrate consumption, product formation and endogeneous respiration reactions. (PHEM model).

In addition to the growth rate of cells, the fermentation process reactions which are of importance, observed externally and useful in non-structured models, can be divided in two classes:

(a) Growth associated reactions in which the reaction rate can be expressed as \( r = -m r_x \) (or \( \alpha r_x \)), \( m \) being a stoichiometric coefficient.

(b) Cell concentration associated reactions in which the reaction rate can be expressed as: \( r = k C_x \) (or \( \beta C_x \)), \( k \) being a rate constant.

The model that follows considers both type of reactions as equivalent for modelling purposes, but for clarity they are treated separately.

2.4.1 A model for pH effects on stoichiometric coefficients for growth associated reactions.

Consider a single cell population growing in a single substrate limited culture, converting part of the substrate to a single product P by a growth associated reaction.

The assumptions of the model are summarised as follows:

(i) Substrate undergoes the following reactions:
(ii) The influence of H and OH ions can be incorporated in the rate expression by multiplicative functions of the type:

\[
\frac{1}{1 + \frac{C_H}{a}} \quad \text{for H ions effect.}
\]

\[
\frac{1}{1 + \frac{b}{C_H}} \quad \text{for OH ions effect.}
\]

(iii) \(m_x, m_o, m_1\) and \(m_2\) are the respective stoichiometric coefficients which are constant in value for all pH.

From the above scheme, the reaction rate for product formation \(r_p\) and substrate consumption \(r_s\) are:

\[
r_s = -m_x \frac{r}{x} - m_o \frac{r}{o} - m_1 \frac{1}{1 + C_H/a} \frac{r}{x} - m_2 \frac{1}{1 + \frac{b}{C_H}} \frac{r}{x}\tag{2.47}
\]

\[
r_p = m_o \frac{r}{o} + m_1 \frac{1}{1 + C_H/a} \frac{r}{x} + m_2 \frac{1}{1 + \frac{b}{C_H}} \frac{r}{x}\tag{2.48}
\]
Rearrangement of Equations (2.47) and (2.48) yields:

\[ r_s = -m \frac{r_x}{s} \]
\[ r_P = \alpha r_x \]  (2.49)

where:

\[ m_s = m_x + m_o + m_1 \frac{1}{1 + C_H/a} + m_2 \frac{1}{1 + b/C_H} \]  (2.50)

\[ \alpha = m_o + m_1 \frac{1}{1 + C_H/a} + m_2 \frac{1}{1 + b/C_H} \]  (2.51)

At constant pH, \( m_s \) and \( \alpha \) are the apparent values of the stoichiometric coefficients observed or estimated in a controlled pH fermentation. These values are independent of the pH effect on the growth rate at any pH.

The expressions given by Equations (2.50) and (2.51) are algebraically equivalent. The following analysis is carried out for \( m_s \) but also applies for \( \alpha \).

Asymptotic or limiting cases.

I. \( a \gg C_H \) over the whole pH operative range, hence:

\[ m_s = m_x + m_o + m_1 \frac{1}{1 + C_H/a} + m_2 \frac{1}{1 + b/C_H} \]  (2.52)

\( m_s \) will show the behaviour depicted in Figure 2.16 over the pH operative range.
FIGURE 2.16 $m_s$ as a function of pH. Case I.

$a \gg c_H$

FIGURE 2.17 $m_s$ as a function of pH. Case II.

$c_H \gg b$. 
II. \( C_H \gg b \) over the whole pH operative range.

\[
m_s = \frac{m + m_1 + m_2}{1 + \frac{1}{C_H/a}}
\]  

(2.53)

This case is shown in Figure 2.17

III. \( a \gg C_H \) and \( C_H \gg b \) over the whole pH operative range.

\[
m_s = m + m_1 + m_2
\]  

(2.54)

The observed \( m_s \) is independent of pH.

IV. \( a \ll C_H \) and \( C_H \ll b \) over the whole pH operative range.

\[
m_s = \frac{m + m_1 a/C_H + m_2 b}{1 + \frac{1}{C_H/a}}
\]  

(2.55)

Figure 2.18 depicts this case.

The general case is depicted in Figures 2.19 and 2.20. The existence of a maximum or a minimum depends on the relative values of the constants \( a \) and \( b \).

In the following Section, the equivalent model for cell concentration associated reaction parameter is proposed, along the same line of thought.
FIGURE 2.18 $m_s$ as a function of pH, Case IV

$a \ll C_H$ and $C_H \ll b$.

FIGURE 2.19 $m_s$ as a function of pH, General case. Possibility of a maximum.
2.4.2 A model for the pH effects on the rate constants of cell concentration associated reactions.

The difference between the model proposed in the preceding Section and the following one is more of definition of terms than of essence. The assumptions are the same and the statement of the model is as follows:

FIGURE 2.20 $m_s$ as a function of pH. General case. Possibility of a minimum.
For product formation, the substrate undergoes the following cell concentration associated reactions:

\[ S \xrightarrow{X, \beta_0} P \] not affected by H or OH ions.

\[ S \xrightarrow{X, \beta_1} P \] affected by H ions.

\[ S \xrightarrow{X, \beta_2} P \] affected by OH ions.

The functional form for H and OH ion effects are the same as those for growth associated reactions. \( \beta_0, \beta_1, \) and \( \beta_2 \) are the respective rate constants. For this type of reactions, the stoichiometric coefficients do not affect the reaction rate expressions.

The reaction rate for the formation of P is:

\[ r_p = \beta C_x \] (2.56)

Equation (2.56) can be expressed as:

\[ r_p = \beta C_x \] (2.57)

where:

\[ \beta = \beta_0 + \beta_1 \frac{1}{1 + C_H/a} + \beta_2 \frac{1}{1 + b/C_H} \] (2.58)

\( \beta \) being the observed rate constant if pH is constant.
It can immediately be deduced that the asymptotic cases analysed for growth associated reactions are the same for the previous case.

Stating the equivalent model for endogeneous respiration reaction is merely a formality and it can be shown that the same assumptions produce the expression:

\[ r_e = k C e^x \] (2.59)

where:

\[ k_e = k_0 + k_1 \frac{1}{1 + C_H/a} + k_2 \frac{1}{1 + b/C_H} \] (2.60)

ke being the observed endogeneous respiration coefficient at constant pH.

Expressions of the type as that given by Equations (2.58) and (2.60) can be derived for all kind of reactions related to cell concentration such as cell decay or enzymic activity.

Qualitative identification of the model is relatively simple and the evaluation of the parameters requires only sufficient observations in the operative range of pH. It is possible to use this model in describing a wide variety of cases which could be of potential use in process optimisation where pH is the variable of prime importance. Experimental evidence to support the model is examined in Chapter 5.
CHAPTER 3

EXPERIMENTAL

3.1 Materials and equipment.

A. The microorganism.

A strain of yeast, Candida lipolytica C.B.S. 5699, supplied by the Centralbureau voor Schimmecultures (Holland) was used in all the experiments. The strain was kept on slopes of nutrient agar at refrigerator temperatures and routine renewal of the culture was performed fortnightly.

B. The nutrient medium.

The nutrient solution was made up as shown in Table 3.1. The formulation of the solution has been developed and used successfully in the Biochemical Engineering Laboratory of Bath University. This medium was used in all the experiments.

The mineral base was prepared in concentrated solution for 100 litres of medium and stored in amber bottles; the phosphates, glucose and nutrient broth were added just prior to sterilisation. The chemicals were supplied by BDH Chemicals LTD and Hopkin & Williams Co. With exception of glucose, all were AnalaR grade.
TABLE 3.1 Nutrient solution formulation.

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration (kg/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>glucose</td>
<td>5.0</td>
</tr>
<tr>
<td>(\text{NH}_4\text{H}_2\text{PO}_4)</td>
<td>5.0</td>
</tr>
<tr>
<td>(\text{KH}_2\text{PO}_4)</td>
<td>0.7</td>
</tr>
<tr>
<td>Nutrient broth (OXOID CMl)</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Mineral base</strong></td>
<td></td>
</tr>
<tr>
<td>(\text{MgSO}_4\cdot7\text{H}_2\text{O})</td>
<td>0.4</td>
</tr>
<tr>
<td>(\text{NaCl})</td>
<td>0.1</td>
</tr>
<tr>
<td>(\text{CaCl}_2\cdot2\text{H}_2\text{O})</td>
<td>0.1</td>
</tr>
<tr>
<td>(\text{ZnSO}_4\cdot7\text{H}_2\text{O})</td>
<td>(7.1 \times 10^{-4})</td>
</tr>
<tr>
<td>(\text{MnSO}_4\cdot4\text{H}_2\text{O})</td>
<td>(8.84 \times 10^{-4})</td>
</tr>
<tr>
<td>(\text{CuSO}_4\cdot5\text{H}_2\text{O})</td>
<td>(4.0 \times 10^{-3})</td>
</tr>
<tr>
<td>(\text{(NH}_4\text{)}_2\text{SO}_4\cdot\text{FeSO}_4\cdot6\text{H}_2\text{O})</td>
<td>(2.0 \times 10^{-4})</td>
</tr>
<tr>
<td>distilled water</td>
<td></td>
</tr>
</tbody>
</table>

C. The fermentor.

A three litre working-volume stirred fermentor (Biotec, Mod. FL103) was used in all the experiments. It consisted of two circular stainless steel plates (lid and bottom) between which a Pyrex glass cylinder (inner diameter 160 mm, height 210 mm) was clamped and sealed by two L-section rubber gaskets. The lid contained ports for ancillary equipment (pH and oxygen probes; feed, titrant and inoculum needles; exhaust, etc.), the bottom contained ports for air supply, sampling and overflow. See Figure 3.1

Stirring was provided by a magnetic-trasmission shaft
The fermentor system.

A peristaltic pumps, B pH electrode, C inoculum port, D oxygen probe
E contact thermometer, F heater, G sampling port, H condenser, I Whatman filters.

FIGURE 3.1
driving a coaxial six-paddle stirrer immersed in the fermentor. Stirring rate was kept within the range 400 to 700 rpm.

D. Aeration.

Air was supplied through a jet port in the bottom of the fermentor after the flow had been measured by a rotameter (Rotameter Mfg. Co.) and passed through a sterilising membrane filter (Sterilising filter holder Mod. YY3009000, prefiter AP2007525; membrane filter Type PHWP09025 0.3 μm pore size, all supplied by Millipore Corp.) The flow rate was manually controlled between 0.75 and 3.0 litres/min (0.25 - 1.00 V/V/min).

E. Dissolved oxygen control and measurement.

Dissolved oxygen was measured by a steam sterilisable oxygen probe (Oxygen meter Series 400, Uniprobe Corp.) placed in the fermentor through a port in the lid. The oxygen meter was calibrated prior to sterilisation with air saturated nutrient solution against a 3% (W/V) sodium sulphite solution as blank at 30 °C, as recommended by manufacturers. At this temperature and a total pressure of one atm. (air), the saturation concentration of dissolved oxygen was 7.3 ppm or 0.073 kg/m³, (no significant difference between the saturation concentration of dissolved oxygen in distilled water and the medium solution was found). After sterilisation, the oxygen meter was recalibrated at 30 °C, 1 atm., with the nutrient solution saturated with air. The zero was set at the check position of the meter, as recommended by the manufacturers.

The dissolved oxygen level was controlled manually by adjusting the stirring rate and/or the air flow rate to ensure a minimum concentration of dissolved oxygen of 50% of saturation at any time in each run.
F. pH control and measurement.

The pH was measured with a steam sterilisable pH glass electrode (EIL Mod. 1117 - 500) submerged into the culture through a port in the lid of the fermentor and controlled by a pH monitor (Pye Unicam, Mod. 539) with ± 0.05 pH units accuracy by automatic addition of 3N sodium hydroxide and N/10 hydrochloric acid solutions through needle ports in the lid of the fermentor.

The electrode - monitor system was calibrated with freshly made buffer solution at pH 4 and 6.8 as recommended by the manufacturers. After sterilisation, the accuracy of the pH being recorded by the monitor was checked externally on a five ml culture sample.

G. Temperature control.

A contact thermometer (Gallekamp Mod. 13404/12/70) was placed in a special compartment in the fermentor for such a purpose and connected to a heater submerged into the culture. In addition, the fermentor was provided with a stream of cooling water through a cooling system submerged in the culture. The temperature was kept constant at 30 °C in all experiments with an accuracy of ± 0.1 °C.

H. Sterilisation.

The fermentor and all related equipment in direct contact with culture broth, nutrient solution, titrants, air flow, exhausted air and electrodes were steam sterilisable. Sterilisation was achieved by autoclaving at 121 °C and 15 psig for 40 minutes with most of the ancilliary equipment already placed in the fermentor. The nutrient solution and titrant containers were capped with rubber bungs and open to the atmosphere through sterilising minifilters (Whatman Minifilter grade 10). Exhaust gases were
bubbled into a 5% (V/V) diluted sodium hypochlorite solution.

The nutrient solutions were sterilised in batches of five litres (4.5 litres containing the glucose, nutrient broth and mineral base and 0.5 litre containing the phosphates, mixed aseptically after sterilisation) by autoclaving at 121 °C and 15 psig for 40 minutes. The nutrient solution was pumped into the fermentor by a peristaltic pump (Multi-minipump, Schuco Scientific LTD) through steam sterilisable silicone-rubber tubing.

I. Foam control.

Foam formation was controlled by addition of antifoam emulsion (Dow Corning Antifoam RD Emulsion, Hopkin & Williams Co.) contained in the nutrient solution.

Previous experiments in the fermentor showed that a concentration of up to 5% (V/V) of antifoam in distilled water does not change the dissolved oxygen concentration in water to a significant extent. Dissolved oxygen decreased by less than 5% of the saturation value in a 5% (V/V) solution of antifoam emulsion in water at 30 °C, with a 450 rpm stirring rate and a 1.5 litres per minute (0.5 V/V/min) air flow rate.

Previous cultures in flasks showed that concentrations of antifoam up to 0.75% did not inhibit growth but did have an adverse effect on the biomass determination. After some further tests in flask cultures, it was decided to use a concentration of 0.1% (V/V) of antifoam in batch culture experiments and 0.05% (V/V) in continuous culture experiments. A careful washing of the biomass after the filtration reduced antifoam in the filter to within the order of error of the balance's sensitivity.
3.2 Analytical techniques.

A. Biomass determination.

Dry biomass was determined by gravimetry. A 20 ml sample was withdrawn from the fermentor and filtered under vacuum (Filter Type PHW P02500, 0.3 μm pore size, Millipore Corp.). The filter was dried and weighed before and after filtration for 24 hours at 105 °C. Biomass was washed after filtration with five 5-ml portions of distilled water.

B. Glucose determination.

Glucose was determined by the o-toluidine colourimetric method (Appendix C1) in the filtrate.

C. Ammonium ion determination.

Ammonium ion in the filtrate was determined by standard Kjedhal method (Appendix C2).

3.3 Operation.

Fermentations were carried out at a constant temperature of 30 °C and at several levels of pH. Batch cultures were carried out at pH values of 3, 4, 5.5, 6 and 7; continuous cultures were carried out at pH values of 4, 5, 5.5 and 6.

3.3.1 Batch cultures.

Three 250-ml volume conical flasks each containing 100 ml of nutrient solution were sterilised and inoculated with the strain,
and incubated at 30 °C for 48 hours in a rotary shake incubator. One of the flasks was chosen by visual inspection to inoculate the fermentor.

The fermentor was set for operation at 30 °C and at the natural pH of the nutrient solution (4.5). After sterilisation, it was inoculated with the flask culture through a septum in the inoculum port in the lid of the fermentor. The fermentor was then adjusted to the required pH over a two hour period and the culture was left running for twelve hours for adaptation. After this time, the fermentor was discharged, keeping 300-500 ml of the culture and reloading with fresh medium at the pH of the run. After stabilisation sampling started. The lag phase was almost eliminated with this technique.

Samples of about 30 ml were taken each hour and 20 ml were used to determine biomass and nutrients as described before. 5 ml were used to check the pH externally.

The oxygen level was kept at a minimum of 50% of saturation by adjusting either the stirring rate or the air flow rate manually as previously described. The end of the growth phase was detected when the oxygen level returned to almost saturation value. The sampling continued during the decaying phase for another 3 or 4 hours.

3.3.2 Continuous cultures.

The culture was started as for the batch cultures, but after the twelve hour adaptation period, fresh sterile medium was continuously added. The concentration of the limiting nutrients in the feed were: for glucose 5.0 kg/m³ and for ammonium ion 0.783 kg/m³ in all the experiments.

Before starting sampling, a minimum of five residence
times were allowed to pass (seven residence times for dilution rates higher than 0.15 hr$^{-1}$). Samples were taken every six hours and steady state was assumed when the relative variation in glucose and ammonium concentration was less than 5% in three consecutive determinations. The data collected during the experiments are presented in Tables 3.2 to 3.10

3.4 Results.

Tables 3.2 to 3.6 present the data collected in batch culture experiments and Tables 3.7 to 3.10 present the data collected in continuous culture experiments.

TABLE 3.2 Experimental results. Batch culture at pH 3.

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>$C_x$ (kg/m$^3$)</th>
<th>$C_g$ (kg/m$^3$)</th>
<th>$C_n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.483</td>
<td>4.554</td>
<td>0.7568</td>
</tr>
<tr>
<td>1</td>
<td>0.564</td>
<td>4.392</td>
<td>0.7452</td>
</tr>
<tr>
<td>2</td>
<td>0.663</td>
<td>4.176</td>
<td>0.7344</td>
</tr>
<tr>
<td>3</td>
<td>0.831</td>
<td>3.996</td>
<td>0.7236</td>
</tr>
<tr>
<td>4</td>
<td>1.04</td>
<td>3.618</td>
<td>0.7074</td>
</tr>
<tr>
<td>5</td>
<td>1.33</td>
<td>3.258</td>
<td>0.6804</td>
</tr>
<tr>
<td>6</td>
<td>1.59</td>
<td>2.898</td>
<td>0.6408</td>
</tr>
<tr>
<td>7</td>
<td>2.00</td>
<td>2.196</td>
<td>0.6228</td>
</tr>
<tr>
<td>8</td>
<td>2.29</td>
<td>1.3932</td>
<td>0.5724</td>
</tr>
<tr>
<td>9</td>
<td>2.72</td>
<td>0.4428</td>
<td>0.5364</td>
</tr>
<tr>
<td>10</td>
<td>2.94</td>
<td>0.0180</td>
<td>0.5148</td>
</tr>
<tr>
<td>11</td>
<td>2.79</td>
<td>0.0180</td>
<td>0.5058</td>
</tr>
<tr>
<td>12</td>
<td>2.73</td>
<td>0.0108</td>
<td>0.5076</td>
</tr>
<tr>
<td>13</td>
<td>2.65</td>
<td>0.0144</td>
<td>0.5058</td>
</tr>
<tr>
<td>14</td>
<td>2.63</td>
<td>0.0072</td>
<td>0.5040</td>
</tr>
</tbody>
</table>
### TABLE 3.3 Experimental results. Batch culture at pH 4.

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>( C_x )</th>
<th>( C_g )</th>
<th>( C_n )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.469</td>
<td>4.824</td>
<td>0.7272</td>
</tr>
<tr>
<td>1</td>
<td>0.570</td>
<td>4.608</td>
<td>0.7182</td>
</tr>
<tr>
<td>2</td>
<td>0.700</td>
<td>4.158</td>
<td>0.7020</td>
</tr>
<tr>
<td>3</td>
<td>0.876</td>
<td>3.996</td>
<td>0.6876</td>
</tr>
<tr>
<td>4</td>
<td>1.111</td>
<td>3.402</td>
<td>0.6804</td>
</tr>
<tr>
<td>5</td>
<td>1.41</td>
<td>2.808</td>
<td>0.6300</td>
</tr>
<tr>
<td>6</td>
<td>1.64</td>
<td>2.412</td>
<td>0.5904</td>
</tr>
<tr>
<td>7</td>
<td>1.98</td>
<td>1.854</td>
<td>0.5652</td>
</tr>
<tr>
<td>8</td>
<td>2.44</td>
<td>1.044</td>
<td>0.5400</td>
</tr>
<tr>
<td>9</td>
<td>2.66</td>
<td>0.0432</td>
<td>0.5004</td>
</tr>
<tr>
<td>10</td>
<td>2.67</td>
<td>0.0216</td>
<td>0.4788</td>
</tr>
<tr>
<td>11</td>
<td>2.57</td>
<td>0.0126</td>
<td>0.4878</td>
</tr>
<tr>
<td>12</td>
<td>2.47</td>
<td>0.0108</td>
<td>0.4752</td>
</tr>
<tr>
<td>13</td>
<td>2.42</td>
<td>0.0108</td>
<td>0.4752</td>
</tr>
</tbody>
</table>

### TABLE 3.4 Experimental results. Batch culture at pH 5.5

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>( C_x )</th>
<th>( C_g )</th>
<th>( C_n )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.415</td>
<td>4.554</td>
<td>0.7290</td>
</tr>
<tr>
<td>1</td>
<td>0.518</td>
<td>4.356</td>
<td>0.7236</td>
</tr>
<tr>
<td>2</td>
<td>0.655</td>
<td>4.356</td>
<td>0.7020</td>
</tr>
<tr>
<td>3</td>
<td>0.852</td>
<td>3.978</td>
<td>0.6948</td>
</tr>
<tr>
<td>4</td>
<td>1.09</td>
<td>3.150</td>
<td>0.6786</td>
</tr>
<tr>
<td>5</td>
<td>1.39</td>
<td>2.592</td>
<td>0.6192</td>
</tr>
<tr>
<td>6</td>
<td>1.81</td>
<td>1.962</td>
<td>0.5922</td>
</tr>
<tr>
<td>7</td>
<td>2.26</td>
<td>1.260</td>
<td>0.5436</td>
</tr>
<tr>
<td>8</td>
<td>2.70</td>
<td>0.576</td>
<td>0.5148</td>
</tr>
<tr>
<td>9</td>
<td>2.88</td>
<td>0.0216</td>
<td>0.5040</td>
</tr>
<tr>
<td>10</td>
<td>2.79</td>
<td>0.0216</td>
<td>0.4914</td>
</tr>
<tr>
<td>11</td>
<td>2.63</td>
<td>0.0162</td>
<td>0.4896</td>
</tr>
<tr>
<td>12</td>
<td>2.51</td>
<td>0.0144</td>
<td>0.4698</td>
</tr>
<tr>
<td>13</td>
<td>2.42</td>
<td>0.0144</td>
<td>0.4644</td>
</tr>
</tbody>
</table>
TABLE 3.5 Experimental results. Batch culture at pH 6.

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>$C_X$ (kg/m$^3$)</th>
<th>$C_g$ (kg/m$^3$)</th>
<th>$C_n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.472</td>
<td>5.112</td>
<td>0.7920</td>
</tr>
<tr>
<td>1</td>
<td>0.513</td>
<td>4.842</td>
<td>0.7848</td>
</tr>
<tr>
<td>2</td>
<td>0.607</td>
<td>4.464</td>
<td>0.7776</td>
</tr>
<tr>
<td>3</td>
<td>0.740</td>
<td>4.158</td>
<td>0.7452</td>
</tr>
<tr>
<td>4</td>
<td>0.912</td>
<td>3.438</td>
<td>0.7128</td>
</tr>
<tr>
<td>5</td>
<td>1.187</td>
<td>2.988</td>
<td>0.6912</td>
</tr>
<tr>
<td>6</td>
<td>1.547</td>
<td>2.286</td>
<td>0.6408</td>
</tr>
<tr>
<td>7</td>
<td>1.923</td>
<td>1.4868</td>
<td>0.5958</td>
</tr>
<tr>
<td>8</td>
<td>2.253</td>
<td>0.6606</td>
<td>0.5562</td>
</tr>
<tr>
<td>9</td>
<td>2.297</td>
<td>0.0918</td>
<td>0.5094</td>
</tr>
<tr>
<td>10</td>
<td>2.203</td>
<td>0.0252</td>
<td>0.5058</td>
</tr>
<tr>
<td>11</td>
<td>2.083</td>
<td>0.0252</td>
<td>0.5040</td>
</tr>
<tr>
<td>12</td>
<td>1.923</td>
<td>0.0198</td>
<td>0.5040</td>
</tr>
</tbody>
</table>

TABLE 3.6 Experimental results. Batch culture at pH 7.

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>$C_X$ (kg/m$^3$)</th>
<th>$C_g$ (kg/m$^3$)</th>
<th>$C_n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.517</td>
<td>4.752</td>
<td>0.8856</td>
</tr>
<tr>
<td>1</td>
<td>0.620</td>
<td>3.510</td>
<td>0.8064</td>
</tr>
<tr>
<td>2</td>
<td>0.932</td>
<td>2.484</td>
<td>0.7362</td>
</tr>
<tr>
<td>3</td>
<td>1.250</td>
<td>1.4238</td>
<td>0.6804</td>
</tr>
<tr>
<td>4</td>
<td>1.570</td>
<td>0.6876</td>
<td>0.6300</td>
</tr>
<tr>
<td>5</td>
<td>1.900</td>
<td>0.0738</td>
<td>0.5688</td>
</tr>
<tr>
<td>6</td>
<td>1.850</td>
<td>0.0054</td>
<td>0.5220</td>
</tr>
<tr>
<td>7</td>
<td>1.613</td>
<td>0.0090</td>
<td>0.5076</td>
</tr>
<tr>
<td>8</td>
<td>1.443</td>
<td>-</td>
<td>0.5040</td>
</tr>
<tr>
<td>9</td>
<td>1.303</td>
<td>-</td>
<td>0.5042</td>
</tr>
</tbody>
</table>
### TABLE 3.7 Experimental results. Continuous culture at pH 4.0

<table>
<thead>
<tr>
<th>Dilution rate (1/hr)</th>
<th>C(_x)</th>
<th>C(_g)</th>
<th>C(_n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.040</td>
<td>1.42</td>
<td>0.0090</td>
<td>0.5364</td>
</tr>
<tr>
<td>0.051</td>
<td>1.58</td>
<td>0.0126</td>
<td>0.5364</td>
</tr>
<tr>
<td>0.075</td>
<td>1.95</td>
<td>0.0108</td>
<td>0.5364</td>
</tr>
<tr>
<td>0.084</td>
<td>1.97</td>
<td>0.0216</td>
<td>0.5382</td>
</tr>
<tr>
<td>0.115</td>
<td>2.03</td>
<td>0.0198</td>
<td>0.5382</td>
</tr>
<tr>
<td>0.134</td>
<td>2.06</td>
<td>0.0684</td>
<td>0.5418</td>
</tr>
<tr>
<td>0.154</td>
<td>2.10</td>
<td>0.1008</td>
<td>0.5436</td>
</tr>
<tr>
<td>0.182</td>
<td>1.97</td>
<td>0.2844</td>
<td>0.5490</td>
</tr>
<tr>
<td>0.200</td>
<td>1.97</td>
<td>0.5490</td>
<td>0.5580</td>
</tr>
<tr>
<td>0.216</td>
<td>1.69</td>
<td>0.9360</td>
<td>0.5688</td>
</tr>
<tr>
<td>0.247</td>
<td>1.43</td>
<td>1.908</td>
<td>0.6400</td>
</tr>
<tr>
<td>0.272</td>
<td>0.735</td>
<td>3.42</td>
<td>0.7110</td>
</tr>
</tbody>
</table>

### TABLE 3.8 Experimental results. Continuous culture at pH 5.0

<table>
<thead>
<tr>
<th>Dilution rate (1/hr)</th>
<th>C(_x)</th>
<th>C(_g)</th>
<th>C(_n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.040</td>
<td>1.97</td>
<td>0.0090</td>
<td>0.5040</td>
</tr>
<tr>
<td>0.051</td>
<td>2.10</td>
<td>0.0072</td>
<td>0.5040</td>
</tr>
<tr>
<td>0.075</td>
<td>2.20</td>
<td>0.0108</td>
<td>0.5040</td>
</tr>
<tr>
<td>0.084</td>
<td>2.28</td>
<td>0.0135</td>
<td>0.5040</td>
</tr>
<tr>
<td>0.115</td>
<td>2.33</td>
<td>0.0144</td>
<td>0.5040</td>
</tr>
<tr>
<td>0.134</td>
<td>2.34</td>
<td>0.0288</td>
<td>0.5076</td>
</tr>
<tr>
<td>0.154</td>
<td>2.29</td>
<td>0.0342</td>
<td>0.5076</td>
</tr>
<tr>
<td>0.180</td>
<td>2.59</td>
<td>0.0900</td>
<td>0.5076</td>
</tr>
<tr>
<td>0.200</td>
<td>2.25</td>
<td>0.3600</td>
<td>0.5148</td>
</tr>
<tr>
<td>0.216</td>
<td>2.26</td>
<td>0.6120</td>
<td>0.5292</td>
</tr>
<tr>
<td>0.247</td>
<td>1.87</td>
<td>1.3140</td>
<td>0.5670</td>
</tr>
<tr>
<td>0.285</td>
<td>1.10</td>
<td>2.7360</td>
<td>0.6534</td>
</tr>
</tbody>
</table>
### TABLE 3.9 Experimental results. Continuous culture at pH 5.5

<table>
<thead>
<tr>
<th>Dilution rate (1/hr)</th>
<th>$C_x$</th>
<th>$C_g$</th>
<th>$C_n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.040</td>
<td>1.38</td>
<td>0.0090</td>
<td>0.5326</td>
</tr>
<tr>
<td>0.051</td>
<td>1.54</td>
<td>0.0216</td>
<td>0.5326</td>
</tr>
<tr>
<td>0.075</td>
<td>1.67</td>
<td>0.0270</td>
<td>0.5326</td>
</tr>
<tr>
<td>0.084</td>
<td>1.76</td>
<td>0.0342</td>
<td>0.5326</td>
</tr>
<tr>
<td>0.115</td>
<td>1.81</td>
<td>0.0450</td>
<td>0.5326</td>
</tr>
<tr>
<td>0.134</td>
<td>1.84</td>
<td>0.0720</td>
<td>0.5346</td>
</tr>
<tr>
<td>0.154</td>
<td>1.80</td>
<td>0.1980</td>
<td>0.5368</td>
</tr>
<tr>
<td>0.182</td>
<td>1.71</td>
<td>0.4680</td>
<td>0.5418</td>
</tr>
<tr>
<td>0.200</td>
<td>1.70</td>
<td>0.8478</td>
<td>0.5508</td>
</tr>
<tr>
<td>0.216</td>
<td>1.50</td>
<td>1.08</td>
<td>0.5616</td>
</tr>
<tr>
<td>0.247</td>
<td>1.33</td>
<td>1.80</td>
<td>0.612</td>
</tr>
<tr>
<td>0.272</td>
<td>0.831</td>
<td>2.736</td>
<td>0.648</td>
</tr>
</tbody>
</table>

### TABLE 3.10 Experimental results. Continuous culture at pH 6.0

<table>
<thead>
<tr>
<th>Dilution rate (1/hr)</th>
<th>$C_x$</th>
<th>$C_g$</th>
<th>$C_n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.040</td>
<td>1.32</td>
<td>0.0144</td>
<td>0.5436</td>
</tr>
<tr>
<td>0.051</td>
<td>1.50</td>
<td>0.0450</td>
<td>0.5418</td>
</tr>
<tr>
<td>0.075</td>
<td>1.60</td>
<td>0.0648</td>
<td>0.5454</td>
</tr>
<tr>
<td>0.084</td>
<td>1.65</td>
<td>0.0990</td>
<td>0.5436</td>
</tr>
<tr>
<td>0.115</td>
<td>1.72</td>
<td>0.1440</td>
<td>0.5490</td>
</tr>
<tr>
<td>0.134</td>
<td>1.60</td>
<td>0.3600</td>
<td>0.5526</td>
</tr>
<tr>
<td>0.154</td>
<td>1.66</td>
<td>0.5760</td>
<td>0.5634</td>
</tr>
<tr>
<td>0.182</td>
<td>1.49</td>
<td>1.062</td>
<td>0.5868</td>
</tr>
<tr>
<td>0.200</td>
<td>1.33</td>
<td>1.404</td>
<td>0.5994</td>
</tr>
<tr>
<td>0.216</td>
<td>1.37</td>
<td>1.80</td>
<td>0.612</td>
</tr>
<tr>
<td>0.247</td>
<td>1.00</td>
<td>2.412</td>
<td>0.648</td>
</tr>
<tr>
<td>0.272</td>
<td>0.651</td>
<td>3.24</td>
<td>0.700</td>
</tr>
</tbody>
</table>
In this Chapter, the procedure for parameter evaluation is discussed. Sections 4.1 and 4.2 describe the parameter evaluation for the continuous culture and the batch culture models respectively. In Section 4.3 the assumption of no mass transfer limitations implicit in the kinetic model is tested.

4.1 Estimation of parameters for the continuous culture model.

To examine the performance of the objective function introduced in Section 2.1, Equation (2.11), for the parameter estimation of a multiparameter kinetic model, the experimental data from the continuous culture were tested in a double substrate interactive model. The models examined were Monod-Monod, Monod- Contois, Contois-Monod and Contois-Contois double substrate kinetic models with glucose and ammonium ion as limiting nutrients.

A multiresponse objective function was used in the optimisation procedure. The system's responses were the substrate concentrations (glucose and ammonium) and the dry biomass concentration. The multiresponse function (see Equation (2.18)) is stated as follows:
Let the model structure equations (MSE) for the continuous culture process model be described by:

\[ C(X,Y,t) = 0 \] (4.1)

where \( C = (C_1, C_2, C_3) \); \( C_1, C_2, \) and \( C_3 \) being the implicit functions derived from the mass balance equations of the continuous culture at steady state. The definitions of \( C_1, C_2 \) and \( C_3 \) stated in Section 2.2, Equation (2.29).

The multiresponse objective function can then be stated as:

\[
J(X) = \sum_{i=1}^{\text{nep}} \sum_{j=1}^{\text{nev}} (C_j(X,Y_{ei},t_i))^2
\] (4.2)

where:

\( C_j \): components of \( C \) (MSE vector).
\( X \): estimated value of parameter vector whose components are:

\[ x_1 : K_s^g, \text{Monod saturation constant for glucose or } B_s^g, \text{Contois constant for glucose.} \]

\[ x_2 : K_n^m, \text{Monod saturation constant for ammonium ion or } B_n^m, \text{Contois constant for ammonium ion.} \]

\[ x_3 : k_e, \text{endogeneous respiration coefficient.} \]

\[ x_4 : \mu_m, \text{maximum specific growth rate.} \]

\[ x_5 : m_g, \text{stoichiometric coefficient for glucose.} \]

\[ x_6 : m_n, \text{stoichiometric coefficient for ammonium ion.} \]
\[ y_{e1} : \text{dependent variable (response) vector, whose components are:} \]
\[ y_{1i} : C_g, \text{glucose concentration.} \]
\[ y_{2i} : C_n, \text{ammonium ion concentration.} \]
\[ y_{3i} : C_x, \text{dry biomass concentration.} \]

The subscript \( i \) corresponding to the \( i \)th experimental observation, \( i = 1, \text{nep} \).

\[ t_i : \text{independent variable vector, whose components are:} \]
\[ t_{1i} : D, \text{dilution rate.} \]

nep : number of experimental points.
nev : number of dependent variables.

The optimisation procedure was carried out by a computer programme developed by the author (see Appendix B5 for flow chart). The programme consisted of the minimisation of the objective function defined by Equation (4.2). The problem presents non-linear characteristics, i.e., the function \( J(\bar{X}) \) is non-linear with respect to the parameters, therefore, a modified Newton algorithm was chosen (Subroutine E04LAF of the Mark 6 Library of the South West Universities Computer Network, SWUCN). The Newton algorithm requires the evaluation of the Hessian matrix of \( J(\bar{X}) \) in addition to the gradient. It was mentioned in Section 2.1.2 that the objective function introduced here has analytical partial derivatives (see Appendix B3) and that the solution of the MSE is not required by the optimisation procedure.

* As the feed concentration of glucose and ammonium were kept constant in all the experiments, they are not included in the independent variable vector.
Another characteristic of the objective function given by Equation (4.2) is that the MSE can be manipulated algebraically to produce variations in the functional form of $J(\lambda)$. To examine the effect of such a possibility, the MSE were presented in four different functional forms, called Mode 1, Mode 2, Mode 3 and Mode 4 by the programme. These are given in Table 4.1 for the Monod-Monod model. Similar expressions were used to test the Monod-Contois, Contois-Monod and Contois-Contois models.

The input data to the programme were:

i) nep experimental values of $y_1$, $y_2$ and $y_3$ corresponding to the respective dilution rate $D$ ($y_4$ in the programme)

ii) $X^1$: initial estimated value of the parameter vector.

The output from the programme was:

i) Value of the parameter vector $X$ at the minimum of $J(\lambda)$.

ii) Value of $J(\lambda)$ at the minimum.

iii) Value of the gradient of $J(\lambda)$ at the minimum.

iv) Value of the function $PI(\lambda)$, performance index, at the minimum of $J(\lambda)$:

$$PI(\lambda) = \sum_{i=1}^{nep} (y_{1e} - y_{1e}'_1)^2 + (y_{2e} - y_{2e}'_1)^2 + (y_{3e} - y_{3e}'_1)^2$$

(4.3)

where $y_{1e}$, $y_{2e}$ and $y_{3e}$ are the corresponding experimental observations. $PI(\lambda)$ was calculated with the value of $X$ at the minimum of $J(\lambda)$. Although $PI(\lambda)$ was not included in the optimisation routine, it was used as one of the criteria to assess the models.

v) The variance-covariance matrix of the parameter vector $X$.

vi) The 99%, 95% and 90% confidence limits for the parameters. (After Wolberg, 1967).
TABLE 4.1 Modes of the MSE for the optimisation of $J(X)$ for the Monod-Monod model.

<table>
<thead>
<tr>
<th>Mode 1</th>
<th>$C_1 = \frac{y_1y_2y_3}{(x_1+y_1)(x_2+y_2)} - (x_3 + y_4)y_3 = 0$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$C_2 = y_4(C_{GF} - y_1) - x_5 \frac{y_1y_2y_3}{(x_1+y_1)(x_2+y_2)} = 0$</td>
</tr>
<tr>
<td></td>
<td>$C_3 = y_4(C_{nF} - y_2) - x_6 \frac{y_1y_2y_3}{(x_1+y_1)(x_2+y_2)} = 0$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mode 2</th>
<th>$C_1 = \frac{y_1y_2y_3}{(x_1+y_1)(x_2+y_2)} - (x_3 + y_4)y_3 = 0$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$C_2 = y_4(C_{GF} - y_1) - x_5(x_3 + y_4)y_3 = 0$</td>
</tr>
<tr>
<td></td>
<td>$C_3 = y_4(C_{nF} - y_2) - x_6(x_3 + y_4)y_3 = 0$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mode 3</th>
<th>$C_1 = x_4y_1y_2y_3 - (x_1+y_1)(x_2+y_2)(x_3 + y_4)y_3 = 0$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$C_2 = y_4(C_{GF} - y_1) - x_5(x_3 + y_4)y_3 = 0$</td>
</tr>
<tr>
<td></td>
<td>$C_3 = y_4(C_{nF} - y_2) - x_6(x_3 + y_4)y_3 = 0$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mode 4</th>
<th>$C_1 = x_4y_1y_2 - (x_1+y_1)(x_2+y_2)(x_3 + y_4) = 0$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$C_2 = y_4(C_{GF} - y_1) - x_5(x_3 + y_4)y_3 = 0$</td>
</tr>
<tr>
<td></td>
<td>$C_3 = y_4(C_{nF} - y_2) - x_6(x_3 + y_4)y_3 = 0$</td>
</tr>
</tbody>
</table>

$x_1$: glucose saturation constant $K_g$.
$x_2$: ammonium ion saturation constant $K_n$.
$x_3$: endogeneous respiration coefficient $k_e$.
$x_4$: maximum specific growth rate $\mu_m$.
$x_5$: glucose stoichiometric coefficient $m_g$.
$x_6$: ammonium ion stoichiometric coefficient $m_n$.
$y_1$: glucose concentration, $C_g$.
$y_2$: ammonium ion concentration, $C_n$.
$y_3$: dry biomass concentration, $C_x$.
$y_4$: dilution rate $D$.
$C_{GF}$: glucose feed concentration.
$C_{nF}$: ammonium ion feed concentration.
The programme succeeded with Modes 3 and 4 and failed with Modes 1 and 2. The results of the mathematical analysis are summarised in Table 4.2 giving the parameter values and 95% confidence limits for the double kinetics Contois-Contois model. The values of \( J(X) \) at the minimum and the performance index \( PI(X) \) for the four model are summarised in Tables 4.3 and 4.4. The variance of the parameters (main diagonal of the variance-covariance matrix) are listed in Tables 4.5 and 4.6 for the runs at pH 4 and pH 6 respectively.

The values of the parameters were fitted to a minimax polynomial of degree 2 (Subroutine E02CAF, Mark 6 Library, SWUCN) as functions of pH in the interval 4 \( \leq \text{pH} \leq 6 \). The coefficients and plots of the polynomials are given in Appendix F1. All the results correspond to the Mode 4 of the MSE in the optimisation programme.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>4</th>
<th>5</th>
<th>5.5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>$B_g$ (kg/kg)</td>
<td>0.02443</td>
<td>0.01029</td>
<td>0.04120</td>
<td>0.09292</td>
</tr>
<tr>
<td></td>
<td>(0 - 3.654)</td>
<td>(0 - 2.196)</td>
<td>(0 - 5.454)</td>
<td>(0 - 5.598)</td>
</tr>
<tr>
<td>$B_n$ (kg/kg)</td>
<td>0.08221</td>
<td>0.105</td>
<td>0.1346</td>
<td>0.1747</td>
</tr>
<tr>
<td></td>
<td>(0 - 5.67)</td>
<td>(0 - 7.974)</td>
<td>(0 - 9342)</td>
<td>(0 - 7.164)</td>
</tr>
<tr>
<td>$k_e$ (1/hr)</td>
<td>0.023</td>
<td>0.0147</td>
<td>0.0167</td>
<td>0.0237</td>
</tr>
<tr>
<td></td>
<td>(0.0025 - 0.042)</td>
<td>(0 - 0.0314)</td>
<td>(0 - 0.0376)</td>
<td>(0.0031 - 0.0443)</td>
</tr>
<tr>
<td>$\mu_m$ (1/hr)</td>
<td>0.2313</td>
<td>0.3542</td>
<td>0.3436</td>
<td>0.3511</td>
</tr>
<tr>
<td></td>
<td>(0 - 2.31)</td>
<td>(0 - 4.6)</td>
<td>(0 - 4.67)</td>
<td>(0 - 2.58)</td>
</tr>
<tr>
<td>$m_g$ (kg/kg)</td>
<td>2.057</td>
<td>1.863</td>
<td>2.365</td>
<td>2.320</td>
</tr>
<tr>
<td></td>
<td>(1.818 - 2.304)</td>
<td>(1.674 - 2.052)</td>
<td>(2.07 - 2.664)</td>
<td>(2.304 - 2.61)</td>
</tr>
<tr>
<td>$m_n$ (kg/kg)</td>
<td>0.1029</td>
<td>0.1065</td>
<td>0.1275</td>
<td>0.1171</td>
</tr>
<tr>
<td></td>
<td>(0.0884 - 0.1174)</td>
<td>(0.0936 - 0.1193)</td>
<td>(0.1078 - 0.1471)</td>
<td>(0.099 - 0.1351)</td>
</tr>
</tbody>
</table>

**TABLE 4.2** Parameter values for the double kinetics Contois-Contois model. Continuous culture. The figures in brackets are the 95% confidence limits, estimated with the values of $X$ and $J(X)$ at the minimum. They represent the limits of confidence for the MSE deviation. When the lower limit was negative, it was set to zero.
TABLE 4.3 Values of $J(X)$, objective function. From Mode 4 in the optimisation programme. The figures are relative values of $J(X)$ with respect to the lowest observed in each row (constant pH).

<table>
<thead>
<tr>
<th>Model</th>
<th>pH</th>
<th>Monod-Monod</th>
<th>Monod-Contois</th>
<th>Contois-Monod</th>
<th>Contois-Contois</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>1.003</td>
<td>1.006</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

TABLE 4.4 Values of PI($X$), performance index. From Mode 4 in the optimisation programme. The figures are relative values of PI with respect to the lowest observed in each row (constant pH).

<table>
<thead>
<tr>
<th>Model</th>
<th>pH</th>
<th>Monod-Monod</th>
<th>Monod-Contois</th>
<th>Contois-Monod</th>
<th>Contois-Contois</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.0</td>
<td>1.2134</td>
<td>10.108</td>
<td>14.517</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>16.452</td>
<td>5.722</td>
<td>559.3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>11.704</td>
<td>4.035</td>
<td>226.06</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>9.456</td>
<td>17.01</td>
<td>245.1</td>
<td>1</td>
</tr>
<tr>
<td>Parameter</td>
<td>Monod-Monod</td>
<td>Monod-Contois</td>
<td>Contois-Monod</td>
<td>Contois-Contois</td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>-------------</td>
<td>---------------</td>
<td>---------------</td>
<td>-----------------</td>
<td></td>
</tr>
<tr>
<td>$K_g$ or $B_g$</td>
<td>3.388</td>
<td>17.11</td>
<td>1</td>
<td>4.64</td>
<td></td>
</tr>
<tr>
<td>$K_n$ or $B_n$</td>
<td>342.0</td>
<td>1</td>
<td>348.0</td>
<td>1.06</td>
<td></td>
</tr>
<tr>
<td>$k_e$</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>$\mu_m$</td>
<td>533.0</td>
<td>1</td>
<td>541.0</td>
<td>1.027</td>
<td></td>
</tr>
<tr>
<td>$m_g$</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>$m_n$</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total relative variance</td>
<td>881.388</td>
<td>22.11</td>
<td>893.0</td>
<td>9.907</td>
<td></td>
</tr>
</tbody>
</table>

**Table 4.5** Variance of parameters at pH 4 (variance-covariance matrix main diagonal components)

The figures are relative values with respect to the lowest observed in each row.
<table>
<thead>
<tr>
<th>Model</th>
<th>Parameter</th>
<th>Monod-Monod</th>
<th>Monod-Contois</th>
<th>Contois-Monod</th>
<th>Contois-Contois</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_g$ or $B_g$</td>
<td>15.29</td>
<td>2.62</td>
<td>10.58</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>$K_n$ or $B_n$</td>
<td>44.6</td>
<td>1</td>
<td>110.94</td>
<td>1.21</td>
</tr>
<tr>
<td></td>
<td>$k_e$</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>$\mu_m$</td>
<td>9.09</td>
<td>1</td>
<td>212.66</td>
<td>1.31</td>
</tr>
<tr>
<td></td>
<td>$m_g$</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>$m_n$</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total relative variance</td>
<td>153.79</td>
<td>7.62</td>
<td>337.18</td>
<td>6.52</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 4.6** Variance of parameters at pH 6 (variance-covariance matrix main diagonal components)

The figures are relative values with respect to the lowest observed in each row.
4.2 Estimation of parameters for the batch culture model.

Data from the batch culture were treated by the same procedure as the data from the continuous culture. The objective functions was set as follows:

$$J(X) = \sum_{i=1}^{nep} \sum_{j=1}^{nev} (C_j(x, y_{ei}, t_i))^2 \quad (4.4)$$

where:
- $C_j$ : components of the MSE vector.
- $X$ : estimated value of the parameter vector whose components are the same as these for the continuous culture parameter vector.
- $Y_{ei}$ : dependent variable vector whose components are (the $i$ subscript corresponding to the $i$th experimental observation)
  - $y_{1i}$ : glucose concentration, $C_g$.
  - $y_{2i}$ : ammonium ion concentration, $C_n$.
  - $y_{3i}$ : dry biomass concentration, $C_x$.
  - $y_{4i}$ : first derivative with respect to time of biomass concentration.
  - $y_{5i}$ : first derivative with respect to time of glucose concentration.
  - $y_{6i}$ : first derivative with respect to time of ammonium ion concentration.
- $t_i$ : independent variable vector whose components are:
  - $t_i$ : time.
The first derivatives of $y_{1i}$, $y_{2i}$ and $y_{3i}$ were generated by a subroutine called by the main programme using a second order approximation, i.e.:

$$\frac{dy}{dt} = \frac{y_{i+1} - y_{i-1}}{2h}$$

(4.5)

where $h$ is the step length between two successive observations.

The MSE were presented in four different algebraic forms called Mode 5, Mode 6, Mode 7 and Mode 8 by the programme. These are listed in the Appendix B6 for the Monod–Monod model.

The programme succeeded in Modes 6 and 7 of the MSE although not for all the data treated. A careful analysis of the data suggested that the inclusion of the variables $y_{4i}$, $y_{5i}$ and $y_{6i}$ (first derivatives of concentration) and the uncertainty of glucose concentration around exhaustion, caused serious problems in the execution of the optimisation routine.

The computer programme output from the set of data in which the programme succeeded gave values of the saturation constant $K_n$ and Contois constant $B_n$ for ammonium equal to zero. From this fact, it was deduced that in the batch culture, the fermentation was single substrate limited with glucose as the limiting nutrient. In view of this, a graphical approach to analysing the batch culture data was used. The additional terms used in the graphical procedure are:

- $r_x$: growth rate
- $r'_x$: apparent growth rate ($r_x - k C_x$)
- $R'_x$: apparent specific growth rate ($r'_x/C_x$)
- $r_g$: glucose consumption rate
- $R'_g$: specific glucose consumption rate, ($r'_g/C_x$)
- $r_n$: ammonium ion consumption rate
- $R'_n$: specific ammonium ion consumption rate, ($r'_n/C_x$)
- $r_e$: endogenous respiration rate ($k C_x$)
In a batch culture, the mass balance equations with no mass transfer effects considered, can be stated as:

\[
\frac{dC_x}{dt} = r_x - kC_x e^x \quad (4.6)
\]

\[
\frac{dC_g}{dt} = -\frac{m r}{g x} \quad (4.7)
\]

\[
\frac{dC_n}{dt} = -\frac{m r}{n x} \quad (4.8)
\]

where \( r_x \) may be expressed in terms of either a Monod function or a Contois function of glucose. The evaluation of parameters was carried out as described below.

A. Stoichiometric coefficient for substrates.

Dividing Equation (4.7) by Equation (4.6) gives:

\[
\frac{dC_g}{dC_x} = -\frac{\frac{m r}{g x}}{\frac{r_x - kC_x e^x}{x}} \quad (4.9)
\]

At low cell concentration and excess of substrate, the term \( kC_x e^x \) can be neglected and integration of Equation (4.9) gives:

\[
\frac{C^o}{g} - C = -m \left(\frac{C^o}{g} - C_x\right) \quad (4.10)
\]

similarly:

\[
\frac{C^o}{n} - C_n = -m \left(\frac{C^o}{n} - C_x\right) \quad (4.11)
\]

Equations (4.10) and (4.11) are expressions derived from the Monod's concept (1949) of growth associated substrate consumption, when the substrate concentration is in excess and the endogeneous respiration activity is not significant. A graph of \( C_g \) (or \( C_n \)) versus \( C_x \) will produce a straight line with slope \( -\frac{m}{g} \) (or \( -\frac{m}{n} \)). The data will deviate from the straight line as \( C_x \) increases,
FIGURE 4.1 Stoichiometric coefficient determination at pH 3.0

\[ m(x) = 1.584 \text{ kg/kg} \]
\[ m_n(o) = 0.072 \text{ kg/kg} \]

FIGURE 4.2 Stoichiometric coefficient determination at pH 4.0

\[ m(x) = 1.89 \text{ kg/kg} \]
\[ m_n(o) = 0.1044 \text{ kg/kg} \]
the limiting substrate concentration decreases and the term $k_e C_x$ becomes significant. Figures 4.1 and 4.2 show the graphs of $C_g$ versus $C_x$ and $C_n$ versus $C_x$ at pH 3 and 4. The additional graphs (pH 5.5, 6 and 7) can be examined in the Appendix D1.

B. Endogeneous respiration coefficient.

Rearrangement of Equations (4,6), (4,7) and (4,8) gives:

\[
- \frac{1}{m} \frac{dC}{dC_x} = 1 + \frac{k_e}{R'_x} \tag{4.12}
\]

and

\[
- \frac{1}{m} \frac{dC}{dC_x} = 1 + \frac{k_e}{R'_x} \tag{4.13}
\]

A plot of $-(dC/dC_x)/m$ versus $(1/R'_x)$ will produce a straight line with slope $k_e$ and intercept 1. The same will apply to the equivalent plot for ammonium ion given by Equation (4.13). Double response data (Johnson and Berthouex, 1975) were used to determine $k_e$, i.e., both quantities $-(dC/dC_x)/m$ and $-(dC/dC_x)/m$ were plotted versus $(1/R'_x)$ Figure (4.3) shows the double response data plot for the determination of $k_e$ at pH 3. Other plots can be examined in Appendix D1.

C. Constants of the growth rate equation.

Monod single kinetics and Contois single kinetics with glucose as the rate limiting substrate were tested. For Monod kinetics, Equations (4,6), (4,7) and (4,8) can be rearranged as follows:
FIGURE 4.3  Determination of endogeneous respiration constant for batch culture at pH 3.0
\[
\frac{1}{R' + k_e} = \frac{K}{\mu_m} \frac{1}{C_g} + \frac{1}{\mu_m} \quad (a)
\]

\[
- \frac{m}{R'_g} = \frac{K}{\mu_m} \frac{1}{C_g} + \frac{1}{\mu_m} \quad (4.14) \quad (b)
\]

\[
- \frac{m}{R'_n} = \frac{K}{\mu_m} \frac{1}{C_g} + \frac{1}{\mu_m} \quad (c)
\]

Although ammonium ion concentration does not limit the growth rate, its response from the system represented by Equation (4.14c) can be included in the determination of \( K_g \) and \( \mu_m \).

In theory, a graph of \((1/R'_x + k_e)\) or \((-m/R'_g)\) or \((-m/R'_n)\) versus \((1/C'_g)\) should produce a straight line with slope \( K_g/\mu_m \) and intercept \((1/\mu_m)\), (Lineweaver-Burk double reciprocal plot). All three responses were used in the determination of \( K_g \) and \( \mu_m \).

The equivalent expressions for Contois single kinetics are:

\[
\frac{1}{R' + k_e} = \frac{B_g}{\mu_m} \frac{C_x}{C_g} + \frac{1}{\mu_m} \quad (a)
\]

\[
- \frac{m}{R'_g} = \frac{B_g}{\mu_m} \frac{C_x}{C_g} + \frac{1}{\mu_m} \quad (4.15) \quad (b)
\]

\[
- \frac{m}{R'_n} = \frac{B_g}{\mu_m} \frac{C_x}{C_g} + \frac{1}{\mu_m} \quad (c)
\]

The left hand side of the above Equations should be plotted versus \((C_x/C'_g)\). Figure (4.4) shows the multiresponse plots for Monod and Contois kinetic models at pH 3. Other graphs can be examined in the Appendix D2. The estimated parameter values are summarised in Table 4.7.
FIGURE 4.4 Determination of growth rate constants for the Batch culture at pH 3.0 (a) Monod kinetics and (b) Contois kinetics. The Y axis in either case are:

- \((*)\) \(\frac{1}{(R'_x + k_e)}\)
- \((x)\) \(-\frac{m}{R'_e} \frac{kg}{kg}\)
- \((o)\) \(-\frac{m}{R'_n} \frac{kg}{kg}\)
<table>
<thead>
<tr>
<th>Parameter</th>
<th>pH</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>m_g kg/kg</td>
<td>3.0</td>
<td>1.584</td>
<td>1.890</td>
<td>1.80</td>
<td>2.286</td>
</tr>
<tr>
<td>m_n kg/kg</td>
<td></td>
<td>0.072</td>
<td>0.104</td>
<td>0.109</td>
<td>0.126</td>
</tr>
<tr>
<td>k_e 1/hr</td>
<td></td>
<td>0.044</td>
<td>0.045</td>
<td>0.05</td>
<td>0.067</td>
</tr>
<tr>
<td>K_g kg/m^3</td>
<td></td>
<td>0.400</td>
<td>0.905</td>
<td>0.840</td>
<td>0.904</td>
</tr>
<tr>
<td>U_m 1/hr</td>
<td></td>
<td>0.290</td>
<td>0.303</td>
<td>0.310</td>
<td>0.339</td>
</tr>
<tr>
<td>B_g kg/kg</td>
<td></td>
<td>0.118</td>
<td>0.311</td>
<td>0.229</td>
<td>0.319</td>
</tr>
<tr>
<td>U_m 1/hr</td>
<td></td>
<td>0.259</td>
<td>0.263</td>
<td>0.289</td>
<td>0.298</td>
</tr>
</tbody>
</table>

**TABLE 4.7** Parameter values for the single kinetics Monod model and single kinetics Contois model for the batch culture.
4.3 A test of the insignificance of mass transfer effects.

The kinetic models examined previously assumed that the rate limiting step was the kinetic process and that the mass transfer effects could be neglected. These assumptions were tested as shown below.

4.3.1 Mass transfer in the continuous culture.

To verify the insignificance of mass transfer effects in the continuous culture, the following criteria were tested:

(a) For the transfer of oxygen into the continuous culture fluid.

If the rate of oxygen transfer from the gas bulk into the culture fluid of the fermentor considerably exceeds the rate of oxygen uptake by the microorganisms, it is unlikely that the process was controlled by the rate of absorption of gaseous species. Hence, the process can be described in terms of the kinetic expressions only.

(b) For the transfer of dissolved species (glucose and oxygen) from the culture fluid to the biological transport (or metabolic) region.

If the difference of concentrations between the external and the internal shell of the non-biological transport zone (i.e., diffusion zone) around a single microorganism is negligible, it is unlikely that the mass transfer through the film affected the process to a significant extent. Therefore, the process can be described by the kinetic expressions in terms of the bulk fluid concentrations.
4.3.1.1 Transfer of oxygen in the continuous culture. Non-biological interphase.

The volumetric rate of oxygen transfer from gas phase to culture fluid is given by:

\[ q_{O_2} = H K_v (C^*_{O_2} - C_{O_2}) \]  \hspace{1cm} (4.16)

or

\[ q_{O_2} = k_a (C^*_{O_2} - C_{O_2}) \]  \hspace{1cm} (4.17)

where:

- \( H \): Henry's law constant for oxygen.
- \( K_v \): volumetric absorption coefficient.
- \( k_a \): volumetric transfer coefficient.
- \( C^*_{O_2} \): oxygen concentration in liquid phase in equilibrium with bulk gas oxygen concentration.
- \( C_{O_2} \): oxygen concentration in bulk liquid.
- \( q_{O_2} \): volumetric rate of oxygen transfer.

A liquid phase oxygen balance over the fermentor at steady state gives:

\[ q_{O_2} - r_{O_2} = 0 \]  \hspace{1cm} (4.18)

where \( r_{O_2} \) is the rate of oxygen uptake by the microorganisms.

Assuming that the oxygen uptake rate is associated with both growth rate and endogeneous respiration rate, the oxygen uptake rate is given by:

\[ r_{O_2} = m_{ox} r_x + m_{oe} k C \]  \hspace{1cm} (4.19)

where \( m_{ox} \) and \( m_{oe} \) are the appropriate stoichiometric coefficients.
It is assumed that the values for \( m_o \) and \( m_{oe} \) reported by Sinclair and Ryder (1975), can be used.

At steady state in a continuous culture:

\[
 r_x = (D + k_e)C_x \tag{4.20}
\]

where \( D \) is the dilution rate. Hence, Equation (4.19) becomes:

\[
 r_o = m DC_x + (m_o + m_{oe})k C \tag{4.21}
\]

The reported values for the stoichiometric coefficients are: \( m_o = 0.709 \) kg/kg and \( m_{oe} = 6.286 \) kg/kg (Sinclair and Ryder, C. utilitis in glycerol-oxygen, 1975). The rate of oxygen uptake by the microorganisms was calculated for each run of the continuous culture. It was assumed that the stoichiometric coefficients \( m_o \) and \( m_{oe} \) are independent of pH.

The fermentor capacity for transfer of oxygen from the gas phase into the culture fluid is given by:

\[
 q_c = k_{la} (C_{O_2}^* - C_{O_2}) \tag{4.22}
\]

The value of \( k_{la} \) depends on the hydrodynamic conditions in the reactor and on the fluid properties. It was found that the average value of \( k_{la} \) can be taken as 224 (1/hr), assuming an efficiency of 50% for the transport of oxygen. (The calculation of \( k_{la} \) is shown in the Appendix E1). If an efficiency of 100% is assumed, the value of \( k_{la} \) is doubled. Finally, the capacity of oxygen transfer depends on the concentration difference \( (C_{O_2}^* - C_{O_2}) \). Figure 4.5 shows the average capacity of the fermentor to transfer oxygen and the rate of oxygen uptake by the microorganisms.
FIGURE 4.5 Rate of oxygen uptake in continuous culture ($r_{O_2}$) at different pH levels and average capacity of oxygen transfer rate of the fermentor ($q_c$) at several levels of oxygen concentration in the culture fluid.
It can be observed that the capacity of the fermentor to transfer oxygen was reasonably higher than the uptake rate by the microorganisms at a dissolved oxygen concentration of 50% of saturation. If the concentration of oxygen increases, the value of \( q_c \) decreases but in such a situation the availability of oxygen in the culture can be excluded from limiting the growth rate. The oxygen concentration was kept to a minimum of 50% of saturation, hence, it can be assumed that there was at least a concentration of 0.0037 kg/m\(^3\) in the fermentor (the saturation concentration of oxygen at 30°C and 0.21 atm is 7.4 x 10\(^{-3}\) kg/m\(^3\)). If the growth and endogeneous respiration rate expressions are considered to be affected by a Monod type function involving oxygen, the value of the term \( \frac{C_{O_2}}{K_{O_2} + C_{O_2}} \), \( K_{O_2} \) being the saturation constant for oxygen, is of the order of 0.97, assuming that the saturation constant for oxygen is of the order of 8 x 10\(^{-5}\) kg/m\(^3\) (Synclair and Ryder, 1975). Therefore, the oxygen availability in the culture fluid does not affect the kinetic process.

4.3.1.2 Transfer of oxygen and glucose from the culture fluid to the cellular material.

The transport of a nutrient \( S \) through a film surrounding a single cell, from the culture fluid to the biological (or metabolic) transport zone can be expressed as:

\[
 r_s = k_{l a} (C_s - C_{si}) 
\]

(4.23)

where:
- \( C_s \): concentration of \( S \) at the external shell of non-biological transport film.
- \( C_{si} \): concentration of \( S \) at the internal shell of non-biological transport film.
- \( k_{l a} \): volumetric mass transfer coefficient.
The rate of substrate uptake is equal to the rate of substrate transport into the biological region, if steady state conditions are assumed to prevail in each region involved in the process. Hence, the mass balance of glucose and oxygen produces the following expressions:

for glucose: \[(k_a)_{lg} (C_g - C_{gi}) - m_r = 0\] (4.25)

for oxygen: \[(k_a)_{lo_2} (C_{o_2} - C_{o_2 i}) - (m_r x + m_{o_2} x k_{o_2}) = 0\] (4.26)

The difference of concentration in the non-biological transport film can then be expressed as:

\[\Delta C_g = C_g - C_{gi} = \frac{m_r x}{(k_a)_{lg}}\] (4.27)

\[\Delta C_{o_2} = C_{o_2} - C_{o_2 i} = \frac{m_r x + m_{o_2} x k_{o_2}}{(k_a)_{lo_2}}\] (4.28)

Estimated values of \(k_a\) for glucose and oxygen are \(7.8 \times 10^2\) (1/hr) and \(2.82 \times 10^3\) (1/hr) respectively (see Appendix E2 for calculations). The difference of concentration \(\Delta C_g\) and \(\Delta C_{o_2}\) are shown as percentage of the actual bulk fluid concentration in Figure 4.6, for the continuous culture.

It is observed that for oxygen, the maximum difference of concentration was about 5.5% of the bulk fluid concentration which was taken as an average of 50% of the saturation value, and it occurs at a dilution rate of about 0.2 hr\(^{-1}\). This value decreased at lower and higher dilution rates. For glucose, the maximum difference of concentration in the film surrounding a single cell was about 6% of the actual bulk concentration at pH 5 and less than 3% at other pH values. It can be concluded that a minimum of 0.945 \(C_{o_2}\) and 0.94 \(C_g\) were available at the biological transport zone and therefore, the kinetic process can be described in terms of the bulk fluid concentrations with no mass transfer limitations.
FIGURE 4.6 Concentration difference in the film surrounding a single cell for oxygen and glucose. Continuous culture at various pH levels.
4.3.2 Mass transfer in the batch culture.

It can be expected that the transfer of gaseous species into the batch culture fluid followed the same pattern as that observed in the continuous culture. Dissolved oxygen was kept to a minimum of 50% of saturation and it increased to almost saturation values at the end of the growth phase. The hydrodynamic conditions in both type of cultures were essentially the same. Therefore, it is quite reasonable to assume that the dissolved oxygen level in the batch culture was well in excess to fulfil the biological requirements and that the transport of it through the film surrounding a single cell did not affect the kinetic process to such an extent to invalidate the independence of the kinetic expressions of oxygen concentration.

To examine the mass transfer of the limiting substrate in the batch culture, the Powell's model was simulated using typical values of the parameters calculated from the batch culture data. It was stated in Section 1.1.3.1 that the specific volumetric rate of substrate removal was given by:

\[
R = \frac{R_{max}}{2(k_2')^2} \left[ (1 + (k_2')^2 + k_3c^*) - \left( (1+(k_2')^2-k_3c^*)^2+4k_3c^* \right)^{\frac{1}{2}} \right] \quad (4.29)
\]

The terms in Equation (4.29) were defined in Section 1.1.3.1, Equations (1.20) to (1.23). The mass balance of substrate and biomass in a batch culture with endogeneous respiration taken into account can be stated as follows:

\[
\frac{dC_s}{dt} = -RC_x \quad (\text{for substrate}) \quad (a)
\]

\[
\frac{dC_x}{dt} = \frac{R}{m} C_x - \frac{k}{c_x} C_x \quad (\text{for biomass}) \quad (4.30)
\]

where \( R \) is the specific rate of substrate removal and \( m \) the stoichiometric coefficient for substrate.

It has been pointed out (Atkinson, 1974) that the quantity \( k_2' \) can be used as a criterion to assess the extent to which mass transfer affects the whole process. Figure 4.7 shows the simulation of the model stated by Equations (4.29) and (4.30) for various values of \( k_2' \).
FIGURE 4.7 Powell's model response. Diffusion with biochemical reaction and endogeneous respiration in a batch culture.

The values of the parameters in the simulation are:

\[ \frac{R_{\text{max}}}{m} = 0.285 \text{ (1/hr)} \], \[ m = 1.6 \text{ (kg/m}^3) \], \[ \frac{1}{k_3} = 0.43 \text{ kg/m}^3 \]

\[ k_e = 0.047 \text{ (1/hr)} \]

The estimated value of \( k_2' \) for the batch culture was found to be \( 3 \times 10^{-2} \) (see Appendix E3 for the calculation of \( k_2' \)). For this value of \( k_2' \) the model response is graphically indistinguishable from the model response when \( k_2' = 0 \), i.e., when there are no mass transfer limitations at all. Hence, assuming that the process can be described by the kinetic expressions in terms of the bulk fluid concentrations only is correct.
In Section 5.1, the optimisation procedure is discussed in conjunction with the kinetic model for the continuous culture. Section 5.2 deals with the kinetic model for the batch culture. The pH effect on the fermentation process is examined in Section 5.3 and some examples of the PHEM model introduced in Section 2.4 are given in Section 5.4.

5.1 The optimisation technique and the kinetic model for the continuous culture.

5.1.1 Model discrimination.

The discrimination of the kinetic model that follows, is based on the results of the mathematical analysis presented in Section 4.1.

Examination of Table 4.3 (values of \( J(X) \) at the minimum), predicts that, from the point of view of the model structure, all four models examined described the process adequately. This result is not surprising if it is taken into account that Monod's function has been shown to describe a wide range of fermentation process adequately and that the Contois function, as other models, represent only a better-data-fit expression. Examination of Table 4.4 (Performance index values), however, produces clear evidence about which of the four models is the 'best one' to describe
the process. There is no doubt that the Contois-Contois double kinetic model is the most suitable, and that the Contois-Monod model shows the largest deviation from the experimental data in all cases.

Inspection of Tables 4,5 and 4,6 (variance of parameters) confirms the prediction of the performance index values; the Contois-Contois model presents the lowest total variance of parameters followed by the Monod-Contois model. The Contois-Monod model parameters show the largest total variance, hence it is the least suitable to represent the process. It can be observed in Tables 4,5 and 4,6 that the parameters $k_e$, $m$ and $m$ present the same variance independent of the model; this is an expected result because these parameters do not depend on the model structure for the growth rate equation.

The model responses for the continuous culture are presented in Figures 5,1 to 5,4 for pH values 4 and 6; the graphs for pH 5 and 5.5 can be examined in Appendix Gl. At pH 4, the Monod-Monod, Monod-Contois and Contois-Contois models are almost indistinguishable graphically. At pH values 5, 5.5 and 6 the visual inspection confirms the result of the previous analysis, i.e., the Contois-Contois model describes the experimental results better than the other three models.

In the following Section, a comparison of the parameter values with others reported in the literature will provide an additional criterion to assess the reliability of the optimisation approach.

5.1.2 Comparison of the model parameters for the continuous culture with other values reported in the literature.

It is not uncommon to find, in the literature, great discrepancies among the values of parameters of models reported by several workers. This is due to the different conditions at
FIGURE 5.1 Model response for the continuous culture at pH 4.

Biomass.

- Monod-Monod
- Monod-Contois
- Contois-Monod
- Contois-Contois

(●) Experimental
FIGURE 5.2 Model response for the continuous culture at pH 4.
Substrates.

- **Monod-Monod**
- **Monod-Contois**
- **Contois-Monod**
- **Contois-Contois**
FIGURE 5.3 Model response for the continuous culture at pH 6.

Biomass.

--- Monod-Monod
----- Monod-Contois
------ Contois-Monod
------- Contois-Contois
(•) Experimental.
FIGURE 5.4 Model response for the continuous culture at pH 6. Substrates.

- Monod-Monod.
- Monod-Contois.
- Contois-Monod.
- Contois-Contois.
which the experiments are carried out. For example, a given microorganism-carbon source system would have a different value of the yield constant if the nutrient solution is enriched by only traces of some growth factors. Topiwala and Sinclair (1971) and Eroshin et. al. (1976) showed that such parameters as the endogeneous respiration coefficient, maximum specific growth rate and yield change with temperature significantly within a short interval. The effect of pH reviewed in Section 1.3 is another important factor to consider when comparing parameter values.

Several values of parameter found in the literature were selected for comparison purposes. There are various microorganisms as well as carbon source listed. Parameter values were nearly always selected from those derived from mathematical modelling.

Examination of Tables 5.1 to 5.6 shows that the parameter values reported here compare favorably with the values reported elsewhere.

5.1.3 The optimisation procedure.

The optimisation technique introduced here, has proved to be highly efficient for the analysis of data from the continuous culture. Such a technique allows a deep analysis of experimental data while avoiding the bias that occurs when analysing data which present excessive experimental error. It was found that the algebraic form of the model structure equations can be manipulated to produce a better behaved function. The algebraic form of Modes 3 and 4 (see Table 4.1) have a lower degree of non-linearity than the algebraic forms of Mode 1 and 2, i.e., the quotients which appear in Modes 1 and 2 do not appear in Modes 3 and 4. It seems that this was the reason for the success of the optimisation procedure for the Modes 3 and 4.
<table>
<thead>
<tr>
<th>$\mu_m$ 1/hr</th>
<th>Culture type</th>
<th>pH</th>
<th>Microorganism and substrate</th>
<th>Model characteristics and reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>continuous</td>
<td>-</td>
<td>A. aerogenes in glucose-ammonium</td>
<td>Contois(glucose) single kinetics, no endogeneous respiration.</td>
</tr>
<tr>
<td>2.0</td>
<td>continuous</td>
<td>-</td>
<td>A. aerogenes in glucose-ammonium</td>
<td>Contois(ammonium) single kinetics, no endogeneous respiration.</td>
</tr>
<tr>
<td>0.55</td>
<td>continuous</td>
<td>-</td>
<td>A. aerogenes in succinate-ammonium.</td>
<td>Contois(succinate) single kinetics, no endogeneous respiration.</td>
</tr>
<tr>
<td>0.2</td>
<td>continuous</td>
<td>5.5</td>
<td>Candida lipolytica in n-dodecane</td>
<td>Monod single kinetics, no endogeneous respiration. Moo-Young et al (1971)</td>
</tr>
<tr>
<td>0.6</td>
<td>continuous</td>
<td>3.5</td>
<td>Candida utilis in sugar (glucose-fructuose)</td>
<td>Monod single kinetics, with endogeneous respiration. Paredes-Lopez et al (1976)</td>
</tr>
</tbody>
</table>

TABLE 5.1 Comparison of parameter values. Maximum specific growth rate. (Cont. next page)
<table>
<thead>
<tr>
<th>( \mu \text{m} )</th>
<th>Culture type</th>
<th>pH</th>
<th>Microorganism and substrate</th>
<th>Model characteristics and reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.210</td>
<td>batch</td>
<td>3.0</td>
<td>T. viride in glucose</td>
<td>Monod single kinetics, endogeneous respiration.</td>
</tr>
<tr>
<td>0.076</td>
<td>batch</td>
<td>3.0</td>
<td>T. viride in glucose</td>
<td>Contois single kinetics, endogeneous respiration. Nihtila and Virkkunen (1977).</td>
</tr>
<tr>
<td>0.039</td>
<td>continuous</td>
<td>2.5</td>
<td>T. viride in glucose</td>
<td>Monod single kinetics, endogeneous respiration.</td>
</tr>
<tr>
<td>0.111</td>
<td>continuous</td>
<td>3.0</td>
<td>T. viride in glucose</td>
<td>Brown and Zainudeen (1977)</td>
</tr>
<tr>
<td>0.154</td>
<td>4.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.312</td>
<td>flask</td>
<td>7.3</td>
<td>C. utilis in glycerine</td>
<td>Lirova et al (1978a)</td>
</tr>
<tr>
<td>0.384</td>
<td>4.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.321</td>
<td>continuous</td>
<td>4.0</td>
<td>C. lipolytica in glucose-ammonium</td>
<td>Contois-Contois double kinetics with endogeneous respiration. This work</td>
</tr>
<tr>
<td>0.354</td>
<td>5.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.343</td>
<td>5.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.351</td>
<td>6.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 5.1** (cont.) Comparison of parameter values. Maximum specific growth rate.
<table>
<thead>
<tr>
<th>B kg/kg</th>
<th>Culture type</th>
<th>pH</th>
<th>Microorganism and substrate</th>
<th>Model characteristics and reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.09</td>
<td>continuous</td>
<td>4</td>
<td>C. utilitis in glycerol-oxygen</td>
<td>Contois(glycerol)-Monod(oxygen) double kinetics with endogeneous respiration. Sinclair and Ryder (1975).</td>
</tr>
<tr>
<td>0.668</td>
<td>batch</td>
<td>3</td>
<td>T. viride in glucose</td>
<td>Contois single kinetics with endogeneous respiration. Nihtila and Virkkunen (1977)</td>
</tr>
<tr>
<td>0.90</td>
<td>continuous</td>
<td>2.6-3.0</td>
<td>A. foetidus in sucrose</td>
<td>Contois single kinetics with endogeneous respiration. Kristiansen and Sinclair (1979)</td>
</tr>
<tr>
<td>0.0244</td>
<td>continuous</td>
<td>4.0</td>
<td>C. lipolytica in glucose-ammonium</td>
<td>Contois-Contois double kinetics with endogeneous respiration. This work.</td>
</tr>
<tr>
<td>0.0103</td>
<td></td>
<td>5.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0412</td>
<td></td>
<td>5.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0929</td>
<td></td>
<td>6.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 5.2** Comparison of parameter values. Contois constant of carbon source.
<table>
<thead>
<tr>
<th>Culture type</th>
<th>pH</th>
<th>Microorganism and substrate</th>
<th>B (carbon source) (kg nitrogen source)</th>
<th>B&lt;sub&gt;C&lt;/sub&gt;/B&lt;sub&gt;N&lt;/sub&gt;</th>
<th>Model characteristics and reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>continuous</td>
<td>4.0</td>
<td>C. lipolytica in glucose-ammonium</td>
<td>4.147</td>
<td>0.237</td>
<td>Contois-Contois double kinetics with endogenous respiration. This work.</td>
</tr>
<tr>
<td>continuous</td>
<td>5.0</td>
<td>A. aerogenes in glucose-ammonium</td>
<td>58.61</td>
<td>0.098</td>
<td>Contois(azlactone) single kinetics. Contois(azlactone) single kinetics. Contois(SM93) single kinetics.</td>
</tr>
<tr>
<td>continuous</td>
<td>5.5</td>
<td>A. aerogenes in succinate-ammonium</td>
<td></td>
<td>0.306</td>
<td>Contois(azlactone) single kinetics. Contois(SM93) single kinetics.</td>
</tr>
<tr>
<td>continuous</td>
<td>6.0</td>
<td>C. lipolytica in glucose-ammonium</td>
<td></td>
<td>0.530</td>
<td>Contois(azlactone) single kinetics. Contois(SM93) single kinetics.</td>
</tr>
</tbody>
</table>

**TABLE 5.3** Comparison of parameter values. Contois constant for nitrogen source.
<table>
<thead>
<tr>
<th>$k_e$ (1/hr)</th>
<th>Culture type</th>
<th>pH</th>
<th>Microorganisms and substrate</th>
<th>Model characteristics and reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.08</td>
<td>continuous</td>
<td>7.1</td>
<td><em>A. aerogenes</em> in glucose</td>
<td>Monod single kinetics.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(30 °C)</td>
<td>Topiwala and Sinclair (1971)</td>
</tr>
<tr>
<td>0.0702</td>
<td>continuous</td>
<td>5.5</td>
<td><em>S. cerevisiae</em> in glucose-riboflavin</td>
<td>Monod-Monod double kinetics.</td>
</tr>
<tr>
<td>0.01</td>
<td>continuous</td>
<td>4.0</td>
<td><em>C. utilis</em> in glycerol-oxygen</td>
<td><em>Contois</em>(glycerol)-Monod(oxygen).</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sinclair and Ryder (1975)</td>
</tr>
<tr>
<td>0.0084</td>
<td>batch</td>
<td>3.0</td>
<td><em>T. viride</em> in glucose</td>
<td>Monod and <em>Contois</em> single kinetics.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Nihtila and Virkkonen (1977)</td>
</tr>
<tr>
<td>0.00196</td>
<td>continuous</td>
<td>2.5</td>
<td><em>T. viride</em> in glucose</td>
<td>Monod single kinetics.</td>
</tr>
<tr>
<td>0.00620</td>
<td></td>
<td>3.0</td>
<td></td>
<td>Brown and Zainudeen (1977).</td>
</tr>
<tr>
<td>0.00815</td>
<td></td>
<td>4.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0223</td>
<td>continuous</td>
<td>4.0</td>
<td><em>C. lipolytica</em> in glucose-ammonium</td>
<td>Contois-Contois double kinetics.</td>
</tr>
<tr>
<td>0.0147</td>
<td></td>
<td>5.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0167</td>
<td></td>
<td>5.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0237</td>
<td></td>
<td>6.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 5.4** Comparison of parameter values. Endogeneous respiration coefficient.
<table>
<thead>
<tr>
<th>Y (kg/kg)</th>
<th>Culture type</th>
<th>pH</th>
<th>Microorganism and substrate</th>
<th>Model characteristics and reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.50</td>
<td>continuous</td>
<td>7.1 (30°C)</td>
<td>A. aerogenes in glucose</td>
<td>Monod single kinetics with endogeneous respiration. Topiwala and Sinclair (1971)</td>
</tr>
<tr>
<td>0.55</td>
<td>continuous</td>
<td>4.0</td>
<td>C. utilitis in glycerol-oxygen</td>
<td>Monod(oxygen )-Contois(glycerol) with endogeneous respiration. Sinclair and Ryder (1975)</td>
</tr>
<tr>
<td>0.430</td>
<td>continuous</td>
<td>2.5</td>
<td>T. viride in glucose</td>
<td>Monod single kinetics with endogeneous respiration. Brown and Zainudeen (1977)</td>
</tr>
<tr>
<td>0.495</td>
<td></td>
<td>3.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.533</td>
<td></td>
<td>4.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.486</td>
<td>continuous</td>
<td>4.0</td>
<td>C. lipolytica in glucose-ammonium</td>
<td>Contois-Contois double kinetics with endogeneous respiration. This work.</td>
</tr>
<tr>
<td>0.536</td>
<td></td>
<td>5.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.422</td>
<td></td>
<td>5.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.431</td>
<td></td>
<td>6.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 5.5** Comparison of parameter values. Yield of carbon source.
<table>
<thead>
<tr>
<th>Y kg/kg</th>
<th>Culture type</th>
<th>pH</th>
<th>Microorganism and substrate</th>
<th>Model characteristics and reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.103</td>
<td>flask</td>
<td>5.3</td>
<td>Scopulariopsis brevicalis in malt extract-ammonium.</td>
<td>Bothast et al. (1975)</td>
</tr>
<tr>
<td>8.503</td>
<td></td>
<td>7.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.000</td>
<td></td>
<td>9.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.416</td>
<td></td>
<td>10.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.416</td>
<td></td>
<td>10.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.14</td>
<td>flasks</td>
<td>4.5-7</td>
<td>C. utilis in glucose-ammonium</td>
<td>Lirova et al. (1978a)</td>
</tr>
<tr>
<td>7.14</td>
<td>continuous</td>
<td>2.3-7.8</td>
<td>C. utilis in glycerol-ammonium</td>
<td>Lirova et al. (1978b)</td>
</tr>
<tr>
<td>9.718</td>
<td>continuous</td>
<td>4.0</td>
<td>C. lipolecta in glucose-ammonium</td>
<td>Contois-Contois double kinetics with endogeneous respiration. This work.</td>
</tr>
<tr>
<td>9.389</td>
<td></td>
<td>5.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.84</td>
<td></td>
<td>5.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.539</td>
<td></td>
<td>6.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE 5.6 Comparison of parameter values. Yield of nitrogen source.
The method developed here differs from other optimisation methods (Nihtila and Virkkunen, 1977; Johnson and Berthouex, 1975) which have been used in the mathematical modelling of fermentation processes in the fact that what is fundamentally tested is the model structure. Therefore, it has not been assumed that the model structure is adequate, as a simple sum of squares function (Equations (1.87) or (1.89)) would assume.

Existence of a minimum of the objective function of a given set of experimental data is not, per se, a proof of the model adequacy and therefore the analysis should be implemented with some other criteria such as the variance of the parameters and the deviation from the experimental data. The final frame of reference is found in the literature, hence, a comparison of parameter values is required.

The failure of the optimisation procedure with data from the batch culture is probably due to either, the mathematical properties of the objective function for such a case, or a failure in the algorithm chosen to solve the minimisation problem. Each set of experimental data generates a particular objective function which depends on the particular value of the dependent variables; the first derivatives of the dry biomass and substrates concentration were estimated with the actual experimental data, introducing additional sources of error. All these values were used to calculate the gradient and the Hessian matrix of the objective function $J(X)$ and if they showed an abnormal behaviour in any of their components, the whole procedure was stopped.

Future improvement to the general scheme of the optimisation procedure would consist of finding out the most adequate algorithm to minimise the objective function generated from batch culture data. It depends on the mathematical properties of such functions and an in-depth analysis of such properties is required. As it was outside of the scope of this work, such a possibility is open to future research.
5.2 The kinetic model for the batch culture.

5.2.1 Model response for the batch culture.

The model for the batch culture stated in Section 2.2.1.2 was simulated with the parameter values estimated in Section 4.2. The following results were found:

i) There was no significant difference between the single kinetics Monod model and the single kinetics Contois model. From this point of view, both models are equivalent.

ii) Both model responses showed a strong deviation from the experimental data.

Figures 5.5 and 5.6 show the model responses for the batch culture at pH 6 and 7. Examination of the graphs suggested that the deviations were due to a fault in the model conception; particularly in the introduction of the endogeneous respiration term. Despite the work of Nihtila and Virkkunen (1977) that the endogeneous respiration concept can be introduced in a batch culture model as it was introduced here, it was found that the inclusion of the endogeneous respiration term in the batch culture model predicts a lower accumulation of biomass than observed.

On the other hand, if endogeneous respiration is neglected, the model will predict a stationary phase with no cellular material consumption as has been observed elsewhere (Monod, 1949; Luedking and Piret, 1959; Constantinides et al., 1970; Tsao and Hanson, 1975; Marchal et al., 1977).

It is known that certain batch cultures show a stationary phase prior to decay phase (Monod, 1949; Dean and Hinshelwood, 1966; Brown and Halsted, 1975), and that endogeneous respiration becomes significant when the culture is under starving conditions (Herbert, 1958; Dawes and Ribbons, 1964).
FIGURE 5.5 Model response for the batch culture at pH 6.

- - - Monod kinetics. ----- Contois kinetics

(●) Biomass, (X) Glucose, (○) Ammonium.
FIGURE 5.6  Model response for the batch culture at pH 7.

--- Monod kinetics. ------ Contois kinetics.

(*) Biomass, (x) Glucose, (o) Ammonium.
In the case examined here, the batch culture did not present stationary phase; the consumption of cellular material was detected immediately after the growth has ceased and it was more active at starving conditions than when there was carbon source still available.

In the following Section, an alternative model for the batch culture is proposed, based on the observations mentioned in the above paragraph.

5.2.2 An alternative model for the batch culture.

Based on the data from the batch culture and on information in the literature discussed in the preceding section, the following model is proposed for the batch culture.

The model assumes that endogenous respiration is present at the end of the growth phase and that it is fully developed when the limiting substrate is exhausted. This implies that the substrate acts as a repressor of the endogeneous respiration activity. In other words, when substrate is available in sufficient quantities to fulfil both synthesis and energetic requirements, the endogeneous respiration activity is not manifested in the form of cell material consumption, but once the substrate has run out, the respiration activity is diverted to consumption of cellular material pools. This is reflected in a decrease of biomass. Hence, the endogeneous respiration rate is given by:

$$ r_e = k_e x \frac{K_{GL}}{K_{GL} + C} $$

where $K_{GL}$ is an inhibition constant for glucose. The inhibition term in Equation (5.1) was proposed by Ierusalimskii (1965) for metabolic activity repression by a certain agent. Although the Ierusalimskii expression has been used in the modelling of inhibited growth by product (Ierusalimskii, 1965; Aiba et al., 1968) it
may be applied to any type of repression of metabolic activity, which in this case is consumption of cellular material by endogeneous respiration inhibited by substrate. Therefore, the model equations for the batch culture are stated as follows:

\[ r' = r - k \frac{X}{e^x} \frac{C}{K + C} \]

\[ r = -m \frac{r}{g} X \]

\[ r = -m \frac{r}{n} X \]

This model predicts the following behaviour of the batch culture: when \( C \gg K \), the endogeneous respiration term is negligible and is not manifested during the exponential growth phase. As the substrate is gradually exhausted, the growth rate diminishes and the endogeneous respiration starts to be significant, hence, by the end of the growth phase, a lower cell concentration than expected if no endogeneous respiration were present is observed. At substrate exhaustion, growth ceases and endogeneous respiration is fully developed. The rate of decay of cellular material in the later phase is given by \( k e^x \).

This model was tested in the batch culture. The results are examined below. The particulars of parameter evaluation can be examined in Appendix D3.

The model response of the batch culture at pH 6 and 7 are shown in Figures 5.7 and 5.8 (other graphs in Appendix G2). If the graphs are compared with those shown before (Figures 5.5 and 5.6) a significant improvement can be appreciated. It can be said that the alternative model for the batch culture is satisfactory. The value of the parameters are summarised in Table 5.7.
FIGURE 5.7 Model response for the batch culture at pH 6. Alternative model. Endogeneous respiration repressed by substrate (glucose).
FIGURE 5.8  Model response for the batch culture at pH 7.  
Alternative model.  Endogeneous respiration repressed by substrate (glucose).
TABLE 5.7 Kinetic parameters of the alternative model for the batch culture. Monod single kinetics with endogeneous respiration repressed by the carbon source (glucose).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>3</th>
<th>4</th>
<th>5.5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_g$ (kg/m$^3$)</td>
<td>0.345</td>
<td>0.995</td>
<td>1.08</td>
<td>1.30</td>
<td>1.29</td>
</tr>
<tr>
<td>$\mu_m$ (1/hr)</td>
<td>0.256</td>
<td>0.292</td>
<td>0.325</td>
<td>0.329</td>
<td>0.555</td>
</tr>
<tr>
<td>$k_e$ (1/hr)</td>
<td>0.044</td>
<td>0.045</td>
<td>0.050</td>
<td>0.067</td>
<td>0.107</td>
</tr>
<tr>
<td>$K_{gi}$ (kg/m$^3$)</td>
<td>1.62</td>
<td>0.63</td>
<td>0.162</td>
<td>0.90</td>
<td>0.0054</td>
</tr>
</tbody>
</table>

A further comment on endogeneous respiration is needed. Pirt (1966) suggested that the endogeneous respiration concept introduced by Herbert (1958) "seems artificial and indirect" and showed that the concept of endogeneous metabolism should be better linked to substrate consumption than to cell material consumption. Additionally, Pirt suggested that the observation of stationary phases after growth in batch cultures supported such an idea.

The results obtained here for the batch culture are in direct opposition to Pirt's idea with respect to the expected stationary phase after the growth has ceased, but does not oppose the concept of endogeneous metabolism being supported by the substrate consumption instead of cellular material consumption during the growth phase.
It is not uncommon to misinterpret the true nature of natural phenomena when physical significance is given to parameters of mathematical models. From the modelling point of view, it seems more honest to say that the fermentation system is satisfactorily described by Herbert's concept in the continuous culture without invalidation of the Pirt's concept of maintenance energy production from substrate consumption during the growth phase, but Herbert's concept is more adequate to describe the batch culture after the growth has ceased.

5.3 The pH effect on the fermentation process.

The pH effect on the continuous culture kinetic process is discussed with those on the batch culture. The pH effects are examined in the light of the model parameter responses to hydrogen ion concentration.

5.3.1 pH effect on growth rate constants.

Two general opinions have been developed to explain the pH effect on the growth rate constants. The bottle-neck concept (i.e., a single metabolic reaction affected by pH, controls the whole fermentation process) developed by the Russian School in the 60's (Ierusalimskii, 1965; Ibragimova et al., 1969; Andreeva, 1969) and a later tendency to associate the pH effect on parameters such as the Monod saturation constant and maximum specific growth rate with membrane transport phenomena (Muzychenko, 1973; Brown and Halsted, 1975; Brown and Zainudeen, 1977). The permease model for membrane transport developed by the Paris School (Cohen and Rickenberg, 1956; Monod, 1956; Cohen and Monod, 1957) seemed to be an adequate solution to link enzyme kinetics with membrane transport phenomena.
It was mentioned in Section 2.3 that the bottle-neck concept is valid from the modelling point of view, but almost unrealistic from the biological counterpart in the light of the strong effects of pH in the whole cell organisation mentioned in Section 1.3. Therefore, enzyme kinetics have limited possibilities to explain the complex responses of the non-structured model parameters with respect to pH. On the other hand, the Muzychenko's model (Section 1.3) has been the only one provided up to now, to suggest a possible explanation of the pH effects on the mentioned parameters, considering membrane transport phenomena. With exception of the example presented in the original paper (1973), no other experimental evidence to support the general framework of the model has been presented.
The permease model, being supported by enzyme kinetics presents the same faults as these and, in addition, is currently under strong scrutiny of its fundamentals (Simoni and Postma, 1975; Hamilton, 1975; Mitchell, 1977).

The pH effect on the growth rate parameters observed in this work confirms the observations made in the preceding paragraphs. Neither an enzyme-like kinetics model nor the Muzychenko's model provided an adequate explanation of the behaviour of such parameters with respect to pH. Therefore, the pH effect is discussed from a qualitative point of view.
A. Maximum specific growth rate.

Figures 5.9 and 5.10 show the pH effect on the growth rate parameters for continuous and batch cultures respectively. The maximum specific growth rate in the continuous culture shows a slight tendency to increase towards low hydrogen ion concentration; this tendency is observed too in the batch culture in the range of pH 4 to 6, although the values of $\mu_m$ are slightly higher for the continuous culture than for the batch culture. Brown and Halsted (1975) reported a $\mu_m$ value of 0.077 hr$^{-1}$ for the batchwise culture of T. viride in glucose at pH 3.28°C and Brown and Zainudeen (1977) reported a $\mu_m$ value of 0.111 hr$^{-1}$ for the continuous culture of the same microorganism-carbon source-pH system at 30°C. The difference of values may be due to the temperature at which the experiments were carried out, which has been shown to affect strongly the maximum specific growth rate (Topiwala and Sinclair, 1971). Pirt and Callow (1960) reported that the values of $\mu_m$ for P. chrysogenum in glucose were almost the same in batch as in continuous culture at a given pH value. These observations suggest that in excess of substrate conditions, the continuous culture and the batch culture show little difference with respect to the growth rate and that the maximum specific growth rate value is essentially the same in the pH range 4 to 6.

B. Contois constants of the continuous culture model.

The Contois constants of the limiting substrates glucose and ammonium were found to be strongly influenced by pH. $B_g$ shows a minimum at pH 5 with more accelerated increase at higher pH values than a lower pH. $B_n$ shows a strictly increasing behaviour in the whole pH range. The increase of $B_n$ at high pH values predicts that the ammonium ion concentration becomes increasingly important in the kinetic expression as pH rises; hence, the double limitation (carbon source-nitrogen source) on growth is stronger at high pH values.
C. Monod saturation constant (glucose) of the alternative model for the batch culture.

The behaviour of the Monod saturation constant of glucose in the batch culture is complex (Figure 5.10). At low pH values, $K_g$ tends to zero, suggesting that the growth rate will be independent of glucose concentration even at very low concentration of substrate. This may occur at a pH value around 3, but the growth will not halt because the maximum specific growth rate does not fall to zero in such a situation. Brown and Zainudeen (1977) found this type of behaviour for the saturation constant of glucose in the continuous culture of glucose at low pH levels, but they found that the growth come to a halt because $\mu_m$ showed the same trend as that of the saturation constant towards low pH values.

At pH values between 4 and 5.5 it seems that $K_g$ is little affected by the change of hydrogen ion level, although the tendency is to increase. Between pH values 5.5 to 6, $K_g$ shows a marked increase and it comes to stable values at around neutral pH levels. The general tendency of $K_g$ is to increase the growth rate dependence on glucose towards high pH levels.

After examining the pH effect on the growth rate constants, the personal opinion of the author is that:

- Up to the present there is not a simple non-structured model to explain the pH effect on the growth rate parameters.

- The most promising mechanism to model mathematically membrane transport is that supported by the Chemiosmotic Theory (Mitchell, 1977; Hamilton, 1975)

- A model considering membrane transport should take into account two barriers that have been identified: the energetic barrier represented by the electrical properties of the membrane (Muzychenko's model) and the material barrier represented by the mass of the membrane itself, which has been found to change with pH (Lirova et al., 1978b).
5.3.2 pH effect on the stoichiometric coefficients.

Figure 5.11 shows the pH effect on the stoichiometric coefficients of the continuous and batch culture. Both cultures show a more efficient utilisation of the carbon source when going towards low pH values than towards high pH values; the same tendency is observed with the nitrogen source stoichiometric coefficient.
Examination of Figure 1.4, Section 1.3.1, shows that the pH dependence of stoichiometric coefficients (inverse of yield) is varied and irregular, hence, a general agreement is not expected.

Batch cultures accumulate more biomass at low pH levels than at high pH levels for the same quantity of substrate consumed. This suggests that maintenance requirements are low at a high concentration of hydrogen ions. The ratio of carbon source to nitrogen source utilisation \( \frac{m_g}{m_n} \) show the same trend in both types of culture in the pH range 4 to 6. This suggest that the chemical composition of the cells is changed with pH as it has been observed elsewhere (Gaudy, 1975). The rise of the ratio of carbon source to nitrogen source utilisation at extremes of the range of pH 3 to 6 suggests that the cellular metabolism is diverted to synthesis of nitrogen free compounds. This observation agrees with the findings of Lirova et al. (1978b); it was reported that there is an increment of lipids content in the biomass at extreme pH values in the form of lipid granules and, presumably, cell wall materials.

The cellular biochemical composition is not contemplated by non-structured models, therefore, a simple explanation is not expected for the change in the ratio \( \frac{m_g}{m_n} \), derived from such models, but it is worthwhile to point out such data to serve as future reference.

5.3.3 pH effect on endogeneous respiration coefficients.

Figure 5.12 shows the effect of pH on endogeneous respiration coefficients for batch and continuous cultures. The curves show that a significant difference exists between the two type of cultures with respect to relative values of the parameter and the tendency of the curves towards low pH values. The behaviour of the endogeneous respiration activity with respect to pH for the continuous culture agrees with that reported by Lirova et al. (1978b) for the continuous culture of C. utilitis in glycerine,
although a tendency to reduce endogeneous metabolism to insignificant levels as pH decreases has been reported by Brown and Zainudeen (1977) for the continuous culture of T. viride in glucose. There is no data of pH effect on $k_e$ for batch cultures to compare with the results obtained here but examination of the results of Brown and Halsted (1975) and Brown and Zainudeen (1977) shows that the endogeneous respiration activity of a given microorganism in a continuous culture, differs from that in a batch culture.

High yield in the continuous culture correspond to low endogeneous respiration activity. This suggests that utilisation
of chemical pools for synthesis and energy production is more efficient at pH values around 5 than at lower or higher pH levels. Table 5.8 shows the values of the maintenance coefficient (Pirt's concept) of glucose in the continuous culture.

**TABLE 5.8 Maintenance coefficient of glucose for the continuous culture.**

<table>
<thead>
<tr>
<th>pH</th>
<th>m&lt;sub&gt;c&lt;/sub&gt; kg/kg/hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.0458</td>
</tr>
<tr>
<td>5</td>
<td>0.0274</td>
</tr>
<tr>
<td>5.5</td>
<td>0.0395</td>
</tr>
<tr>
<td>6</td>
<td>0.0549</td>
</tr>
</tbody>
</table>

The maintenance coefficient (Pirt's concept) m<sub>c</sub> is related to the stoichiometric coefficient m<sub>c</sub> and endogeneous respiration coefficient (Herbert's concept) k<sub>e</sub>, by the relationship: m<sub>c</sub> = m<sub>c</sub> k<sub>e</sub>, m<sub>c</sub> given in kg of substrate consumed for maintenance purposes per kg of cells per hour.

Endogeneous respiration coefficients for the batch culture were determined by the cellular mass consumption rate after the growth has ceased and substrate was exhausted, therefore, a connection between high yields and low endogeneous respiration activity is not direct. Despite what is stated above, it can be said that in the batch culture, as in the continuous culture, when the substrate was utilised more efficiently for synthesis, the cell material consumption to provide energy for survival was more efficient too. The efficiency of the metabolic networks in that respect increased at low pH values in the batch culture. It should be pointed out that examination of the results of Brown and Zainudeen (1977) and Lirova et. al. (1978b) shows that high yields correspond to high endogeneous respiration activity and in the former case, correspond to high maintenance requirements which is in opposition to the results found in this work.
5.3.4 pH effect on glucose inhibition constant for endogeneous respiration in the batch culture.

Functions containing inhibition terms have been incorporated into non-structured models to account for deviations observed experimentally. Therefore, the observation that an environmental variable affects the inhibition constants demonstrates once again, that non-structured models are crude approximations to describe natural processes. Figure 5.13 shows the pH effect on the inhibition constant $K_{gi}$ of glucose for the repression of endogeneous metabolism in the batch culture. The value of $K_{gi}$ at pH 6, shows a great discrepancy with the trend of the curve at other pH values.

After examining the behaviour of $K_{gi}$ with pH, it is the opinion of the author that a simple biological interpretation is not feasible. However, the shape of the curve indicates that at low pH values, the endogeneous metabolism was inhibited by glucose concentration to a lesser extent that at high pH values.
5.4 Some examples of the PHEM model.

In this Section, some examples of the PHEM model, proposed in Section 2.4 are given. These are referred to growth associated and cell concentration associated reaction parameters. The identification of the expression which describes the pH effect on the parameters of the reaction rate expression given here, was carried out by comparison with the curves generated by the limiting cases of the PHEM model given in Section 2.4.1
<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>KINETIC EXPRESSION</th>
<th>EXPRESSION DERIVED FROM THE PHEM MODEL</th>
<th>FIGURE</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha )</td>
<td>( r_p = \alpha r_x / \beta C_x )</td>
<td>( \alpha = 2.2 + 4.0/(1 + 8 \times 10^{-5}/C_H) )</td>
<td>5.14</td>
<td>Lactic acid formation by <em>L. delbrueckii</em> in glucose. Luedking and Piret (1959)</td>
</tr>
<tr>
<td>( \beta )</td>
<td>( \beta = 0.025 + 0.55/(1 + C_H/7.94 \times 10^{-6}) )</td>
<td></td>
<td>5.14</td>
<td>( r_p ): lactic acid formation rate</td>
</tr>
<tr>
<td>( \lambda )</td>
<td>( r_p = \lambda r_x )</td>
<td>( \lambda = 15.2/(1 + C_H/3.98 \times 10^{-4}) )</td>
<td>5.15</td>
<td>Citric acid formation by <em>A. foetidus</em> in glucose. Kristiansen and Sinclair (1979)</td>
</tr>
<tr>
<td>( m_n )</td>
<td>( r_s = -m r_{nx} )</td>
<td>( m_n = 0.094 + 0.03/(1 + 2.81 \times 10^{-8}/C_H) )</td>
<td>5.16</td>
<td>Ammonium ion consumption by <em>S. brevicaulis</em> in malt extract-ammonium. Bothast et al (1975).</td>
</tr>
<tr>
<td>( m_g )</td>
<td>( r_s = -m r_{gx} )</td>
<td>( m_g = 1.87 + 1.13/(1 + 4.89 \times 10^{-3}/C_H) )</td>
<td>5.17</td>
<td>Glucose consumption by <em>T. viride</em> Brown and Zainudeen (1977)</td>
</tr>
<tr>
<td>( k_e )</td>
<td>( a ), ( r_e = k e C_x )</td>
<td>( k_e = 0.0126 + 1.03 \times 10^{-10} + \frac{C_H}{9.718 \times 10^{-3}} )</td>
<td>5.18</td>
<td><em>C. lipolytica</em> in glucose ammonium. This work.</td>
</tr>
<tr>
<td></td>
<td>( b ), ( r_e = k e x g e / K g_i C g_i + C_g )</td>
<td>( k_e = 0.044 + 0.074/(1 + C_H/4.26 \times 10^{-7}) )</td>
<td>5.19</td>
<td>(a) ( r_e ): endogeneous respiration rate in continuous culture.</td>
</tr>
</tbody>
</table>

**TABLE 5.9** Some examples of the PHEM model.
Evaluation of the constants involved in each expression was performed by graphical analysis of the properties of the pH dependant functions involved in the model in conjunction with the experimental data provided in each example.

The examples of the PHEM model are summarised in Table 5.9 and Figures 5.14 to 5.19. The curves shown in the graphs are the pH dependant functions given in Table 5.9 for each case.

Examination of the graphs shows that the model describes satisfactorily the pH effect on the parameters in question.
FIGURE 5.16 The pH effect on the stoichiometric coefficient (nitrogen source) of the substrate consumption
equation: \( r_s = -m_r x \).
Bothast et al. (1975).

FIGURE 5.17 The pH effect on the stoichiometric coefficient (carbon source) of the substrate consumption
equation: \( r_s = -m_r x \).
The pH effect on the endogeneous respiration coefficient of the continuous culture of *C. lipolytica*. This work.

The pH effect on the endogeneous respiration coefficient of the batch culture of *C. lipolytica*. This work.
The PHEM model expressions differ from the enzyme-like kinetics expressions in the fact that the former expressions are composed of additive functions of pH (see Equations 2.50 and 2.51) while the enzyme-like kinetic expressions are composed of multiplicative functions of pH. With some exceptions, the multiplicative functions of pH used in enzyme kinetics (see Section 2.3) tend invariably to zero at one extreme (sometimes at both extremes) of the pH range, hence their failure to predict lower non-zero stationary values of the parameters than the maximum values observed.

Four parameters (Figures 5.14(a), 5.16, 5.17 and 5.19) out of the seven examples given here, tend to non-zero stationary values when going towards a certain extreme of the pH range. Examination of Table 5.9 shows that the pH dependent functions which describe these parameters contain a term which is independent of pH. The PHEM model assumes that this term corresponds to the reactions which are not affected by H or OH ions.

The general scheme of the PHEM model suggests parallel metabolic pathways in which one of the routes is pH independent and the other (or others) can be affected by H or OH ions. This is in direct contradiction of Jerusalimskii's bottle-neck concept in which a single metabolic reaction is assumed to control the whole process. The idea of parallel metabolic pathways has been used to model a certain effect observed in the lactic acid fermentation by L. delbrueckii in glucose (Tanner and Overley, 1974) and identification of possible metabolic routes has been performed recently for the citric acid fermentation by C. lipolytica in glucose (Aiba et al., 1979). Therefore, the scheme assumed by the PHEM model has some biological basis. Finally, although the PHEM model cannot explain, per se, the biological mechanisms of the fermentation reactions, it does describe the parameter dependence of pH of the examples given here, with good approximation.
CONCLUSIONS

The optimisation technique proved to be highly efficient for the model discrimination and parameter evaluation of the continuous culture, providing a routine method to obtain valuable information from fermentation systems that can be used in process design and optimisation.

An in-depth mathematical analysis of the properties of the objective function proposed in this work, is required to detect the true causes of the failure of the optimisation procedure with data from the batch culture.

The continuous culture of Candida lipolytica can be described by a double kinetic Contois-Contois interactive model with glucose and ammonium ion as the limiting growth substrates. The batch culture of Candida lipolytica in the same medium, can be described by a single kinetic Monod model with glucose as the rate limiting substrate and endogeneous respiration repressed by the carbon source.

All the model parameters of continuous and batch cultures were found to be affected significantly by the concentration of hydrogen ion in the culture fluid. The pH effect on growth rate constants of the continuous and batch cultures, cannot be explained by any current non-structured model.

Conditions for growth and substrate utilisation efficiency were better at pH about 5 than at higher or lower pH levels in the continuous culture.
Conditions for growth and substrate utilisation efficiency improved at low pH values (3 to 4) with respect to neutral pH values (6 to 7) in the batch culture.

The PHEM model taking into account the effect of pH on growth rate associated and cell concentration associated reaction parameters, is adequate to describe some experimental values of such parameters reported in the literature. The pH effect on the endogeneous respiration coefficient of the batch and continuous culture of Candida lipolytica reported in this work, can be described by the PHEM model introduced here.
APPENDICES
Some enzyme kinetics mechanisms.

In the following list, Michaelis-Menten kinetics are assumed unless otherwise stated. In all equilibrium reactions (double arrowed reactions), the equilibrium constant is defined as a dissociation constant in agreement with the general use, e.g., in the equilibrium reactions:

\[
\begin{align*}
Y & \underset{K_1}{\leftrightarrow} (XY) \underset{K_2}{\leftrightarrow} (XYZ)
\end{align*}
\]

the equilibrium constants are defined as:

\[
K_1 = \frac{[X][Y]}{[XY]} \quad \text{and} \quad K_2 = \frac{[XY][Z]}{[XYZ]}
\]

where the brackets stand for the concentration of species X, XY and XYZ.

\(E_0\) represents total enzyme concentration.


\[
r_p = \frac{k E_0 (S_1)(S_2)}{(K_1 + S_1)(K_2 + S_2)}
\]
B. Double kinetics. Compulsory-order-ternary complex mechanism.

(Briggs-Haldane kinetics).

\[
E + S_1 \xleftarrow{k_1} (ES_1) + S_2 \xrightarrow{k_3} (ES_1S_2) \rightarrow P + E
\]

\[r_p = \frac{k E_0(S_1)(S_2)}{K + K_1S_1 + K_2S_2 + (S_1)(S_2)}\]

where: \(K = k_2(k + k_4)/(k_1k_3), \ K_1 = (k + k_4)/k_3, \ K_2 = k/k_1\).

if \(k_2 = k\), the reaction rate is given by:

\[r_p = \frac{k E_0(S_1)(S_2)}{(S_1 + k/k_1)(S_2 + \frac{k_4 + k}{k_3})}\]

C. Haldane type inhibition by excessive substrate concentration.

\[r_p = \frac{k E_0(S)}{K_1 + S + S^2/K_2}\]

D. Competitive inhibition by H ions.

\[E \xleftarrow{K_1} (ES) \xrightarrow{k} E + P\]
E. Competitive inhibition by OH ions.

\[ r_p = \frac{kEoS}{K_1(1 + H/K_H) + S} \]

F. Uncompetitive inhibition by H or OH ions.

\[ r_p = \frac{kEoS}{K_1(1 + K_{OH}/H) + S} \]

\[ K_{OH} = 10^{-14}/K_{OH} \]

if \( I = H \),

\[ r_p = \frac{kE_0(S)(1 + H/K_H)}{S + K_1/(1 + H/K_H)} \]

if \( I = OH \),

\[ r_p = \frac{kE_0(S)(1 + K'_{OH}/H)}{S + K_1/(1 + K'_{OH}/H)} \]
G. Non-competitive inhibition by H or OH ions.

\[
\begin{align*}
E & \xrightleftharpoons[K_1]{K_I} (ES) \xrightarrow{k} E^* + P \\
I & \xrightleftharpoons[K_1]{K_I} S \xrightarrow[k]{(E)} (EIS)
\end{align*}
\]

if \( I = H \), \[ r_p = \frac{k E_0 (S) / (1 + H/K_H)}{S + K_1} \]

if \( I = OH \), \[ r_p = \frac{k E_0 (S) / (1 + K'_{OH}/H)}{S + K_1} \]

H. Competitive inhibition by H and OH ions.

\[
\begin{align*}
E & \xrightleftharpoons[K_H]{K_{OH}} (EH) \\
S & \xrightarrow[K_1]{(E)} (ES) \xrightarrow{k} P + E \\
& \xrightarrow[K_{OH}]{(E)} (EOH)
\end{align*}
\]

\[ r_p = \frac{k E_0 (S)}{K_H (1 + H/K_{OH} + K_{OH}/H) + (S)} \]
I. Non-competitive inhibition by H and OH ions.

\[
\begin{align*}
(EOH) & \xrightarrow{K_{OH}} OH \quad \xrightarrow{K_H} (EH) \\
S & \xrightarrow{K_1} \quad \xrightarrow{K_1} S \\
(EOHS) & \xrightarrow{K_{OH}} OH \quad \xrightarrow{K_H} (EHS) \\
E & \xleftarrow{K} \quad \xleftarrow{K} E \\
(P + E) & \xrightarrow{k} \\
\end{align*}
\]

\[
\frac{kE_o}{S} = \frac{k}{1 + H/K_H + K_{OH}/H}
\]

J. Competitive inhibition by H and OH ions and substrate ionisation by H ions.

\[
\begin{align*}
(SH) & \xrightarrow{H/K_H} S + E \\
K_1 & \xrightarrow{K_{OH}} OH \quad \xrightarrow{K_H} (EOH) \\
(ES) & \xrightarrow{k} P + E \\
\end{align*}
\]

\[
r_p = \frac{kE_o}{K_{p_1}p_2 + S_o}, \quad \text{where} \quad S_o = (SH) + S,
\]

\[
p_1 = (1 + H/K_H^s) \quad \text{and} \quad p_2 = (1 + H/K_H^e + K_{OH}/H)
\]
K. Substrate ionisation by H and OH ions.

\[
\begin{align*}
(SH) & \xrightarrow{H} S + E \xrightarrow{K_1} (ES) \xrightarrow{k} P + E \\
(SOH) & \xrightarrow{OH} \quad r_p = \frac{k E_o S_o}{K_1 (1 + H/K_H + K'_{OH}/H) + S_o}
\end{align*}
\]

where

\[S_o = (S) + (SH) + (SOH)\]

L. Competitive inhibition by H and OH ions and substrate ionisation by H and OH ions.

\[
\begin{align*}
(EH) & \xrightarrow{H} E + S \xrightarrow{K_H} (SH) \\
(EOH) & \xrightarrow{OH} \quad r_p = \frac{k E_o S_o}{K_1 P_1 P_2 + S_o}
\end{align*}
\]

where

\[S_o = (S) + (SH) + (SOH),\]

\[P_1 = (1 + H/K_H + K'_{OH}/H) \quad \text{and} \quad P_2 = (1 + H/K_H + K'_{OH}/H)\]
Competitive inhibition by H and OH ions and ionisation of (ES) complex by H and OH ions.

\[ r = \frac{k_E S}{P_1 P_2} \]

where:

\[ P_1 = \frac{1}{1 + \frac{H}{K_H^e} + \frac{H}{K_H^{e/H}}} \]

\[ P_2 = \frac{1}{1 + \frac{H}{K_H^s} + \frac{H}{K_{OH}^s/H}} \]
Extreme values of a function of several variables.

Let $J$ be a real function of the vectorial variable $X$, i.e.

$$J = J(X)$$

or

$$\mathbb{R}^n \xrightarrow{J} \mathbb{R}$$

where: $\mathbb{R}$ : the real numbers field

$\mathbb{R}^n$ : real vector space of dimension $n$ over $\mathbb{R}$.

$J$ : unidimensional real function of vectorial variable $X$

$X$ : vectorial variable of dimension $n$, $X \in \mathbb{R}^n$.

1. Gradient of $J(X)$.

The gradient of $J(X)$, $\text{grad}(J(X))$, is defined as:

$$\text{grad}(J(X)) = \left( \frac{\delta J}{\delta x_1}, \frac{\delta J}{\delta x_2}, \ldots, \frac{\delta J}{\delta x_n} \right)$$

2. Hessian matrix of $J(X)$.

The Hessian matrix, $H$, of $J(X)$ is defined as:

$$H(X) = \frac{\delta^2 J(X)}{\delta x_j \delta x_i} \quad i, j = 1, n$$

where $x_i$, $x_j$ are the components of $X$. 
3. Critical points of $J(X)$.

$x^0$ is a critical point of $J(X)$ if:

$$\nabla J(x^0) = 0$$

4. Extreme points of $J(X)$.

Let $x^0$ be a critical point of $J(X)$. If:

(a) $x^T H(x^0)x > 0$ for all $x \neq 0$ in $\mathbb{R}^n$, then $x^0$ is a relative minimum.

(b) $x^T H(x^0)x < 0$ for all $x \neq 0$ in $\mathbb{R}^n$, then $x^0$ is a relative maximum.

(c) $x^T H(x^0)x$ has both positive and negative values as $x$ ranges through $\mathbb{R}^n$, then $x^0$ is a saddle point.

$x^T$ stands for the transpose of $x$.
APPENDIX B2

Variance-covariance matrix of $\bar{X}$.

Let $\bar{X}^0$ be the value of the parameter vector at the minimum of $J(\bar{X})$. Let $S$ be the value of $J(\bar{X})$ at $\bar{X}^0$. The variance-covariance matrix $V$ of $\bar{X}$ at $\bar{X}^0$ can be approximated as:

$$ V = \frac{2S}{\text{nep} - \text{np}} H^{-1}(\bar{X}^0) $$

where $H^{-1}$ is the inverse of the Hessian matrix of $J(\bar{X})$, nep is the number of experimental points and np is the number of parameters.

The components of $V$ are:

Main diagonal: $v_{ii}, i = 1, \text{np}$ variance of the parameter $x_i$

Other components: $v_{ij}, i,j = 1, \text{np}, i \neq j$ covariance of parameter $x_i$ with respect to parameter $x_j$

The standard deviation $S_d$ of the $x_j$ parameter is given by:

$$ S_d(x_j) = (v_{ii})^{1/2} $$

The $(1 - \alpha)\%$ confidence limits of the parameter $x_j$ are given by:

$$ x_j^0 - t S_d(x_j) \leq x_j \leq x_j^0 + t S_d(x_j) $$

where $t$ is the value of the $t$-distribution (Student) with a level of significance $\alpha$ and nep - np degrees of freedom. $x_j^0$ is the value of the jth component of $\bar{X}^0$.

First and second partial derivatives of the Model Structure Equations of the continuous culture, Contois-Contois kinetics, Mode 4 in the optimisation programme.

The algebraic form of the MSE for the Contois-Contois model are given below.

\[
C_1 = x_4 y_1 y_2 - (x_1 y_3 + y_1)(x_2 y_3 + y_2)(x_3 + y_4)
\]

\[
C_2 = y_4(C_{gF} - y_1) - x_5(x_3 + y_4)y_3
\]

\[
C_3 = y_4(C_{nF} - y_2) - x_6(x_3 + y_4)y_3
\]

where:

- \(y_1\): glucose concentration
- \(y_2\): ammonium ion concentration
- \(y_3\): dry biomass concentration
- \(y_4\): dilution rate
- \(x_1\): glucose Contois constant
- \(x_2\): ammonium ion Contois constant
- \(x_3\): endogeneous respiration coefficient
- \(x_4\): maximum specific growth rate
- \(x_5\): glucose stoichiometric coefficient
- \(x_6\): ammonium ion stoichiometric coefficient

Each component of the MSE vector \(C = (C_1, C_2, C_3)\) has six first partial derivatives respect to the parameters \(x_1, x_2, x_3, x_4, x_5\) and \(x_6\). They are given below. Only those partial derivatives different from zero are listed.
First partial derivatives of $C_1$ different from zero.

\[
\begin{align*}
\frac{\delta C_1}{\delta x_1} &= - (x_2 y_3 + y_2)(x_3 + y_4)y_3 \\
\frac{\delta C_1}{\delta x_2} &= - (x_1 y_3 + y_1)(x_3 + y_4)y_3 \\
\frac{\delta C_1}{\delta x_3} &= - (x_1 y_3 + y_1)(x_2 y_3 + y_2) \\
\frac{\delta C_1}{\delta x_4} &= y_1 y_2
\end{align*}
\]

First partial derivatives of $C_2$ different from zero.

\[
\begin{align*}
\frac{\delta C_2}{\delta x_3} &= - x_5 y_3 \\
\frac{\delta C_2}{\delta x_5} &= - (x_3 + y_4)y_3
\end{align*}
\]

First partial derivatives of $C_3$ different from zero.

\[
\begin{align*}
\frac{\delta C_3}{\delta x_3} &= - x_6 y_3 \\
\frac{\delta C_3}{\delta x_6} &= - (x_3 + y_4)y_3
\end{align*}
\]

Each component of the MSE vector has 36 second partial derivatives. The following list presents only those different from zero.
Second partial derivatives of $C_1$ different from zero.

\[ \frac{\delta^2 C_1}{\delta x_2 \delta x_1} = - (x_3 + y_4)y_3^2 \]

\[ \frac{\delta^2 C_1}{\delta x_3 \delta x_1} = - (x_2y_3 + y_2)y_3 \]

\[ \frac{\delta^2 C_1}{\delta x_1 \delta x_2} = - (x_3 + y_4)y_3^2 \]

\[ \frac{\delta^2 C_1}{\delta x_1 \delta x_3} = - (x_1y_3 + y_1)y_3^2 \]

Second partial derivatives of $C_2$ different from zero.

\[ \frac{\delta^2 C_2}{\delta x_5 \delta x_3} = - y_3 \]

Second partial derivatives of $C_3$ different from zero.

\[ \frac{\delta^2 C_3}{\delta x_6 \delta x_3} = - y_3 \]

\[ \frac{\delta^2 C_3}{\delta x_3 \delta x_6} = - y_3 \]
Algebraic solution of the Model Structure Equations of the continuous culture at steady state. Contois-Contois double kinetics model.

Algebraic arrangement of the MSE of the continuous culture at steady state produces the following expression for the Contois-Contois double kinetics model:

\[
C_g = C_e - \frac{m C(D + k_e)}{g x} \quad \frac{(D + k_e)}{g F}
\]

\[
C_n = C_e - \frac{m C(D + k_e)}{n x} \quad \frac{(D + k_e)}{n F}
\]

where \( C_x \) is the solution of the quadratic equation:

\[
A C_x^2 + B C_x + C = 0
\]

where:

\[
A = - \left[ \left( \mu_m - D - k_e \right) m m (1 + k_e/D)^2 - (D + k_e) B B + m B (D + k_e)(1 + k_e/D) \right]
\]

\[
B = (\mu_m - D - k_e)(1 + k_e/D)(m C_g + m C_p) + (D + k_e)(C_n B + C_p B)
\]

\[
C = - (\mu_m - D - k_e) C_x C_F
\]

\[ C_x \] is given by:

\[
C_x = \frac{-B + \left( B^2 - 4AC \right)^{\frac{1}{2}}}{2A}
\]
Computer programme flow chart of the optimisation procedure.

The computer programme was composed of a main programme and several subroutines. These are described below. Subroutines DERIV1, DERIV2, FUNCT2, HESS2, MODEL1 and STAT were developed by the author. Subroutines E04LAF and FO1AAF are available in the Mark 6 library of SWUCN (South West Universities Computer Network) but the flow charts are not available for inspection. E04LAF and FO1AAF are described in terms of their parameters.

A. Main Programme.

The programme described here calculates:

(a) The minimum of the function $J(X)$

$$J(X) = \sum_{i=1}^{nep} \sum_{j=1}^{nev} \left(C_j(X,Y_i)\right)^2$$

where: $C_j$ are the components of the model structure equations vector given in APPENDIX B3. nev number of dependant variables and nep is the number of experimental points.

(b) The solutions $(Y^*)$ of the model structure equations at the point $X^*$, corresponding to the minimum of $J(X)$.

(c) The value of the performance index $PI(X)$, at the point $X$, corresponding to the minimum of $J(X)$.

$$PI(X) = \sum_{i=1}^{nep} \sum_{j=1}^{nev} \left(Y^*_{ij} - Y_{ij}\right)^2$$
where $y_t$ are the solution of the model structure equations corresponding to the $j$th experimental value of the dilution rate.

(d) The variance-covariance matrix of the parameters at the minimum of $J(x)$.

(e) The 99%, 95% and 90% confidence limits of the parameters (components of $x$) at the minimum of $J(x)$.

Constants, parameters and variables of the programme.

Data supplied by the user.

$NP$: integer, $NP$ specifies the number of parameters of the model (dimension of $x$).

$NEV$: integer, $NEV$ specifies the number of experimental variables. $NEV$ is the sum of the number of independant variables and dependant variables which are given in a common array. For example, in the continuous culture $NEV = 4$, corresponding to 3 dependant variables ($C_g$, $C_n$, $C_o$) and 1 independant variable (dilution rate).

$NEP$: integer, $NEP$ specifies the number of experimental points.

$X$: real array of dimension ($NP$), $X$ contains the initial estimated of the parameter vector. See APPENDIX B3 for the definition of the components of $X$.

$Y$: real array of dimension ($NEP$, $NEV$), $Y$ contains the values of the $NEV$ experimental variables corresponding to the $NEP$ experimental points:

$Y(J,1) =$ glucose concentration at the $J$th experimental point.

$Y(J,2) =$ ammonium ion concentration at the $J$th experimental point.
\( Y(J,3) \) = dry biomass concentration at the Jth experimental point.

\( Y(J,4) \) = dilution rate at the Jth experimental point.

\( J = 1, 2, \ldots, \text{NEP} \)

MODE : integer, MODE specifies the algebraic form of the model structure equations. See table 4.1

TS : real array of dimension (3), TS contains the values of t (t-distribution) at \((1 - \alpha)\%\) of confidence and \(\text{NEP} - \text{NP}\) degrees of freedom.

\( TS(1) = t \) for \( \alpha = 0.01 \)
\( TS(2) = t \) for \( \alpha = 0.05 \)
\( TS(3) = t \) for \( \alpha = 0.10 \)

Internal variables.

J : real, \( J \) contains the value of \( J(X) \) at the current value of the parameter vector \( X \).

G : real array of dimension (NP), \( G \) contains the components of the gradient of \( J(X) \) at the current value of the parameter vector \( X \).

IFAIL : integer, IFAIL contains a key number for interpretation of results from the optimisation subroutine. See description of E04LAF.

PI : real, performance index.

PJ : real, auxiliary quantity to calculate the performance index.

D : real, dilution rate at the Jth experimental point. \((J = 1, \text{NEP})\)

YT : real array of dimension (3), YT contains the theoretical solution of the model structure equations \( (\text{MSE}) \) at the point \( D \), corresponding to the value of the parameter vector \( X \), at the minimum of \( J(X) \).

\( YT(1) \) = glucose concentration.
\( YT(2) \) = ammonium ion concentration.
\( YT(3) \) = dry biomass concentration.
Flow chart of the Main Programme of the Optimisation Procedure.

START

READ NP, NEV, NEP, X, Y, MODE, TS

SET PARAMETERS OF OPTIMISATION SUBROUTINE

COMPUTE INITIAL VALUES OF J(X) AND G(X) (CALL FUNCT2)

WRITE X, J(X), G(X)

MINIMISE J(X) (CALL E04LAF)

COMPUTE FINAL VALUES J(X) AND G(X) (CALL FUNCT2)

WRITE X, J(X) AND G(X)

A
WRITE IFAIL

PI = 0

DO 35 I = 1, NEP

D = Y(I,4)

COMPUTE SOLUTIONS OF THE MSE AT D
(CALL MODELL)

WRITE D,
YT

\[
P_{J} = \sum_{I=1}^{3} (YT(L) - Y(I,L))^2
\]

PI = PI + PJ

WRITE PI

CALCULATE VARIANCE-COVARIANCE MATRIX
AND CONFIDENCE LIMITS OF THE
PARAMETERS. (CALL STAT)

STOP
B. Subroutine DERIV1

DERIV1 calculates the value of the model structure
equations $C_1$, $C_2$ and $C_3$ and their first partial derivatives
with respect to the parameter vector components, at the point
$X$, $Y$.

Description of arguments of DERIV1.

SUBROUTINE DERIV1($X,Y,C1,C2,C3,DC1,DC2,DC3$)

$X$ : real array of dimension (NP), on entry $X$ contains the current
value of the parameter vector $X$, DERIV1 does not change
the value of $X$.

$Y$ : real array of dimension (NEV), on entry $Y$ contains the
experimental values of the system responses. Unchanged on exit.

$Y(1)$ = glucose concentration.
$Y(2)$ = ammonium ion concentration.
$Y(3)$ = dry biomass concentration.
all the above values corresponding to:
$Y(4)$ = dilution rate.

$C1$ : real, on exit $C1$ contains the value of the first component ($C_1$)
of the MSE vector at the point ($X,Y$).

$C2$ : real, on exit $C2$ contains the value of the second component ($C_2$)
of the MSE vector at the point ($X,Y$).

$C3$ : real, on exit $C3$ contains the value of the third component ($C_3$)
of the MSE vector at the point ($X,Y$).

$DC1$ : real array of dimension (NP), on exit $DC1$ contains the
first partial derivatives of $C_1$ at the point ($X,Y$).

$DC2$ : real array of dimension (NP), on exit $DC2$ contains the
first partial derivatives of $C_2$ at the point ($X,Y$).

$DC3$ : real array of dimension (NP), on exit $DC3$ contains the
first partial derivatives of $C_3$ at the point ($X,Y$).

$C_1$, $C_2$ and $C_3$ and their first partial derivatives are
defined in the APPENDIX B3 for the Contois-Contois model and
mode 4. DERIV1 is called by FUNCT2 and HESS2.
C. Subroutine DERIV2

DERIV2 calculates the value of the second partial derivatives of the model structure equations $C_1$, $C_2$ and $C_3$ with respect to the parameter vector components, at the point $(X,Y)$.

Description of the arguments of DERIV2.

SUBROUTINE DERIV2(X,Y,D2C1,D2C2,D2C3)

X : real array of dimension (NP), on entry X contains the current value of the parameter vector. DERIV2 does not change the value of X.

Y : real array of dimension (NEV), on entry Y contains the experimental values of the system responses. Unchanged on exit.

Y(1) = glucose concentration.
Y(2) = ammonium ion concentration.
Y(3) = dry biomass concentration.

all above values corresponding to:

Y(4) = dilution rate.

D2C1 : real array of dimension (NP,NP), on exit D2C1 contains the second partial derivatives of $C_1$ at the point $(X,Y)$.

D2C2 : real array of dimension (NP,NP), on exit D2C2 contains the second partial derivatives of $C_2$ at the point $(X,Y)$.

D2C3 : real array of dimension (NP,NP), on exit D2C3 contains the second partial derivatives of $C_3$ at the point $(X,Y)$.

$C_1$, $C_2$ and $C_3$ and their second partial derivatives are defined in the APPENDIX B3 for the mode 4 of the Contois-Contois model.

DERIV2 is called by HESS2.
SUBROUTINE DERIV1  flow chart.

SUBROUTINE DERIV2  flow chart.
Subroutine FUNCT2.

FUNCT2 calculates the value of the function \( J(X) \) and the gradient of \( J(X) \) at the current value of the parameter vector \( X \).

Description of the arguments of FUNCT2.

SUBROUTINE FUNCT2(NP,X,J,DJX)
COMMON // NEV,NEP,Y

NP : integer, NP specifies the number of parameters. Unchanged on exit.

X : real array of dimension (NP), on entry X contains the current value of the parameter vector. Unchanged on exit.

J : real, on exit J contains the value of the function \( J(X) \) at the current value of the parameter vector.

DJX : real array of dimension (NP) on exit DJX contains the gradient of \( J(X) \) at the current value of the parameter vector.

NEV : integer, NEV specifies the number of experimental variables. Unchanged on exit.

NEP : integer, NEP specifies the number of experimental points. Unchanged on exit.

Y : real array of dimension (NEP,NEV), Y contains the values of the NEV experimental variables, corresponding to the NEP experimental points. Unchanged on exit.

FUNCT2 is called by E04LAF. FUNCT2 calls DERIV1.
ENTRY

J = 0

DO 10 I = 1, NP

DJX(I) = 0

10

DO 50 I = 1, NEP

DO 20 K = 1, NEV

YA(K) = Y(I, K)

20

COMPUTE $C_1$, $C_2$, $C_3$, $(\partial C_1/\partial x_i)$, $(\partial C_2/\partial x_j)$, $(\partial C_3/\partial x_j)$

j = 1, NP AT THE POINT (X, YA).

(CALL DERIV1)

F = $C_1^2 + C_2^2 + C_3^2$

J = J + F

DO 30 L = 1, NP

DFX(L) = $2C_1(\partial C_1/\partial x_L) + 2C_2(\partial C_2/\partial x_L) + 2C_3(\partial C_3/\partial x_L)$

30

DJX(L) = DJX(L) + DFX(L)

CONTINUE

50

RETURN

SUBROUTINE FUNCT2 flow chart.
E. Subroutine HESS2

HESS2 calculates the second partial derivatives of $J(X)$ at the current value of the parameter vector $X$.

Description of the arguments of HESS2.

SUBROUTINE HESS2(NP,X,HESLC,LH,HESDC)
COMMON // NEV,NEP,Y

NP : integer, NP specifies the number of parameters. Unchanged on exit.

X : real array of dimension (NP), on entry X contains the current value of the parameter vector. Unchanged on exit.

HESLC : real array of dimension LH, on exit HESLC contains the components of the lower triangle of the Hessian matrix of $J(X)$ given by rows.

LH : integer, LH specifies the length of the array HESLC. Unchanged on exit.

HESDC : real array of dimension (NP), on exit HESDC contains the components of the main diagonal of the Hessian matrix of $J(X)$.

NEV : integer, NEV specifies the number of experimental variables. Unchanged on exit.

NEP : integer, NEP specifies the number of experimental points. Unchanged on exit.

Y : real array of dimension (NEP,NEV), Y contains the values of the NEV experimental variables corresponding to the NEP experimental points. Unchanged on exit.

HESS2 is called by E04LAF. HESS2 calls DERIV1 and DERIV2.

In the flow chart, $D2FX(I,J)$ is given by:

$$D2FX(I,J) = 2*(C1*D2C1(I,J) + C2*D2C2(I,J) + C3*D2C3(I,J) + DC1(I)*DC1(J) + DC2(I)*DC2(J) + DC3(I)*DC3(J)).$$
ENTRY

DO 20 I = 1, NP

DO 10 J = 1, I

D2JX(I,J) = 0

CONTINUE

DO 60 M = 1, NEP

DO 30 N = 1, NEV

YA(N) = Y(M,N)

DO 20 I = 1, NP

DO 30 N = 1, NEV

COMPUTE C1, C2, C3, DC1, DC2, DC3 AT THE POINT (X,YA) (CALL DERIV1)

COMPUTE D2C1, D2C2, D2C3 AT THE POINT (X,YA) (CALL DERIV2)

DO 50 I = 1, NP

DO 40 J = 1, I

COMPUTE D2FX(I,J)

D2JX(I,J) = D2JX(I,J) + D2FX(I,J)

CONTINUE

CONTINUE
SUBROUTINE HESS2 flow chart (Cont.)

A

DO 70 I = 1, NP

HESDC(I) = D2JX(I,I) 70

DO 90 I = 2, NP

IA = I - 1

DO 80 J = 1, IA

IL = (I - 2)*(I - 1)/2 + J

HESLC(IL) = D2JX(I,J) 80

CONTINUE 90

RETURN
F. Subroutine MODELL.

MODELL calculates the solution of the model structure equations with the current value of the parameter vector, at the point D (dilution rate).

Description of the arguments of MODELL.

SUBROUTINE MODELL(Y,D)
COMMON / PARAM / X

Y : real array of dimension (3), on exit Y contains the solutions (i.e., model responses) of the model structure equations at the point D.

D : real, on entry D contains the value of the dilution rate at which the model response is required. Unchanged on exit.

X : real array of dimension (NP), X contains the current value of the parameter vector. MODELL does not change the value of X.

MODELL is called by the Main Programme.

In the flow chart, A, B and C are the coefficients of the quadratic equation which defines the value of Y(3) \( C_x \) defined in APPENDIX B4.
SUBROUTINE MODEL1 flow chart.

ENTRY

COMPUTE COEFFICIENTS OF THE QUADRATIC EQUATION, A, B, C IN TERMS OF THE PARAMETER VECTOR VALUE.

RAD = B^2 - 4 AC

RETURN

RAD < 0

RETURN

RAD ≥ 0

COMPUTE Y(3)

COMPUTE Y(2)

COMPUTE Y(1)

RETURN
G. Subroutine STAT.

STAT calculates and prints:

(a) The variance-covariance matrix of the parameter vector at the current value of \( X \).
(b) The 99\%, 95\% and 90\% confidence limits of the parameters.

Details of the calculations are given in APPENDIX B2.

Description of the arguments of STAT.

SUBROUTINE STAT(NP,NEP,S,X,T)

NP : integer, on entry NP specifies the number of parameters. Unchanged on exit.

NEP : integer, on entry NEP specifies the number of experimental points. Unchanged on exit.

S : real, on entry S contains the value of \( J(X) \) at the current value of the parameter vector. Unchanged on exit.

X : real array of dimension (NP), on entry X contains the current value of the parameter vector. Unchanged on exit.

T : real array of dimension (3), on entry T contains the values of \( t \) (t-distribution) at \( (1 - \alpha)\% \) of confidence with \( NEP - NP \) degrees of freedom. Unchanged on exit.

\[ T(1) = t \quad \text{for} \quad \alpha = 0.01 \]
\[ T(2) = t \quad \text{for} \quad \alpha = 0.05 \]
\[ T(3) = t \quad \text{for} \quad \alpha = 0.10 \]

STAT calls HESS2 and FOIAAF. STAT is called by the Main Programme.

Internal variables:

VARX : real array of dimension (NP,NP), VARX contains the components of the variance-covariance matrix of \( X \).

A : real array of dimension (NP,NP), A contains the components of the Hessian matrix of \( J(X) \).
HESDC : real array of dimension (NP), HESDC contains the components of the main diagonal of the Hessian matrix of \( J(X) \) at the current value of the parameter vector.

HESLC : real array of dimension (LH), HESLC contains the components of the lower triangle of the Hessian matrix of \( J(X) \) given by rows at the current value of the parameter vector.

LH : integer, LH specifies the dimension of array HESLC.

UNIT : real array of dimension (NP,NP), UNIT contains the components of the inverse matrix of \( A \) (Inverse of the Hessian matrix of \( J(X) \)) at the current value of the parameter vector.

SD : real, SD contains the standard deviation of the parameter \( X(I) \).

XLB : real array of dimension (3), XLB contains the lower \((1 - \alpha)\)% confidence limits of \( X(I) \): \((I = 1, NP)\)
- XLB(1) for \( \alpha = 0.01 \)
- XLB(2) for \( \alpha = 0.05 \)
- XLB(3) for \( \alpha = 0.10 \)

XUB : real array of dimension (3), XUB contains the upper \((1 - \alpha)\)% confidence limits of \( X(I) \): \((I = 1, NP)\)
- XUB(1) for \( \alpha = 0.01 \)
- XUB(2) for \( \alpha = 0.05 \)
- XUB(3) for \( \alpha = 0.10 \)
SUBROUTINE STAT flow chart.
RETURN

VARX(I,J) = 2*S*UNIT(I,J)/(NEP - NP)

WRITE VARX(I,K) K = 1, NP

WRITE X(UB(J), X(I), XUB(J), J = 1, 3

RETURN
Subroutine E04LAF.

E04LAF is a modified Newton algorithm for finding a minimum of a function \( F(X_1, X_2, \ldots, X_N) \) subjected to fixed upper and lower bounds on the independent variables \( X_1, X_2, \ldots, X_N \), when first and second derivatives of \( F \) are available. It is intended for functions which have continuous first and second derivatives (although it will usually work even if the derivatives have occasional discontinuities).

This routine is applicable to a problem of the form:

\[
\text{Minimise } \quad F(X_1, X_2, \ldots, X_N)
\]

subjected to \( L_j \leq X_j \leq U_j \quad (J = 1, 2, \ldots, N) \)

when first and second derivatives of \( F \) are available.

Special provision is made for problems which actually have no bounds on the \( X_j \), problems which have non-negative bounds and problems in which \( L_1 = L_2 = \ldots = L_N \) and \( U_1 = U_2 = \ldots = U_N \). The user must supply a subroutine \textsc{funct2} to calculate the value of \( F(X) \) and its first derivatives at any point \( X \), and a subroutine \textsc{hess2} to calculate the second derivatives.

From a starting point supplied by the user, there is generated, on the basis of estimates of the curvature of \( F(X) \), a sequence of feasible points which is intended to converge to a local minimum of the constrained function.
Description of the arguments of E04LAF

SUBROUTINE E04LAF(N,IBOUND,BL,BU,X,F,G,IW,LIW,W,LW,IFAIL)

N : integer, N specifies the number of independent variables.
   Unchanged on exit.

IBOUND : integer, on entry IBOUND specifies whether the facilities
   for dealing with bound of specific form is to be used.
   It must be set to one of the following values (Unchanged
   on exit):

   IBOUND = 0  if the user will supply all the $L_j$ and $U_j$
               individually.
   IBOUND = 1  if there are no bounds in any $X_j$.
   IBOUND = 2  if all the bound are of the form: $0 \leq X_j$.
   IBOUND = 3  if $L_1 = L_2 = \ldots = L_N$ and $U_1 = U_2 = \ldots = U_N$.

BL : real array of dimension at least (N), to contain the
    lower bounds $L_j$. If IBOUND is set to 0, the user
    must set BL(J) to $L_j$ (J = 1, 2, ..., N) (if a lower bound is
    not specified for any $X_j$, the corresponding BL(J) should
    be set to $-10^6$). If IBOUND is set to 3, the user must
    set BL(1) equal to $L_1$, E04LAF will then set the remaining
    elements of BL equal to BL(1).

BU : real array of dimension at least (N), to contain the upper
    bounds $U_j$. If IBOUND is set to zero, the user must set
    BU(J) to $U_j$ (J = 1, 2, ..., N) (if any upper bound is not
    specified for any $X_j$, the corresponding BU(J) should be
    set to $10^6$). If IBOUND is set to 3, the user must set
    BU(1) to $U_1$; E04LAF will then set the remaining elements
    of BU equal to BU(1).
**X**: real array of dimension at least (N). Before entry, X(J) must be set by the user to a guess at the Jth component of the position of the minimum (J = 1, 2, ..., N). The routine checks the gradient and the Hessian matrix at the starting point, and is more likely to detect any error in the user's programming if the initial X(J) are non-zero and mutually distinct. On exit, X contains the lowest point found during calculations. Thus, if IFAIL = 0 on exit, X(J) is the Jth component of the position of the minimum.

**F**: real, on exit F contains the value F(X) corresponding to the final point stored in X.

**G**: real array of dimension at least (N). On exit, G(J) contains the values of (∂F/∂X_j) corresponding to the final point stored in X(J), (J = 1, 2, ..., N); the G(J) not on a bound should normally be close to zero.

**IW**: integer array of dimension at least (N + 2) used as workspace.

**LIW**: integer, on entry LIW must specify the actual length of IW as declared in the calling (sub)programme. LIW ≥ N + 2. Unchanged on exit.

**W**: real array of dimension at least (N(N + 7)) or (10) if N = 1. Used as workspace.

**LW**: integer, on entry LW must specify the actual length of W, as declared in the calling (sub)programme. LW ≥ Max(N(N+7),10). Unchanged on exit.

**IFAIL**: Integer. Before entry IFAIL must be set to 0 or 1. For this routine, because the value of the output parameters may be useful even if IFAIL ≠ 0, users are recommended to set IFAIL to 1 before entry. It is essential to test the value of IFAIL on exit; unless the routine detects an
error or gives a warning, IFAIL contains 0 on exit.

IFAIL = 0 Successful calling. X holds the position of the minimum of F(X).

IFAIL = 1 Parameters outside of expected range. This failure will occur if, on entry, N < 1, IBOUND < 0, IBOUND > 3, IBOUND = 0 and BL(J) > BU(J) for some J, IBOUND = 3 and BL(1) > BU(1), LIW < N + 2, or LW ≤ Max(10,N(N + 7)).

IFAIL = 3 The conditions for a minimum have not all been met but a lower point could not be found and the algorithm has failed.

IFAIL = 4 Not used.

IFAIL = 5 There is some doubt whether the point X found by E04LAF is a minimum. The degree of confidence in the result decreases as IFAIL increases. Thus, when IFAIL = 5 it is probable that the final X gives a good estimated of the position of the minimum, but when IFAIL = 8, it is very unlikely that the routine has found a minimum.

IFAIL = 6 E04LAF is a minimum. The degree of confidence in the result decreases as IFAIL increases. Thus, when IFAIL = 5 it is probable that the final X gives a good estimated of the position of the minimum, but when IFAIL = 8, it is very unlikely that the routine has found a minimum.

IFAIL = 7 In the search for a minimum, the modulus of one of the variables has become very large (∼10^6). This indicates that there is a mistake in FUNCT2 or HESS2, that the user's problem has not finite solution or that the problem needs re-scaling.

IFAIL = 8 It is very likely that the user has made a mistake forming the gradient.

IFAIL = 9 In the search for a minimum, the modulus of one of the variables has become very large (∼10^6). This indicates that there is a mistake in FUNCT2 or HESS2, that the user's problem has not finite solution or that the problem needs re-scaling.

IFAIL = 10 It is very likely that the user has made a mistake forming the second derivatives.
I. Subroutine FOIAAF.

FOIAAF calculates the approximate inverse of a real matrix $A$ by Crout's method. The routine calculates the inverse $X$ of a real matrix $A$ by solving the set of linear equations $AX = I$ using Crout's method with partial pivoting and an additional precision accumulation of inner products. The matrix $A$, provided is not singular, is factorised into the form $A = LU$ where $L$ is a lower tringular matrix and $U$ is Unit upper tringular matrix. The columns of the inverse of $A$ are found by forward and backward substitution in the equation $Ly = e$ and $Ux = y$ where $e$ is a column of the identity matrix $I$.

Description of the arguments of FOIAAF.

SUBROUTINE FOIAAF(A,IA,N,UNIT,IUNIT,WKSPCE,IFAIL)

A : real array of dimension $(IA,p)$ $p \geq N$. Before entry, $A$ must contain the elements of the real matrix. On successful exit, it contains the Crout factorisation $A = LU$.

IA : integer, IA specifies the first dimension of array $A$ as declared in the calling (sub)programme. $IA \geq N$. Unchanged on exit.

N : integer, $N$ specifies the order of the matrix $A$, unchanged on exit.

UNIT : real array of dimension $(IUNIT,p)$ where $p \geq N$. On successful exit, $UNIT$ contains the inverse of $A$.

IUNIT : integer, IUNIT specifies the first dimension of array $UNIT$. Unchanged on exit.

WKSPCE : real array of dimension at least $(N)$ used as workspace.

IFAIL : integer, before entry $IFAIL$ must be assigned a value. The recommended value for this subroutine is 0. Unless the routine detects an error, $IFAIL$ contains 0 on exit.

$IFAIL = 1$ The matrix $A$ is singular or almost singular possible due to rounding errors.
APPENDIX B6.

Modes of the model structure equations of the Monod-Monod model for the batch culture.

The terms appearing in the model structure equations are:

\[ x_1 : \text{Monod saturation constant for glucose, } K_g. \]
\[ x_2 : \text{Monod saturation constant for ammonium ion, } K_n. \]
\[ x_3 : \text{endogeneous respiration coefficient, } k_e. \]
\[ x_4 : \text{maximum specific growth rate, } \mu_m. \]
\[ x_5 : \text{stoichiometric coefficient for glucose, } m^g. \]
\[ x_6 : \text{stoichiometric coefficient for ammonium ion, } m^n. \]

\[ y_1 : \text{glucose concentration, } C_g. \]
\[ y_2 : \text{ammonium ion concentration, } C_n. \]
\[ y_3 : \text{dry biomass concentration, } C_x. \]

In Modes 5, 6 and 7:

\[ y_4 : \text{observed growth rate, } r_x. \]
\[ y_4 : \text{glucose consumption rate, } r_g. \]
\[ y_6 : \text{ammonium ion consumption rate, } r_n. \]

In mode 8,

\[ y_4 : \text{observed specific growth rate, } (r_x/C_x). \]
\[ y_5 : \text{specific glucose consumption rate, } (r_g/C_x). \]
\[ y_6 : \text{specific ammonium ion consumption rate, } (r_n/C_x). \]
Mode 5
\[ C_1 = x^4 \frac{y_1 y_2 y_3}{(x_1 + y_1)(x_2 + y_2)} - (x_3 y_3 + y_4) = 0 \]
\[ C_2 = -x^4 x^5 \frac{y_1 y_2 y_3}{(x_1 + y_1)(x_2 + y_2)} - y_5 = 0 \]
\[ C_3 = -x^4 x^6 \frac{y_1 y_2 y_3}{(x_1 + y_1)(x_2 + y_2)} - y_6 = 0 \]

Mode 6
\[ C_1 = x^4 \frac{y_1 y_2 y_3}{(x_1 + y_1)(x_2 + y_2)} - (x_3 y_3 + y_4) = 0 \]
\[ C_2 = -x^5 (x_3 y_3 + y_4) - y_5 = 0 \]
\[ C_3 = -x^6 (x_3 y_3 + y_4) - y_6 = 0 \]

Mode 7
\[ C_1 = x^4 y_1 y_2 y_3 - (x_3 y_3 + y_4)(x_1 + y_1)(x_2 + y_2) = 0 \]
\[ C_2 = -x^5 (x_3 y_3 + y_4) - y_5 = 0 \]
\[ C_3 = -x^6 (x_3 y_3 + y_4) - y_6 = 0 \]

Mode 8
\[ C_1 = x^4 y_1 y_2 - (x_3 + y_4)(x_1 + y_1)(x_2 + y_2) = 0 \]
\[ C_2 = -x^5 (x_3 + y_4) - y_5 = 0 \]
\[ C_3 = -x^6 (x_3 + y_4) - y_6 = 0 \]
APPENDIX B7

Description of the subroutine E02CAF for the polynomial fit of the model parameters as function of pH.

Given a set of data points \((X_i, Y_i), i = 1, \ldots, N\), in the arrays \(X\) and \(Y\), both of dimension \(N\), the routine uses the exchange algorithm to compute an \(M\)th polynomial:

\[
P(X) = a_1 + a_2 X + a_3 X^2 + \ldots + a_{M+1} X^M
\]

such that \(\max \left| P(X_i) - Y_i \right| \) is a minimum. The coefficients of \(p(X)\) are stored in the array \(A\) of dimension \(M_1\), where \(M_1 = M + 1\) and the routine returns in \(REF\) a number whose absolute value is the final reference deviation.

Description of the arguments of E02CAF.

SUBROUTINE E02CAF(X,Y,N,A,M1,REF)

\(X\) : real array of dimension at least \((N)\). On entry \(X\) contains the values of the \(X\) coordinates (abcissae). The \(X\) coordinates must form a strictly increasing monotonic sequence. Unchanged on exit.

\(Y\) : real array of dimension at least \((N)\), on entry, \(Y\) contains the values of the \(Y\) coordinates (ordinates). Unchanged on exit.

\(N\) : integer, \(N\) specifies the number of data points. Unchanged on exit.

\(A\) : real array of dimension at least \((M_1)\) \((M_1 < 100)\), on exit, the coefficients of the final polynomial are stored in \(A\) with the independent term in \(A(1)\).

\(M_1\) : integer. On entry, \(M_1\) specifies a number greater than the degree of the polynomial to be found. \(M_1 < N\) and \(M_1 < 100\).

\(REF\) : real, on exit \(REF\) usually contains the final reference deviation.
Glucose determination.


Principle: Glucose reacts with o-toluidine in glacial acetic acid in presence of heat to yield a blue-green N-glucosylamine, the absorbance of which is measured at 625 nm.

Reagents: O-toluidine reagent(stabilised). To 5.0 g of thiourea, 90.0 ml of o-toluidine was added, dissolved and diluted to one litre with glacial acetic acid. The reagent was stored in an amber bottle at refrigerator temperature.

Standards. 0.2, 0.3 and 0.4 g of pure anhydrous glucose were dissolved in 500 ml of water, containing 1.5 g of benzoic acid and diluted to one litre. The standards were stored at refrigerator temperature in amber bottles.

Procedure: Samples were diluted in proportions 20:1 and 10:1 when required to bring down concentrations to standard level. Test tubes were prepared as follows:
Blank: 0.5 ml dist. water + 5.0 ml o-toluidine reagent.
Standard: 0.5 ml standard + 5.0 ml o-toluidine reagent.
Unknown: 0.5 ml sample + 5.0 ml o-toluidine reagent.

After thoroughly mixed, the tubes were placed in a bath of boiling water and cooled in a bath of cold water after ten minutes. Absorbance was read against the blank at 625 nm immediately after cooling.

Sample conc. = \frac{\text{Absorbance sample}}{\text{Absorbance stand.}} \times \text{conc. standard}

Reagent and standard performance were checked monthly in an absorbance-concentration chart prepared for such a purpose.

All the reagents were analytical grade supplied by BDH Chemicals.
Ammonium ion determination.

Ammonium ion was determined by the Kjedhal method described in "Practical Chemistry Course Manual". University of Bath. School of Chemistry, 1977. Pp 22-23.

Principle: The principle of the method is to heat the sample solution with concentrated sodium hydroxide to distil the resulting ammonia into a boric acid solution where it is titrated with standard hydrochloric acid.

Reagents:

Concentrated sodium hydroxide solution. 400 g of sodium hydroxide were dissolved in cold water and diluted to one litre. The solution was stored at room temperature.

Boric acid solution. 20 g of boric acid were dissolved in water and diluted to one litre. The solution was stored at room temperature.

Standard hydrochloric acid solution. HCl solutions N/100 and N/500 were prepared from concentrated volumetric solutions supplied by BDH chemicals (CVS AnalaR reagents).

Indicator. A mixture of 0.1 g bromocresol green, 0.07 g methyl red and 0.01 g thymol blue in 100 ml of ethanol was used as indicator.

Procedure: 5 ml of sample were placed in a Markham Still Distillation apparatus containing 10 ml of the sodium hydroxide solution and heated by steam.
The resulting ammonia was collected in 20 ml of boric acid solution containing few drops of the indicator. The distillation was carried out until the indicator turned from red (acid) to green (basic) and for five more minutes. The ammonium hydroxide was titrated with the standard HCl solution. The end point was a change from green, through steel gray to pink or violet.

\[
\text{Conc. sample} = \frac{(\text{volume titrant})(\text{Conc. titrant})}{(\text{volume sample})}
\]
APPENDIX D. Parameter evaluation of the batch culture model.

APPENDIX D1. Plots for the evaluation of stoichiometric and endogeneous respiration coefficients of the batch culture model. Figures D.1 to D.4

APPENDIX D2. Plots for the evaluation of kinetic parameters of the batch culture model. Figures D.5 to D.8

APPENDIX D3. Evaluation of kinetic parameters of the alternative model for the batch culture.

The kinetic parameters for the alternative model for the batch culture were evaluated as follows:

A. Endogeneous respiration constant $k_e$.

In view of the fact that the endogeneous respiration coefficient for the batch culture, evaluated as described in Section 4.2, was highly determined by the experimental values of the cell decay phase, no new determination of $k_e$ was performed. Therefore, $k_e$ values for the batch culture are these listed in Table 4.7

B. Saturation constant and maximum specific growth rate.

During growth phase, the endogeneous respiration term can be neglected. Hence, Equation (5.2) can be
expressed as:

\[
\frac{r'}{r^*} = r^*
\]

\[
r = - m \frac{r}{x}
\]

\[
r_n = - m \frac{r}{n x}
\]

For Monod single kinetics, the above Equations can be rearranged as (Lineweaver-Burk form):

\[
\frac{1}{R^*} = \frac{K_g}{\mu_m} \frac{1}{C_g} + \frac{1}{\mu_m}
\]

\[
- \frac{m}{R^*} = \frac{K_g}{\mu_m} \frac{1}{C_g} + \frac{1}{\mu_m}
\]

\[
- \frac{m}{R^*} = \frac{K_g}{\mu_m} \frac{1}{C_g} + \frac{1}{\mu_m}
\]

\[K_g \text{ and } \mu_m \] were determined by a multiresponse plot of \((1/R^*), -(m/R^*)\) and \(-(m/R^*)\) versus \((1/C_g)\).

The plots are given in Figures D.9 and D.10.

C. Inhibition constant \(K_{gi}\) for glucose.

Having estimated the values of \(m_g, m_n, k_e, K_g\) and \(\mu_m\), the only parameter to determine is \(K_{gi}\). Equation (5.2) (for biomass) can be rearranged as follows:
\[ \frac{1}{R - R'} = \frac{1}{k_e} + \frac{1}{k K_g i g} \]

Therefore, a plot of \( \frac{1}{R - R'} \) versus \( C \) should give a straight line with slope \( \frac{1}{k K_g i g} \) and intercept \( \frac{1}{k_e} \). The graphical determination presented excessive noise in the responses due to the uncertainty of glucose concentration value around the exhaustion of such a substrate. In view of this, and based on the advantage that there was only one parameter to determine, an exhaustive enumeration procedure (see section 1.4.2 (1)) was performed. The values of the parameters are summarised in Table 5.7, Section 5.2.2.
FIGURE D.1  Stoichiometric coefficients determination. Batch Culture at (a) pH 5.5 and (b) pH 6.0
FIGURE D.2  Stoichiometric coefficients determination. Batch culture at pH 7.
FIGURE D.3  Endogeneous respiration coefficients determination.
Batch culture at (a) pH 4.0 and (b) pH 5.5
The X - Y coordinates are:

X : 1/R' \_x  hr

Y : \( \times - \frac{(dC / dC_g)}{m_g} \) dimensionless

\( \circ - \frac{(dC / dC_n)}{m_n} \) dimensionless.
Figure D.4 Endogeneous respiration coefficients determination.
Batch culture at (a) pH 6.0 and (b) pH 7.0
See Figure D.3 for symbols.
FIGURE D.5  Determination of growth rate constants for (a) Monod kinetics and (b) Contois kinetics for the Batch culture at pH 4. The Y axis represents:

- $1/(R'_x + k_e)$ hr
- $- (m_g/R'_g)$ hr
- $- (m_n/R'_n)$ hr
FIGURE D.6 Determination of growth rate constants for (a) Monod kinetics and (b) Contois kinetics for the Batch culture at pH 5.5. See Figure D.5 for symbols.
FIGURE D.7 Determination of growth rate constants for (a) Monod kinetics and (b) Contois kinetics for the Batch culture at pH 6. See figure D.5 for symbols.
FIGURE D.8 Determination of growth rate constants for (a) Monod kinetics and (b) Contois kinetics for the Batch culture at pH 7. See figure D.5 for symbols.
FIGURE D.9 Determination of the growth rate constants of the Alternative model for the Batch Culture at (a) pH 3 and (b) pH 4. The Y axis represents:

- \( \frac{1}{R_x' \, \text{hr}} \)
- \( - \frac{(m_{\text{g}})}{R_x' \, \text{hr}} \)
- \( - \frac{(m_{\text{n}})}{R_x' \, \text{hr}} \)

\( \mu_m = 0.256 \, \text{1/hr} \)
\( K_g = 0.345 \, \text{kg/m}^3 \)

\( \mu_m = 0.292 \, \text{1/hr} \)
\( K_g = 0.995 \, \text{kg/m}^3 \)
FIGURE D.10  Determination of the growth rate constants of the
Alternative model for the Batch culture at (a) pH 5.5
(b) pH 6., (c) pH 7.  See figure D.9 for symbols.
APPENDIX E. Estimation of mass transfer parameters.

APPENDIX E1 Estimation of $k_a$ for oxygen transfer from gas phase to culture fluid in the continuous culture.

The volumetric mass transfer coefficient was calculated using the relationship:

$$k_a = \frac{K_v H}{V}$$

where:
- $k_a$ : volumetric mass transfer coefficient.
- $K_v$ : volumetric absorption coefficient.
- $H$ : Henry's law constant.

$K_v$ was calculated by the method described by Aiba et al., (1973). $K_v$ is given by the correlation:

$$K_v = 0.0635 \left( \frac{P_g}{V} \right)^{0.95} (u_s)^{0.67}$$

where:
- $P_g$ : Power input of gassed system (HP)
- $V$ : Volume of liquid ($m^3$)
- $u_s$ : Superficial velocity of gas (m/hr)
- $K_v$ in kgmol/hr/m$^3$/atm

The correlation is applicable if:

$$\left( \frac{P_g}{V} \right) > 0.1 \text{ HP/m}^3$$

and

$$\left( \frac{H_L}{D_t} \right) = 1 \text{ (see Figure E.1)}$$
The geometric characteristics of the fermentor, involved in the calculations are depicted in Figure E.1.

FIGURE E.1 Geometric characteristics of the fermentor.

\[ H_L = 0.158 \text{ m} \]
\[ D_t = 0.158 \text{ m} \]
\[ D_i = 0.0698 \text{ m} \]

\[ \frac{H_L}{D_i} = 2.272 \]
\[ \frac{D_t}{D_i} = 2.272 \]

The values used in the calculation are given below.

Viscosity of fluid \( \mu = 3.02 \text{ kg/m/hr} \) (water at 30 °C)
Density of fluid \( \rho = 1250 \text{ kg/m}^3 \)
Henry's law constants \( H(0_2) = 28.37 \text{ atm/m}^3/\text{kg} \) (at 30 °C)
Average stirring rate \( n = 500 \text{ rpm} = 3 \times 10^4 \text{ rev/hr} \)
Average air flow rate \( F = 2.0 \text{ l/min} = 0.12 \text{ m}^3/\text{hr} \)
Liquid volume \( V = 2.8 \text{ l} = 0.0028 \text{ m}^3 \)

Reynolds number \( N_{Re} = \frac{n \frac{D}{D_1}^2 \rho}{\nu} = 6.074 \times 10^4 \)

Aeration number \( N_a = \frac{F}{n \frac{D}{D_1}^3} = 1.17 \times 10^{-2} \)

Other quantities involved in the calculation are:

- \( P \) : power input required by ungassed system \( \text{HP} \)
- \( P_g \) : power input required by gassed system \( \text{JHP} \)

\[
N = \frac{P}{n \frac{D}{D_1}^3 \rho} \quad \text{Power number}
\]

Calculations. The figure and equation numbers refer to these given in the reference.

(a) From Figure 6.5 (\( N_p \) versus \( N_{Re} \))

\[
N_p = 6.0, \text{ hence} \quad P = N_p \frac{3}{n} \frac{D}{D_1}^5 \rho = 0.00977 \text{ HP (power input requirement of ungassed system)}
\]

(b) \( P \) is corrected by a correction factor \( f_c \) to account for geometric differences. \( f_c \) is given by:

\[
f_c = \left( \frac{(D/L_1 \text{ act}) (H/L_1 \text{ act})}{(D/L_1 \text{ st}) (H/L_1 \text{ st})} \right)^{\frac{1}{2}} = 0.757
\]
where the 'act' subscript stands for the actual geometric characteristic of the fermentor and the 'st' subscript stands for the geometric characteristic of the reference (standard) reactor.

(c) The corrected value of $P$ is:

$$ P = f_c(0.00977) = 0.00739 \text{ HP} $$

(d) From Figure 6.6 ($P / P_g$ versus $N_a$)

$$ P / P_g = 0.92, \text{ hence} $$

$$ P_g = 0.92P = 0.0068 \text{ HP (input power requirement of gassed system)} $$

(e) $P_g / V = 2.428 \text{ HP/m}^3 > 0.1$

(f) From equation (6.37)

$$ K_v = 0.0635(P_g/V)^{0.95} (u_s)^{0.67}, $$

$$ K_v = 0.494 \text{ kgmol(O}_2\text{/hr/m}^3\text{/atm or} $$

$$ K_v = 15.795 \text{ kg(O}_2\text{/hr/m}^3\text{/atm} $$

(g) Assuming 50% efficiency.

$$ K_v = 7.897 \text{ kg(O}_2\text{/hr/m}^3\text{/atm} $$

(h) Finally:

$$ k_{la} = K_v H(O_2) = 224 \text{ l/hr (50% efficiency)} $$
APPENDIX E2. Estimation of \((k_1 a)_{glucose}\) and \((k_1 a)_{oxygen}\) for the transfer of dissolved species from the culture fluid to the biological transport region.

The mass transfer coefficient \((k_1)\) for each species was calculated using the correlation (see Table 1.2):

\[
Sh = \frac{k_1 d}{D} = 2.0 + 0.31(\Delta \rho \ g/ \ \nu \ D)^{1/3}
\]

where:
- \(Sh\): Sherwood number,
- \(k_1\): mass transfer coefficient,
- \(d\): cell diameter,
- \(D\): diffusivity of the substrate,
- \(\Delta \rho\): density difference between cellular material and culture fluid,
- \(g\): acceleration of gravity,
- \(\nu\): viscosity of culture fluid.

\(\Delta \rho\) was assumed to be negligible, hence:

\[
k_1 = 2.0 \ (D/d)
\]

The volumetric area of transfer \((a)\) was calculated as:

\[
a = (a)_{c} \ n_{c}
\]

where:
- \(a_{c} = 4 \pi r_{c}^2\): area of transfer of individual cell \((r_{c}\) radius of individual cell\)
- \(n_{c}\): cell density (number of microorganisms/m^3)
The values used in the calculation are:

\[ D(\text{glucose}) = 2.48 \times 10^{-6} \quad \text{m}^2/\text{hr} \quad (\text{at } 30 \, ^\circ\text{C}) \]

\[ D(\text{oxygen}) = 9 \times 10^{-6} \quad \text{m}^2/\text{hr} \quad (\text{at } 30 \, ^\circ\text{C}) \]

\[ n_c = 2 \times 10^{13} \quad \text{microorganisms/m}^3 \quad (\text{yeast}) \]

(Aiba et al, 1973)

\[ r_c = 2.5 \times 10^{-6} \quad \text{m} \]

\[ d = 5 \times 10^{-6} \quad \text{m} \quad (\text{Aiba et al, 1973}) \]

Therefore:

\[ (k_{1g}) = 4.968 \times 10^{-1} \quad \text{m/hr} \]

\[ (k_{1o2}) = 1.8 \quad \text{m/hr} \]

\[ a_c = 7.85 \times 10^{-11} \quad \text{m}^2/\text{microorganism} \]

\[ a = a_c n_c = 1.57 \times 10^3 \quad \text{m}^2/\text{m}^3 \]

Finally

\[ (k_{1a})_{g} = 7.798 \times 10^2 \quad \text{1/hr} \]

\[ (k_{1a})_{o2} = 2.826 \times 10^3 \quad \text{1/hr} \]
APPENDIX E3. Estimation of parameter $k'_2$ of the Powell's model for the batch culture.

$k'_2$ is given by:

$$k'_2 = \left( \frac{-\alpha \rho}{\beta D_m} \right)^{\frac{1}{2}}$$

where:

$$\alpha = \mu_m f_0 \left( \frac{V_m}{a_i} \right)$$

$$\varnothing = r_i^2 \left( \frac{1}{r_i} - \frac{1}{r_m} \right)$$

$\beta$: saturation constant of glucose.

$\mu_m$: maximum specific growth rate

$r_i$: internal radius of diffusion shell.

$r_m$: external radius of diffusion shell.

$\rho_o$: density of cellular material

$V_m$: wet volume of cell ($V_m = \frac{4}{3} \frac{r_m^3}{r_i}$)

$a_i$: surface area at internal radius of diffusion shell.

The value of $\varnothing$ was approximated to:

$$\varnothing \approx r_i^2 \left( \frac{1}{r_i} - 0 \right) = r_i$$

assuming that the thickness of diffusion layer is infinite. To calculate $V_m$ it was assumed that: $r_m \approx r_i$, hence:

$$V_m \approx \frac{4}{3} \pi r_i^3$$
Therefore:

\[
k'_2 = \left( \frac{\mu_m \beta V_{m i} \rho_m r_i}{\beta D_m a_i} \right)^{\frac{1}{3}} = \left( \frac{\mu_m \rho_m r_i^2}{3 \beta D_m} \right)^{\frac{1}{3}}
\]

The following values were used:

\[
\begin{align*}
\mu_m &= 0.285 \text{ 1/hr} \\
\beta &= 0.43 \text{ kg/m}^3 \\
r_i &= 2.5 \times 10^{-6} \text{ m} \\
D_m &= 2.48 \times 10^{-6} \text{ m/hr (glucose in water at 30°C)} \\
\rho_o &= 1000 \text{ kg/m}^3 
\end{align*}
\]

Hence:

\[
k'_2 = 3 \times 10^{-2}
\]
APPENDIX F1. Coefficients and plots of the polynomial fit of the Contois-Contois double kinetic model parameters of the continuous culture.

The parameters of the continuous culture model were fitted to a second degree polynomial:

\[ p(pH) = a_0 + a_1(pH) + a_2(pH)^2 \]

in the range \( 4 \leq pH \leq 6 \). The coefficients of the polynomials are given in Table F.1 and the plots are given in Figures F.1 and F.2.

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>( a_0 )</th>
<th>( a_1 )</th>
<th>( a_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( B_g ) (kg/kg)</td>
<td>1.027</td>
<td>-0.4413</td>
<td>0.04763</td>
</tr>
<tr>
<td>( B_n ) (kg/kg)</td>
<td>0.4522</td>
<td>-0.1853</td>
<td>0.02318</td>
</tr>
<tr>
<td>( k_0 ) (1/hr)</td>
<td>0.2233</td>
<td>-0.08404</td>
<td>0.008456</td>
</tr>
<tr>
<td>( \mu_m ) (1/hr)</td>
<td>0.001572</td>
<td>0.1298</td>
<td>-0.01209</td>
</tr>
<tr>
<td>( m_g ) (kg/kg)</td>
<td>4.769</td>
<td>-1.389</td>
<td>0.1678</td>
</tr>
<tr>
<td>( m_n ) (kg/kg)</td>
<td>-0.05513</td>
<td>0.05313</td>
<td>-0.003867</td>
</tr>
<tr>
<td>( Y_g ) (kg/kg)</td>
<td>-0.2421</td>
<td>0.3606</td>
<td>-0.04240</td>
</tr>
<tr>
<td>( Y_n ) (kg/kg)</td>
<td>21.09</td>
<td>-3.774</td>
<td>0.2658</td>
</tr>
</tbody>
</table>
FIGURE F.1  The minimax polynomial
\[ p(pH) = a_0 + a_1(pH) + a_2(pH)^2 \quad 4 \leq pH \leq 6 \]

For the kinetic parameters of the continuous culture model. Values of the coefficients are given in Table F.1.
FIGURE F.2 The minimax polynomial

\[ p(pH) = a_0 + a_1(pH) + a_2(pH)^2 \quad 4 \leq pH \leq 6 \]

For stoichiometric coefficients (\( m \)) and yield constants (\( 1/m \)) of the continuous culture model.

Values of the coefficients are given in Table F.1
APPENDIX F2. Coefficients and plots of the polynomial fit of the parameters of the Alternative model for the batch culture.

The parameters of the alternative model for the batch culture were fitted to a third degree polynomial:

\[ p(\text{pH}) = a_0 + a_1(\text{pH}) + a_2(\text{pH})^2 + a_3(\text{pH})^3 \]

in the range \(3 \leq \text{pH} \leq 7\). The coefficients of the polynomials are given in Table F.2 and the plots are given in Figure F.3.

**TABLE F.2** Coefficients of the polynomials of the parameter of the Alternative Model for the batch culture

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>(a_0)</th>
<th>(a_1)</th>
<th>(a_2)</th>
<th>(a_3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(K_g) ((\text{kg/m}^3))</td>
<td>-4.310</td>
<td>2.743</td>
<td>-0.4714</td>
<td>0.02793</td>
</tr>
<tr>
<td>(\mu_m) ((\text{1/hr}))</td>
<td>-1.178</td>
<td>0.9852</td>
<td>-0.2184</td>
<td>0.01612</td>
</tr>
<tr>
<td>(k_e) ((\text{1/hr}))</td>
<td>0.003187</td>
<td>0.04708</td>
<td>-0.01564</td>
<td>0.001583</td>
</tr>
<tr>
<td>(K_{gi}) ((\text{kg/m}^3))</td>
<td>10.10</td>
<td>-4.78</td>
<td>0.7764</td>
<td>-0.04288</td>
</tr>
<tr>
<td>(m_G) ((\text{kg/kg}))</td>
<td>-0.7785</td>
<td>1.932</td>
<td>-0.4926</td>
<td>0.042</td>
</tr>
<tr>
<td>(m_n) ((\text{kg/kg}))</td>
<td>-0.4639</td>
<td>0.3582</td>
<td>-0.07578</td>
<td>0.0054</td>
</tr>
<tr>
<td>(Y_g) ((\text{kg/kg}))</td>
<td>1.206</td>
<td>-0.4374</td>
<td>0.1029</td>
<td>-0.008392</td>
</tr>
<tr>
<td>(Y_n) ((\text{kg/kg}))</td>
<td>65.35</td>
<td>-32.62</td>
<td>6.372</td>
<td>-0.4201</td>
</tr>
</tbody>
</table>
FIGURE F.3 The minimax polynomial

\[ p(pH) = a_0 + a_1(pH) + a_2(pH)^2 + a_3(pH)^3 \quad 3 \leq pH \leq 7 \]

For the parameters of the Alternative model of the Batch culture. Values of the coefficients are given in Table F.2.
APPENDIX G. Model response Plots for Batch and Continuous cultures.

APPENDIX G1. Model response for the continuous culture at pH 5 and 5.5 Figures G.1 to G.4

APPENDIX G2. Model response for the Batch culture. Alternative Model for the Batch Culture at pH 3.4 and 5.5 Figures G.5 to G.7
FIGURE G.1 Model response for the continuous culture at pH 5.
Biomass.

- Solid line: Monod - Monod.
- Dashed line: Monod - Contois.
- Dashed-dotted line: Contois - Monod.
- Dotted line: Contois - Contois.
FIGURE G.2 Model response for the continuous culture at pH 5.

Substrates:
- Monod - Monod.
- Monod - Contois.
- Contois - Monod.
- Contois - Contois.
FIGURE G.3 Model response for the continuous culture at pH 5.5
Biomass.

- - - - - - Monod - Monod.
- - - - - - - - Monod - Contois.
- - - - - - - - Contois - Monod.
- - - - - - - - - - - - Contois - Contois.
FIGURE G.4 Model response for the continuous culture at pH 5.5

Substrates,
- Monod - Monod,
- Monod - Contois,
- Contois - Monod,
- Contois - Contois.
FIGURE G.5  Model response for the Batch culture at pH 3.
Alternative Model for the Batch culture.
FIGURE G.6  Model response for the Batch culture at pH 4.

Alternative Model for the Batch culture.
FIGURE G.7  Model response for the batch culture at pH 5.5.
Alternative model for the Batch Culture.
REFERENCES


47. HAROLD, F. M., Curr. Top. Bioenerg., 6(1977)83.


100. TARASOVA, S. S.; BIRYUKOV, V. V. and MAKAREVICH, V. G., Antibiotiki(Moscow), 22(1977)291. (Russ.)

101. TARASOVA, S. S.; BIRYUKOV, V. V.; MAKAREVICH, V. G. and UPITER, G. D., Antibiotiki(Moscow), 22(1976)387. (Russ.)


