An Investigation into the Use of Batch Experiments in the Determination of the Kinetics of Ferrous-Iron Oxidation by *Leptospirillum ferrooxidans*

by

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Summary

The objectives of this research were to investigate the effect of temperature and pH on the bacterial ferrous-iron oxidation by *Leptospirillum ferrooxidans* in batch culture and compare these results with those previously obtained in continuous culture. An additional objective was to assess the validity of a variable maintenance equation by a re-evaluation of previously published literature and to evaluate this equation for use during bacterial ferrous-iron oxidation with more than one limiting factor.

The ferrous-iron oxidation kinetics of a predominantly *Leptospirillum ferrooxidans* culture were studied in batch bioreactors. The inoculum for the batches was obtained from steady state continuous cultures of predominantly *Leptospirillum ferrooxidans* grown on ferrous-iron medium at a dilution rate of 0.04 h^{-1}. The continuous cultures were grown at the conditions (temperature and pH) at which the batches were operated. The batches were started with a inoculum of 500 ml and 500 ml of a salts medium containing 12 $g.\ell^{-1}$ ferrous-iron. The batches were run at temperatures ranging from 30 to 40°C and pH values ranging from pH 1.10 to pH 1.70. The growth rate and the oxygen and ferrous-iron utilisation rates of the bacteria were monitored by means of off-gas analysis and redox potential measurement.

The re-evaluation of previously published data proved that the bio-oxidation of ferrous-iron can be represented using a variable maintenance equation proposed by Pirt (1982). An estimation of the maximum bacterial specific ferrous-iron utilisation rate and the maximum bacterial specific growth rate can be obtained from batch or continuous experiments that have been limited by more than one factor e.g. ferrous-iron, arsenic, ammonia, phosphate, sulfate, growth rate. The maximum bacterial specific ferrous-iron utilisation rates and the maximum bacterial specific growth rates calculated from the variable maintenance model are very similar to those values previously published and affirms the validity of the equation.

The batch growth curves follow expected trends and have a very long exponential phase and a very short deceleration phase. The bacterial growth during the exponential phase follows first order kinetics with respect to biomass concentration and is independent of the substrate concentration.

It was not possible to determine the maximum bacterial yields and the maintenance coefficients during the batch bacterial ferrous-iron oxidation using the constant maintenance energy equation of Pirt (1965). The reason for this is that at the beginning of a batch the ferrous-iron concentration is very high and the culture is limited by the growth rate, while at

the end of a batch it is limited by ferrous-iron and inhibited by the ferric-iron product. A variable maintenance energy equation, proposed by Pirt (1982), had to be used to calculate the maximum bacterial yields and the constant- and variable maintenance coefficients.

The maximum bacterial yields on ferrous-iron and oxygen and the respective constant, and variable maintenance coefficients did not vary significantly with temperature or pH. The average maximum yield and constant maintenance coefficient calculated were similar to those reported previously for *Leptospirillum ferrooxidans* in continuous culture and for *Acidithiobacillus ferrooxidans* in continuous and batch culture. The maximum bacterial specific ferrous-iron utilisation rate and the maximum bacterial specific growth rate calculated from the variable maintenance equation are very similar to those previously published.

The constant maintenance equation is only valid for bacterial ferrous-iron oxidation if the culture is only limited by the substrate during the whole experiment. If at any stage the culture is limited by another factor then the bioenergetics must be represented using the variable maintenance equation. The variable maintenance equation should therefore replace the constant maintenance equation when quantifying the bioenergetics of bacterial ferrous-iron oxidation and the bioleaching of sulfide minerals. The variable maintenance equation is especially relevant to the bioleaching of sulfide minerals at low redox potentials (high ferrous-iron concentrations) where the bacterial culture may not be limited by the energy source (ferrous-iron).

The growth rate during the deceleration phase in a batch culture is limited by the substrate, ferrous-iron, and follows Michaelis-Menten based kinetics. The kinetic parameters calculated from batch experiments were very similar to those calculated by previous researchers in continuous experiments. The variation of temperature and pH, however produced different results in batch and continuous experiments.

The continuous experiments are more accurate for representing bacterial ferrous-iron oxidation kinetics limited by ferrous-iron because batch experiments have transient conditions and the limitation by ferrous-iron occurs over a very short time, hence a limited number of data points can be obtained. Batch experiments, however are useful for initial short-term analysis of a system and to provide an initial indication of the kinetic modelling parameters.

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Nomenclature

A	Arrhenius constant	h^{-1}
$a_{Fe^{2+}}$	activity of ferrous-iron species	dimensionless
$a_{Fe^{3+}}$	activity of ferric-iron species	dimensionless
c	variable maintenance equation constant	h
c_X	concentration of bacteria	$mmolC.\ell^{-1}$
c_{X0}	initial concentration of bacteria	$mmolC.\ell^{-1}$
d	diameter	mm
D	dilution rate	h^{-1}
E	redox potential of the solution (Pt-Ag/AgCl)	mV
E_0	redox potential of the solution at equilibrium	mV
$E_0^{'}$	equilibrium redox potential for Ag/AgCl electrode	mV
E_a	activation energy	$kJ.mol^{-1}$
F	Faraday constant	$C.mol^{-1}$
$[Fe^{2+}]$	concentration of ferrous-iron	$mmol\ Fe^{2+}.\ell^{-1}$
$[Fe^{2+}]_t$	threshold concentration of ferrous-iron	$mmol\ Fe^{2+}.\ell^{-1}$
$[Fe^{3+}]$	concentration of ferric-iron	$mmol\ Fe^{3+}.\ell^{-1}$
h	height	mm
k	variable maintenance equation constant	h
K	kinetic constant in bacterial ferrous-iron oxidation	dimensionless
K_{As}	arsenic inhibition constant	$mmolAs.\ell^{-1}$
$K_{As^{3+}}$	arsenite inhibition constant	$mmolAs^{3+}.\ell^{-1}$
K_{ci}	Michaelis-Menten cell inhibition constant	$mmolC.\ell^{-1}$
$K_{Fe^{2+}}$	ferrous-iron based kinetic constant in bacterial ferrous-iron oxidation	dimensionless
K_{H1}	first pH based kinetic constant	$mmolH_2.\ell^{-1}$
K_{H2}	second pH based kinetic constant	$mmolH_2.\ell^{-1}$
K_I	Michaelis-Menten product (ferric-iron) inhibition constant	$mmol\ Fe^{3+}.\ell^{-1}$
K_m	Michaelis-Menten kinetic constant	$mmol\ Fe^{2+}.\ell^{-1}$
$K_m^{'}$	Michaelis-Menten kinetic constant for pH effect	$mmol\ Fe^{2+}.\ell^{-1}$
K_{O_2}	oxygen based kinetic constant in bacterial ferrous-iron oxidation	dimensionless
K_p	Michaelis-Menten product inhibition constant	$mmol.\ell^{-1}$
K_S	Monod equation substrate (ferrous-iron) saturation constant	$mmol\ Fe^{2+}.\ell^{-1}$

K_{Si}	Monod equation substrate inhibition term	mmol $Fe^{2+}.\ell^{-1}$
$m_{Fe^{2+}}$	maintenance coefficient on ferrous-iron	mmol Fe^{2+}.(mmol C)$^{-1}.h^{-1}$
$m^{v}_{Fe^{2+}}$	variable maintenance coefficient on ferrous-iron	mmol Fe^{2+}.(mmol C)$^{-1}.h^{-1}$
m_{O_2}	maintenance coefficient on oxygen	mmol O_2.(mmol C)$^{-1}.h^{-1}$
$m^{v}_{O_2}$	variable maintenance coefficient on oxygen	mmol O_2.(mmol C)$^{-1}.h^{-1}$
m_S	maintenance coefficient on the substrate	mmol S.(mmol C)$^{-1}.h^{-1}$
m^{v}_S	variable maintenance coefficient on the substrate	mmol S.(mmol C)$^{-1}.h^{-1}$
$[O_2]$	dissolved oxygen concentration	mmol $O_2.\ell^{-1}$
$[O_2]_t$	threshold dissolved oxygen concentration	mmol $O_2.\ell^{-1}$
$q_{Fe^{2+}}$	bacterial specific ferrous-iron utilisation rate	mmol Fe^{2+}.(mmol C)$^{-1}.h^{-1}$
q_{O_2}	bacterial specific oxygen utilisation rate	mmol O_2.(mmol C)$^{-1}.h^{-1}$
$q^{max}_{Fe^{2+}}$	maximum bacterial specific ferrous-iron utilisation rate	mmol Fe^{2+}.(mmol C)$^{-1}.h^{-1}$
$q^{max}_{O_2}$	maximum bacterial specific oxygen utilisation rate	mmol O_2.(mmol C)$^{-1}.h^{-1}$
q_s	bacterial specific substrate utilisation rate	mmol S.(mmol C)$^{-1}.h^{-1}$
q^{max}_S	maximum bacterial specific substrate utilisation rate	mmol S.(mmol C)$^{-1}.h^{-1}$
R	Universal gas constant	kJ.K^{-1}.mol^{-1}
$r_{Fe^{2+}}$	ferrous-iron production rate	mmol $Fe^{2+}.\ell^{-1}.h^{-1}$
r_{CO_2}	carbon dioxide production rate	mmol $CO_2.\ell^{-1}.h^{-1}$
r_{O_2}	oxygen production rate	mmol $O_2.\ell^{-1}.h^{-1}$
r_S	substrate production rate	mmol $S.\ell^{-1}.h^{-1}$
r_X	biomass production rate	mmol $C.\ell^{-1}.h^{-1}$
R^2	correlation coefficient	dimensionless
S	substrate concentration	mmol $S.\ell^{-1}$
S_t	threshold substrate concentration	mmol $S.\ell^{-1}$
t	time	s
T	absolute temperature	K
V	ferrous-iron utilisation rate	mmol $Fe^{2+}.\ell^{-1}.h^{-1}$
V_{max}	maximum ferrous-iron utilisation rate	mmol $Fe^{2+}.\ell^{-1}.h^{-1}$
$Y_{Fe^{2+}X}$	bacterial yield on ferrous-iron	mmol C.(mmol Fe^{2+})$^{-1}$
$Y^{max}_{Fe^{2+}X}$	maximum bacterial yield on ferrous-iron	mmol C.(mmol Fe^{2+})$^{-1}$
Y_{O_2X}	bacterial yield on oxygen	mmol O_2.(mmol C)$^{-1}$
$Y^{max}_{O_2X}$	maximum bacterial yield on oxygen	mmol O_2.(mmol C)$^{-1}$
Y_{SX}	bacterial yield on the substrate	mmol C.(mmol S)$^{-1}$
Y^{max}_{SX}	maximum bacterial yield on the substrate	mmol C.(mmol S)$^{-1}$
z	number of electrons involved in a reaction	dimensionless
α	constant	mmol$Fe^{2+}.\ell^{-1}$
β	constant	mmolC.ℓ^{-1}
χ	constant	dimensionless
μ	bacterial specific growth rate	h^{-1}
μ^{max}	maximum bacterial specific growth rate	h^{-1}

Chapter 1

Introduction

Bioleaching is now an established technology for the pre-treatment of refractory gold ores and concentrates, and the leaching of whole ore copper heaps. Processes for the bioleaching of copper, nickel and zinc bearing ores and concentrates by mesophilic and thermophilic micro-organisms are currently under development. In many cases, bioleaching offers economic, environmental and technical advantages over pressure oxidation and roasting (Poulin and Lawrence, 1996; van Aswegen, 1993). Commercial bioleaching plants are in operation in South Africa, Australia, Brazil and Ghana to treat pyrite/arsenopyrite concentrates (Dew *et al.*, 1997, Dew, 1995; van Aswegen, 1993) and heap leaching is used in the U.S.A. and Bulgaria (Brierley *et al.*, 1995, Brierley J.A., 1997, Groudev *et al.* 1995).

In order to optimise the plant operating parameters, it is necessary to have a complete mechanism and a kinetic model for the bioleaching of sulfide minerals. The mechanism of sulfide mineral bioleaching is not fully understood, and hence a full kinetic model has not been formulated.

Recent work on the bioleaching of pyrite has provided strong evidence that the bioleaching of sulfide minerals occurs via a multiple sub-process mechanism (Boon *et al.*, 1995). In the multiple sub-process mechanism the sulfide mineral is chemically oxidised by the ferric-iron present in the bioleaching medium according to:

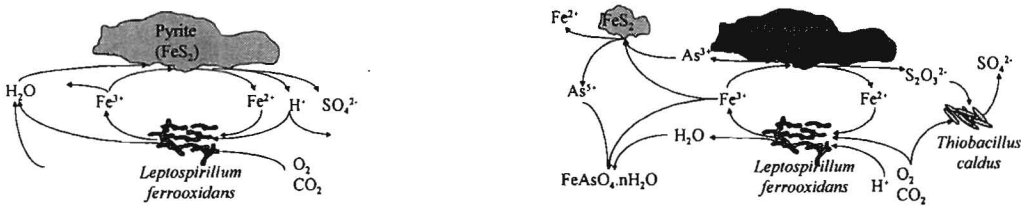
$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \qquad (1.1)$$

$$FeAsS + 5Fe^{3+} \rightarrow As^{3+} + 6Fe^{2+} + S^0 \qquad (1.2)$$

The ferrous-iron produced by this reaction is subsequently oxidised to the ferric form by the ferrous-iron oxidising bacteria:

$$4Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O \qquad (1.3)$$

Polysulfides and sulfur or thiosulfate produced by the ferric leaching reactions are oxidised to sulfate by sulfur oxidising micro-organisms (Schippers and Sand, 1999). Diagrammatic representations of the bioleaching of both pyrite and arsenopyrite are shown in Figure 1(a) and 1(b), respectively.



a) b)

Figure 1.1: Diagrammatic representation of the bioleaching of (a) pyrite and (b) arsenopyrite via the multiple sub-process mechanism. The bacteria are shown unattached for reasons of clarity; however, the mechanism is the same for both attached and unattached micro-organisms (after Breed and Hansford, 1999[b]).

A multiple sub-process mechanism suggests that the overall process can be expressed as a number of interconnected sub-processes. The kinetics of the respective sub-processes may be studied separately, and the results used to predict the performance of bioleach reactors for a variety of different minerals, micro-organisms and operating conditions.

Bacterial ferrous-iron oxidation combined with chemical ferric leaching has been used to successfully model the bioleaching of pyrite and arsenopyrite (Breed and Hansford, 1999[b]; Breed, 2000). It is therefore evident that bacterial ferrous-iron oxidation is an important sub-process in the bioleaching of sulfide minerals.

A number of rate equations for bacterial ferrous-iron oxidation have been proposed by various authors (Nemati *et al.*, 1998; Boon, 1996). These can be broadly classified as either empirical or based on Michaelis-Menten/Monod kinetics. Empirical models use tools such as the logistic equation to model the kinetics, while Michaelis-Menten/Monod based rate equations assume that the rate limiting reactions can be represented using traditional enzyme kinetics.

Most of the ferrous-iron kinetic studies performed to date have been carried out using *Acidithiobacillus ferrooxidans**, at temperatures in the region of 30°C and pH values in the region of pH 2.0. Apart from the work performed by Lacey and Lawson (1970), in batch culture, and Nemati and Webb (1997), by initial rates method, however, the effect of temperature on ferrous-iron oxidation by *Acidithiobacillus ferrooxidans* has not been studied extensively. There is however very little data available on the effect of pH on the ferrous-iron oxidation kinetics of *Acidithiobacillus ferrooxidans*.

Boon (1996) proposed a modified Michaelis-Menten based rate equation in terms of the bacterial specific oxygen utilisation rate. The rate equation incorporated terms for both ferric-iron inhibition and a threshold ferrous-iron concentration. Boon (1996) showed that the

* The genus *Thiobacillus* has recently been reclassified and the species *Thiobacillus ferrooxidans*, *Thiobacillus thiooxidans* and *Thiobacillus caldus* have been renamed *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Acidithiobacillus caldus* respectively (Kelly and Wood, 2000).

kinetics could be related to the ferric/ferrous-iron ratio or redox potential, which is consistent with the chemiosmotic theory proposed by Ingledew (1986).

Furthermore, although most of the kinetic studies have been carried out with *Acidithiobacillus ferrooxidans* it has been shown that *Leptospirillum ferrooxidans* is at least as important, if not more so than *Acidithiobacillus ferrooxidans* in commercial bioleach reactors (Rawlings *et al.*, 1999; Boon, 1996, Rawlings, 1995; Norris *et al.*, 1988). The rate equation proposed by Boon (1996) has subsequently been used to describe the kinetics of *Acidithiobacillus ferrooxidans* (Boon, 1996) and *Leptospirillum ferrooxidans* (van Scherpenzeel *et al.*, 1998; van Scherpenzeel, 1996; Breed *et al.*, 1999; Breed and Hansford, 1999[a]). This suggests that the ferrous-iron oxidation kinetics of both *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* may be described using the similar rate equations.

The effects of temperature and pH on the ferrous-iron oxidation kinetics of *Leptospirillum ferrooxidans* have been investigated in continuous culture (Breed *et al.*, 1999; Breed and Hansford, 1999[a]).

Batch experiments require less time and reagents, but from most work performed to date it is apparent that there is confusion as to whether batch experiments are reliable when determining the bacterial ferrous-iron oxidation kinetics (Kelly and Jones, 1978; Macdonald and Clark, 1970).

The bacterial growth rate is usually related to the ferrous-iron oxidation rate by the growth yield, Y_{SX}, and the constant maintenance coefficient, m_S, in the Pirt Equation (Pirt, 1965). The Pirt Equation is based on the assumption that for a given amount of biomass the maintenance energy is constant and independent of the growth rate. Deviations from the results predicted by this equation have been found particularly when cultures are energy sufficient and limited by factors other than the energy source. To account for these differences, Neijssel and Tempest (1976) developed a relationship that incorporated variable maintenance energy. Pirt (1982) modified this relationship. The variable maintenance relationship is based on the assumptions that the maintenance energy term includes a portion that decreases with an increase in the specific growth rate and this growth dependent maintenance energy falls to zero as the growth rate approaches the maximum rate.

Differences between the calculated maximum bacterial yield and constant maintenance coefficients and those predicted by the Pirt Equation for ferrous-iron oxidation by *Acidithiobacillus ferrooxidans* have been noted by Jones and Kelly (1983) in continuous culture and Boon (1996) in batch culture. Breed *et al.* (1999), Breed and Hansford (1999[a]), and van Scherpenzeel (1996) reported similar deviations for the ferrous-iron oxidation by *Leptospirillum ferrooxidans* in continuous culture.

Based on the above it is apparent that bacterial ferrous-iron oxidation is an important sub-process in the bioleaching of sulfide minerals. This has been investigated in batch and continuous culture. Continuous experiments take a long time and are resource intensive, but differences have been noticed between the maximum bacterial yields determined during batch and continuous culture. The batch ferrous-iron oxidation was therefore investigated and compared to continuous experimental results.

The specific objectives of the research detailed in this thesis are to:

- Re-evaluate published data of other workers using the variable maintenance equation and assess the validity of the equation.
- Describe the results of an investigation into the effect of temperature and pH on the bacterial ferrous-iron oxidation by *Leptospirillum ferrooxidans* in batch culture.
- Evaluate the variable maintenance equation for use during bacterial ferrous-iron oxidation.
- Compare the bacterial ferrous-iron oxidation results obtained in batch culture with those obtained at the same conditions in continuous culture.
- Evaluate the validity of batch experiments in determining the kinetics of bacterial ferrous-iron oxidation.

The structure of the thesis is outlined below:

- Brief review of the pertinent literature.
- The theoretical aspects used to evaluate the data.
- The materials and methods used for batch temperature and pH work.
- Re-evaluation of previously published literature with respect to the implementation of the variable maintenance equation.
- Results and discussion. The results and discussion section is divided into the following sections: validation of the raw data, bacterial growth rate, yield and maintenance and kinetics of bacterial ferrous-iron oxidation.
- Finally, conclusions are drawn and recommendations are made.

Chapter 2

Literature Review

In order for bioleaching to compete with other pre-treatment processes, it needs to be optimised with regard to the parameters that affect the process. Furthermore, there is a need for mechanistically based kinetic models that can be used to derive performance equations that can be used in the design, optimisation and control of bioleaching processes. This has led to a large amount of research into the mechanisms and kinetics of bioleaching (Rawlings, 1997; Boon, 1996; Barrett *et al.* 1993; Rossi, 1990).

The existence of a multiple sub-process mechanism implies that the bacterial and chemical sub-processes may be studied separately. This research may be concentrated on a single sub-process and it was decided to focus the literature review on bacterial ferrous-iron oxidation. Extensive literature reviews on bacterial ferrous-iron oxidation, however have been written by Nemati *et al.* (1998) and Boon (1996). The material in this literature review has concentrated on the following topics:

- A description of the micro-organisms responsible for bacterial ferrous-iron oxidation.
- A description of the mechanism of the bacterial ferrous-iron oxidation.
- A description of general yield and maintenance and their applicability to bacterial ferrous-iron oxidation.
- A review of the kinetic models used to describe bacterial ferrous-iron oxidation.

2.1 Acidophilic Ferrous-iron Oxidation Micro-organisms

The micro-organisms that are responsible for ferrous-iron oxidation in bioleaching environments are acidophiles i.e. organisms that live in environments at pH values lower than pH 4.0. These micro-organisms are classified according to the optimal temperature for growth. Three classes of micro-organisms are proposed on this basis: mesophiles, moderate thermophiles and extreme thermophiles. The temperature range and most important micro-organisms for each class are listed in Table 2.1.

All the classes of bacteria have been reviewed elsewhere (Breed, 2000; Norris, 1997; Hallberg, 1995; Barrett *et al.*, 1993) hence in the scope of this thesis only mesophilic ferrous-iron oxidising micro-organisms will be reviewed.

The mesophilic ferrous-iron oxidising bacteria most often isolated from mining environments are *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans*. As stated previously, most of the kinetic studies have been carried out with *Acidithiobacillus ferrooxidans*, but it has recently been shown that *Leptospirillum ferrooxidans* is at least as important, if not more important than *Acidithiobacillus ferrooxidans* in commercial bioleach reactors (Rawlings *et al.*, 1999; Boon, 1996; Rawlings, 1995; Norris *et al.*, 1988).

Table 2.1: Table of the class of organism and temperature range for the most important ferrous-iron oxidising autotrophic micro-organisms (Hallberg, 1995; unless otherwise stated)

Class of Organism	Temperature Range	Micro-organism
Mesophiles	20 - 45 °C	*Acidithiobacillus ferrooxidans* *Leptospirillum ferrooxidans*
Moderate Thermophiles	45 - 55 °C	*Sulfobacillus thermosulfidooxidans* *Leptospirillum thermoferrooxidans*[*]
Extreme Thermophiles	>55 °C	*Sulfolobus acidocaldarius* *Acidianus brierleyi* *Metallosphaera sedula*[†] *Sulfolobus metallicus*[‡]

Acidithiobacillus ferrooxidans and *Leptospirillum ferrooxidans* are both Gram-negative, obligate autotrophs. *Acidithiobacillus ferrooxidans* is rod shaped and the cells range in diameter from 0.3 to 0.8 μm and in length from 0.9 to 2 μm (Barrett *et al.*, 1993). *Leptospirillum ferrooxidans* is usually spiral shaped and 0.5 to 3 μm in length (van Scherpenzeel, 1996). *Leptospirillum ferrooxidans* cells may appear to assume a cocoid shape in environments very high in ferric-iron as a result of the ferric-iron being incorporated in the EPS layer (Barrett *et al.*, 1993). This has possibly contributed to them being mistakenly identified as being *Acidithiobacillus ferrooxidans* during some previous investigations where the shape has been used as a means of identification.

Acidithiobacillus ferrooxidans is a facultative aerobe. It is able to obtain energy aerobically by using either ferrous-iron or reduced sulfur compounds and it is able to reduce ferric-iron to ferrous-iron anaerobically by using reduced sulfur compounds as the electron donor (Brock and Gustafson, 1976). *Leptospirillum ferrooxidans* however is an obligate aerobe and gains energy solely by oxidising aqueous ferrous-iron (Barrett *et al.*, 1993).

The optimum reported conditions (temperature and pH) for the growth and ferrous-iron oxidation of *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* are displayed in Table 2.2.

[*] Barrett *et al.* (1993)

[†] Norris (1997)

[‡] Searby and Hansford, unpublished data.

Table 2.2: Table of optimum temperature and pH values reported for the oxidation of ferrous-iron by *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans*

Bacteria	Optimum Temperature (°C)	Optimum pH
Acidithiobacillus ferrooxidans (Barrett *et al.*, 1993)	28 - 35	2.0 - 2.5
Leptospirillum ferrooxidans (van Scherpenzeel, 1996)	35	1.5 - 2.0
Leptospirillum ferrooxidans (Breed, 2000)	40	1.5

2.2 Mechanism of Ferrous-iron Oxidation

Acidithiobacillus ferrooxidans is able to derive the energy required for growth from the oxidation of ferrous- to ferric-iron, using oxygen as the oxidant. The coupling of ATP synthesis and ferrous-iron oxidation in *Acidithiobacillus ferrooxidans* is explained using the chemiosmotic theory (Ingledew, 1986).

Ingledew (1986) suggested that the ferrous- to ferric-iron half reaction occurs at the outside of the cell envelope:

$$Fe^{2+} \rightarrow Fe^{3+} + e^{-} \qquad (2.1)$$

The electrons that are produced from this half reaction are transported via an electron transport chain through the cytoplasmic membrane to the cytoplasm where they are consumed by the oxygen half reaction:

$$\frac{1}{2}O_2 + 2H^{+} + 2e^{-} \rightarrow H_2O \qquad (2.2)$$

Oxygen therefore acts as the final electron acceptor after the electron donated by the ferrous-iron has passed through the electron transport chain. Ingledew (1986) proposed that the electron transport chain consisted of rusticyanin, cytochrome c and cytochrome a, as shown in Figure 2.1. The electron transport chain results in a trans-membrane electrical potential. The trans-membrane electrical potential and the pH gradient produce a proton motive force.

The scheme shows the consumption of hydrogen ions in the cell cytoplasm. Ingledew (1986) proposed that if the leakage of protons did not occur through the cell membrane, then ATP could be formed by protons driven through ATP-synthetase due to the proton electrochemical gradient.

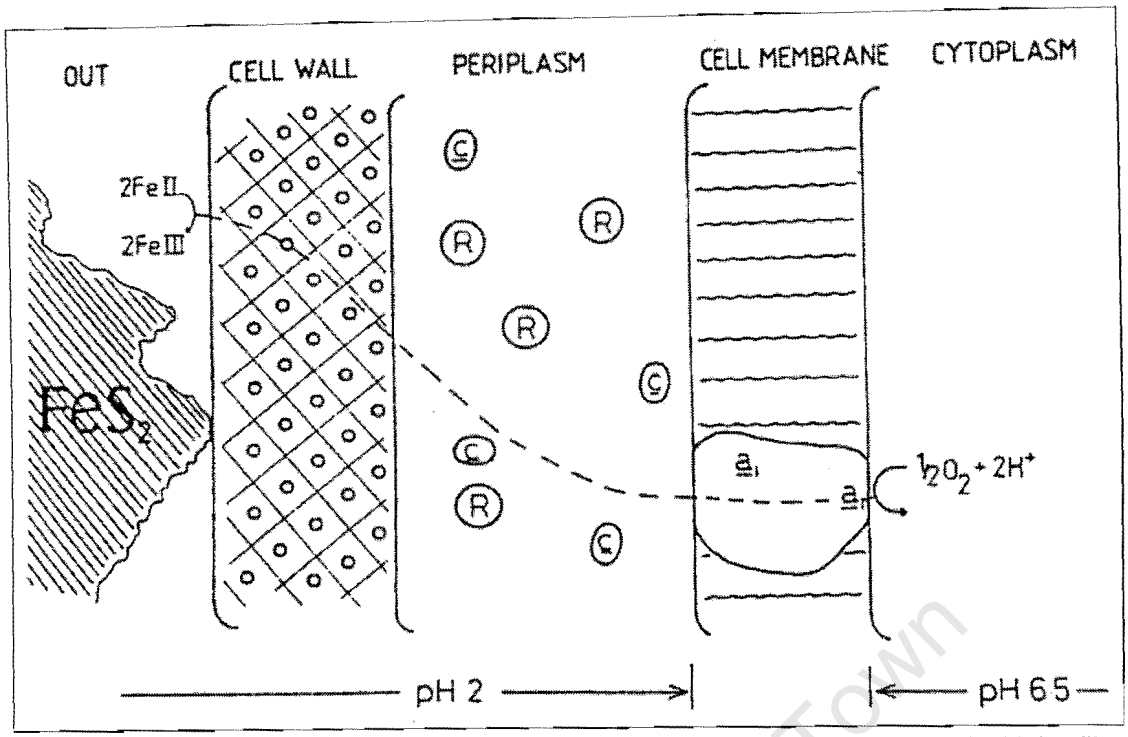


Figure 2.1: Schematic representation of the ferrous-iron oxidation by *Acidithiobacillus ferrooxidans*, (®) rusticyanin, (©) cytochrome c, (a_1) cytochrome a, the small circles in the cell wall represent bound ferric-iron (after Ingledew, 1986).

It has been noted that if the oxygen half reaction (Equation 2.2) occurred outside the cell envelope, then a proton pump would be required (Ingledew, 1986). The reason for this is that the external pH is very low (<2) and the bacteria have to maintain a pH of approximately 6 in the cell cytoplasm.

Rusticyanin, which is an important component in the electron transport chain, is a small blue copper protein that is present at relatively high concentrations in *Acidithiobacillus ferrooxidans*, up to 5% of the cell protein (Ingledew, 1986). Ingledew (1986) reported that rusticyanin is directly, but slowly, reducible by ferrous-iron when pure. Cavazza and Bruschi (1995) reported that rusticyanin does not appear to be the initial electron acceptor, but they reported that the initial electron acceptor may be iron:rusticyanin oxidoreductase.

2.3 Yield and Maintenance

2.3.1 General

The cell concentration is often very difficult to measure, hence it is common to evaluate the growth rate from the substrate utilisation rate. Growth rate is usually related to the substrate utilisation rate by the growth yield, Y_{SX}, which is defined to be the amount of biomass produced per substrate consumed:

$$Y_{sx} = \frac{\text{quantity of biomass produced}}{\text{quantity of substrate used}} \qquad (2.3)$$

or

$$Y_{sx} = \frac{\text{rate of biomass production}}{\text{rate of substrate utilisation}} = \frac{r_x}{-r_s} \quad (2.4)$$

It is often assumed that the growth yield is constant, however this assumption is only valid within strict limits. Some of the factors that have been found to affect the yield include temperature, pH, growth rate, inhibitory ions, growth-limiting substrate, and maintenance energy.

Pirt (1965) postulated that bacteria require energy for both growth and maintenance. The balance for energy source utilisation is given by:

$$\left\{\begin{array}{c}\text{overall rate of} \\ \text{substrate utilisation}\end{array}\right\} = \left\{\begin{array}{c}\text{rate of substrate utilisation} \\ \text{for maintenance}\end{array}\right\} + \left\{\begin{array}{c}\text{rate of substrate utilisation} \\ \text{for growth}\end{array}\right\}$$

If it assumed that the maintenance energy requirement is constant and independent of the growth rate and the maximum growth yield, Y_{sx}^{max}, occurs when the maintenance energy, m_s, is zero, then:

$$\frac{\mu c_x}{Y_{sx}} = \frac{\mu c_x}{Y_{sx}^{max}} + m_s c_x \quad (2.5)$$

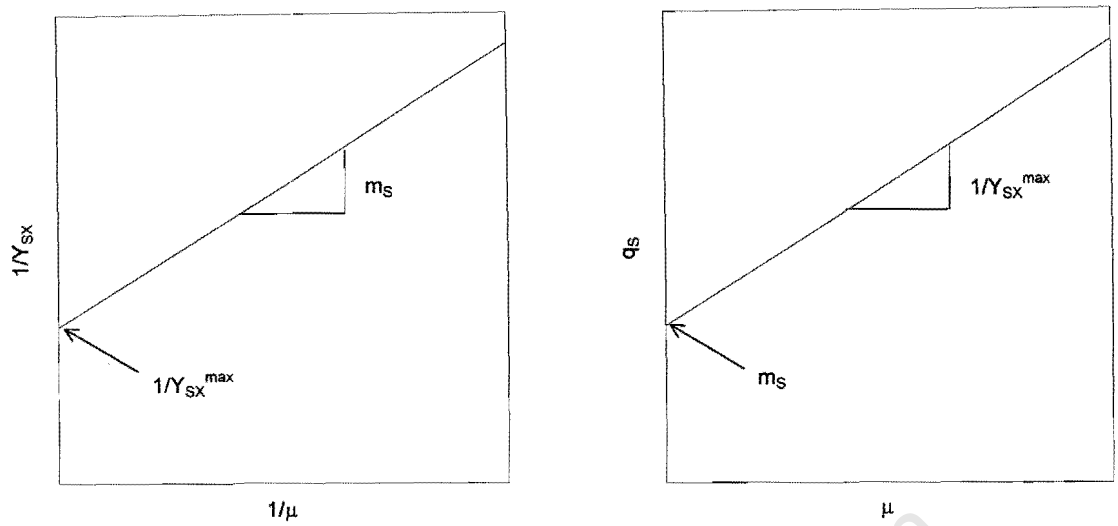
hence:

$$\frac{1}{Y_{sx}} = \frac{1}{Y_{sx}^{max}} + \frac{m_s}{\mu} \quad (2.6)$$

alternatively, multiplying this equation by μ:

$$q_s = \frac{\mu}{Y_{sx}^{max}} + m_s \quad (2.7)$$

From Equations 2.6 and 2.7 it is possible to obtain the maximum growth yield and the maintenance coefficient from linear graphs of $\frac{1}{Y_{sx}}$ vs. $\frac{1}{\mu}$ or q_s vs. μ respectively as shown in Figures 2.2a and b.



a) b)

Figure 2.2: Plots used to determine the maximum growth yield and the maintenance coefficient using a) Equation 2.6 and b) Equation 2.7.

The linear relationship between the specific substrate utilisation rate, q_S, and the specific growth rate, μ, as shown in Equation 2.7 and the constant maintenance energy requirement has been confirmed by many researchers for different heterotrophic micro-organisms (Hempfling and Mainzer, 1975; Pirt, 1975; Schulze and Lipe, 1964). The validity of this relationship, however, has been questioned as a result of deviations from the constant maintenance equation (Equation 2.7) when the micro-organisms are energy sufficient, i.e. when the growth is limited by something other than the energy source (Downs and Jones, 1975; Neijssel and Tempest, 1975; Stouthamer and Bettenhausen, 1975 and Kuenen, 1979).

Neijssel and Tempest (1976) suggested that the maintenance requirement was dependent on the specific growth rate:

$$\frac{1}{Y_{SX}} = \frac{1}{Y_{sx}^{max}} + \frac{m_s}{\mu} + cm_s \qquad (2.8)$$

where c is a second constant that defines the variation in the maintenance requirement with growth rate.

Pirt (1982) modified the equation developed by Neijssel and Tempest (1976) by assuming that the maintenance energy term included a portion that decreased with an increase in the specific rate of growth:

$$q_S = \frac{\mu}{Y_{sx}^{max}} + m_S + m_S^v(1-k\mu) \qquad (2.9)$$

In Equation 2.9, m_s is the constant maintenance energy term and $m_s^v(1-k\mu)$ is the growth rate dependent maintenance energy. Pirt (1982) also postulated that the growth rate dependent maintenance energy requirement decreased to zero as the specific growth rate, μ, approached the maximum value, μ^{max}. This implies that:

$$k = \frac{1}{\mu^{max}} \tag{2.10}$$

Combining Equations 2.9 and 2.10 yields:

$$q_S = \left(\frac{1}{Y_{SX}^{max}} - \frac{m_S^v}{\mu^{max}}\right)\mu + m_S + m_S^v \tag{2.11}$$

A graphical representation of Equation 2.11 is shown in Figure 2.3. The Figure contains two lines representing experimental data under different energetic conditions. The solid line represents data from energy-limited experiments and the broken line represents data from energy-sufficient experiments. The constant maintenance energy requirement, m_S, is the minimum energy that is required to maintain the vital functions of the micro-organisms. It is therefore apparent that under conditions in which the growth is determined by substrate limitation, the maintenance energy of the organisms is the lowest and constant. The constant maintenance coefficient can thus be obtained from the y-intercept the solid line. The variable maintenance coefficients can be calculated from energy sufficient experiments, thus it can be determined from the y-intercept of the broken line. The variable maintenance coefficient is dependent on which factor is limiting. These factors include; phosphate, PO_4^{3-}, sulfate, SO_4^{2-}, ammonium, NH_4^+, heavy metals and growth rate. Because the variable maintenance requirement falls to zero under energy limited conditions, the maximum growth yield can be determined from the slope of the solid line.

From Figure 2.3 it can be seen that the maximum bacterial specific substrate utilisation rate and the maximum bacterial specific growth rate are defined by the intersection of the solid and the broken lines.

Pirt (1982) confirmed the validity of Equation 2.11 using the continuous data for *Klebsiella aerogenes* published by Neijssel and Tempest (1976). The data was obtained from experiments in which the limiting substances were either phosphate, sulphate, ammonia or glucose. The constant maintenance coefficient was calculated from the experiment that was glucose limited, while the variable maintenance coefficients were calculated from the experiments limited by the other factors. Phosphate limitation was found to cause the largest variable maintenance coefficient followed by the sulfate limitation, and finally limitation by ammonia. The variable maintenance coefficients calculated for ammonia and sulfate however were very similar.

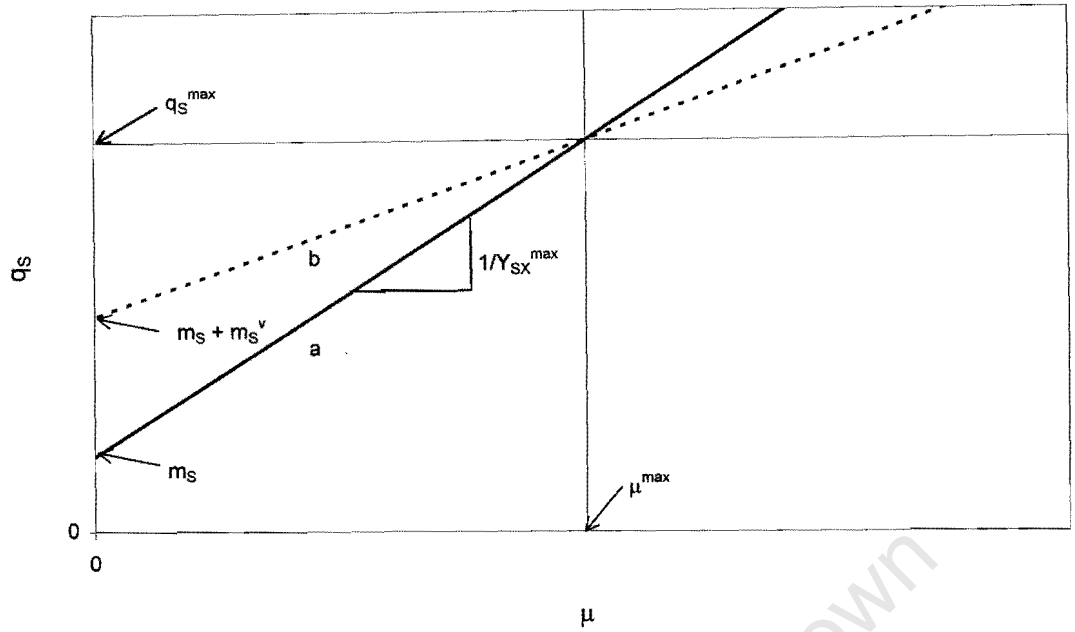


Figure 2.3: Two possible relations of specific rate of substrate utilisation (q_S) to the specific growth rate (μ) with a) constant maintenance and b) variable maintenance.

From the above discussion it is therefore apparent that the bioenergetics of bacteria can only be represented by the equation proposed by Pirt (1965) (Equation 2.7) if the culture is limited by a single substrate for the duration the entire experiment. If the limiting substrate varies during the experiment then the bioenergetics might need to be represented by the equation with variable maintenance requirement proposed by Pirt (1982) (Equation 2.11).

2.3.2 Yield and Maintenance for Ferrous-iron Oxidising Micro-organisms

Most of the early researchers who investigated bacterial ferrous-iron oxidation by *Acidithiobacillus ferrooxidans* did not take maintenance into account and assumed that the yield coefficient was a constant (Lacey and Lawson, 1970; Liu *et al.*, 1988; Macdonald and Clark, 1970; Shrihari *et al.*, 1990):

Macdonald and Clark (1970) determined that the bacterial yield on ferrous-iron in continuous culture was independent of the dilution rate and was 4.7×10^{10} cells/g of iron oxidised.

Kelly and Jones (1978) discovered that ferrous-iron oxidation can be uncoupled from carbon dioxide utilisation in *Acidithiobacillus ferrooxidans* in batch culture and the bacteria had a mechanism by which the energy from iron oxidation can be dissipated.

Jones and Kelly (1983) investigated the bacterial ferrous-iron oxidation of *Acidithiobacillus ferrooxidans* in continuous culture at 30°C and a pH of 1.6. They modelled the bioenergetics using Equation 2.6 and found two different relationships, depending on whether competitive or non-competitive product inhibition was present. The results obtained by the authors are situated in Table 2.3.

Table 2.3: Table of maximum bacterial yield on ferrous-iron and the maintenance coefficients obtained by Jones and Kelly (1983)

	Y_{SX}^{max} (g dry weight.(g Fe^{2+})$^{-1}$)	m_S (g Fe^{2+}.(g dry weight)$^{-1}$.h^{-1})
Competitive Inhibition	0.36 - 0.38	≤0.04
Non-competitive Inhibition	1 - 1.33	0.38 - 0.43

Boon (1996) investigated the bacterial oxidation of ferrous-iron by *Acidithiobacillus ferrooxidans* in continuous and batch culture. Boon also reported the work of van Scherpenzeel (1996) for *Leptospirillum ferrooxidans* in continuous culture. The results presented for *Acidithiobacillus ferrooxidans* by Boon (1996) are listed in Table 2.4. Boon attempted to model the bioenergetics of the ferrous-iron oxidation by *Acidithiobacillus ferrooxidans* by means of the Pirt Equation with constant maintenance energy (Equation 2.6). Only the continuous data could be modelled successfully by this equation. The Pirt Equation (Equation 2.6) was fitted through the initial and the final sections of the batch data separately.

Table 2.4: Values of the average maximum bacterial yield on ferrous-iron, $Y_{Fe^{2+}X}^{max}$, and the maintenance coefficient on ferrous-iron, $m_{Fe^{2+}}$, reported by Boon (1996) using *Acidithiobacillus ferrooxidans* at 30°C and a pH of 1.8*

Conditions	$Y_{Fe^{2+}X}^{max}$ (molC.(molFe^{2+})$^{-1}$)	$m_{Fe^{2+}}$ (molFe^{2+}.molC^{-1}.h^{-1})
Batch (initial)	0.032	8
Batch (final)	0.0123	0.8
Continuous	0.012	0.4

Based on the maximum yield and maintenance coefficients presented by Boon (1996) listed in Table 2.4 it is apparent that the only real difference is that the results reported for the initial section of the batch are higher than those calculated for the final section of the batch and the continuous data.

The ferrous-iron oxidation by *Leptospirillum ferrooxidans* has been investigated by a number of researchers and the maintenance and yield results are displayed in Table 2.5. All of the researchers used the Pirt Equation (Equation 2.7). The maximum bacterial yield on ferrous-iron and the maintenance coefficient on ferrous-iron calculated by all the researchers are of the same order of magnitude and are of the same order of magnitude as those calculated for *Acidithiobacillus ferrooxidans*.

It was found that the maximum bacterial yields and the maintenance coefficients calculated for *Leptospirillum ferrooxidans* in continuous culture did not show any appreciable variation with changes in either temperature (Breed *et al.*, 1999) or pH (Breed and Hansford, 1999[a]) over the temperature range 30 to 40 °C or the pH range 1.1 to 1.7.

* The maximum yields and maintenance coefficients on ferrous-iron were calculated from the "degree of reduction" balance, Equation 3.5.

Table 2.5: Values of the average maximum bacterial yield on ferrous-iron, $Y^{max}_{Fe^{2+}X}$, and the maintenance coefficient on ferrous-iron, $m_{Fe^{2+}}$ calculated from continuous culture data for *Leptospirillum ferrooxidans*

Reference	Temp. (°C)	pH	$Y^{max}_{Fe^{2+}X}$ (molC.(molFe^{2+})$^{-1}$)	$m_{Fe^{2+}}$ (molFe^{2+}.molC^{-1}.h^{-1})
Breed and Hansford (1999)	40	1.1-1.7	0.0074	1.0029
Breed *et al.* (1999)	35-40	1.7	0.0059	0.797
van Scherpenzeel *et al.* (1998)	30	1.5-1.6	0.011	0.444
van Scherpenzeel (1996) Low Dilution Rates*	30	1.5-1.6	0.0109	0.0125
van Scherpenzeel (1996) High Dilution Rates*	30	1.5-1.6	0.0125	0.888

The values reported by van Scherpenzeel *et al.* (1998) are average parameters over all of the dilution rates. van Scherpenzeel (1996) however found evidence that the maintenance coefficient and the maximum bacterial yield on ferrous-iron and oxygen had different values depending on the specific growth rate (dilution rate). The boundary between the two was at a specific growth rate of 0.03 h^{-1}.

Breed *et al.* (1999) and Breed and Hansford (1999[a]) suggested that the maximum bacterial yield on ferrous-iron and oxygen had a dependency on the specific growth rate (dilution rate) for *Leptospirillum ferrooxidans* in continuous culture. The authors concluded that the constant maintenance model proposed by Pirt (1965) might not be applicable to their systems.

From these investigations it appears that the bacterial ferrous-iron oxidation by *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* in batch and continuous culture does not appear to obey the constant maintenance model proposed by Pirt (1965). If a bacterial culture is not limited by the energy source, but is limited by another factor, then the maximum growth yield calculated using the constant maintenance model is a gross overestimate of the actual value.

2.4 Bacterial Ferrous-Iron Oxidation Kinetics

The multiple sub-process mechanism suggests that during the bioleaching of sulfide minerals the role of the micro-organisms is the oxidation of ferrous-iron to ferric-iron and the oxidation of the sulfur moiety to sulfate. It has been proposed that ferrous-iron oxidation is the rate controlling sub-process in the bioleaching of sulfide minerals (Boon, 1996). This assumption enabled Breed (2000) to use the kinetics of bacterial ferrous-iron oxidation combined with the kinetics of chemical ferric leaching to successfully model the bioleaching of pyrite and arsenopyrite. It is therefore evident that bacterial ferrous-iron oxidation is an important sub-process in the bioleaching of sulfide minerals.

* The maximum yields and maintenance coefficients on ferrous iron were calculated from the "degree of reduction" balance, Equations 3.5.

A number of kinetic models for bacterial ferrous-iron oxidation have been proposed (Nemati *et al.*, 1998; Boon 1996) and a table of these is displayed in Table 2.6. They can be classified as either empirically based, or based on Michaelis-Menten/Monod kinetics. The empirical models use tools such as the logistic equation to model the ferrous-iron oxidation kinetics (La Motta, 1976; Pinches *et al.*, 1988).

2.4.1 Basic Growth Kinetics

Michaelis-Menten-type models are based on the assumption that the rate limiting reactions involve the formation of an enzyme-substrate complex. According to this assumption, the substrate, S, combines with the enzyme, E, to form an enzyme-substrate complex, ES, which decomposes in the final step to form the product, P, and free enzyme.

$$E + S \leftrightarrow ES \rightarrow E + P \tag{2.12}$$

The Michaelis-Menten rate equation for uninhibited growth with ferrous-iron as the growth limiting substrate is:

$$V = \frac{V_{max}}{1 + \frac{K_m}{[Fe^{2+}]}} \tag{2.13}$$

On the other hand, the Monod-type rate equations are based on the assumption that bacterial growth on ferrous-iron can be described by the Monod equation:

$$\mu = \frac{\mu^{max}}{1 + \frac{K_S}{[Fe^{2+}]}} \tag{2.14}$$

The Monod equation has a similar form to that of the Michaelis-Menten expression with only one growth limiting substrate and all other essential nutrients in excess. It also assumes that the maintenance energy requirement and the endogenous metabolism are negligible.

The Michaelis-Menten and the Monod based models can both be modified to account for the changes in the conditions under which the organisms are grown, e.g. temperature, pH, inhibitory substances, and more than one limiting substrate.

Schnaitman *et al.* (1969) modelled the ferrous-iron oxidation kinetics of *Ferrobacillus ferrooxidans** in fed batch culture using Michaelis-Menten kinetics. The rate of reaction was found to be directly proportional to the concentration of cells added and followed zero order kinetics. The kinetic constant (K_S) remained constant over the pH range 2.4 to 3.6, however the change in V_{max} was significant over the pH range.

Lacey and Lawson (1970) measured the ferrous-iron oxidation kinetics of *Acidithiobacillus ferrooxidans* in batch culture. They assumed that the maintenance energy was negligible and

**Ferrobacillus ferrooxidans* was later renamed to *Thiobacillus ferrooxidans* (Kelly and Tuovinen, 1972).

that the substrate utilisation rate could be directly related to the substrate utilisation rate via a yield constant and derived a model based on the Monod equation.

Macdonald and Clark (1970) performed both batch and continuous ferrous-iron oxidation experiments with *Acidithiobacillus ferrooxidans* and used the Monod equation to describe their results. They found, however that stringent precautions had to be taken to eliminate growth of bacteria on the internal surfaces of the reactor when modelling continuous experiments. Batch experiments were found to be unsuitable for the determination of the model parameters, because the growth rate changes significantly only during the last few hours of a batch test.

Guay *et al.* (1977) also performed ferrous-iron oxidation experiments in both batch and continuous culture with *Acidithiobacillus ferrooxidans*, however they only modelled the continuous experiments using the Monod model. They reported that the maximum rate of ferrous-iron oxidation, V_{max}, increased with an increase in the concentration of ferrous-iron in the feed. This increase in V_{max} was however accompanied by a decrease in the dilution rate at which washout occurred, *i.e.* the dilution rate at which washout occurred decreased with an increase in the concentration of ferric-iron in the bioreactor.

2.4.2 Modified Monod Kinetics

The Monod model makes the assumption that there is no energy is used for cell maintenance. If maintenance energy exists then there is a discrete rate of substrate utilisation that can take place without growth. This implies that the Monod equation should be written for the specific rate of substrate utilisation rather than the specific growth rate:

$$q_S = \frac{-r_S}{c_X} = \frac{q_S^{max}}{1 + \frac{K_S}{[Fe^{2+}]}} \tag{2.15}$$

The specific substrate utilisation rate is then related to the specific growth rate by the growth yield and maintenance coefficient. Lacey and Lawson (1970) first introduced this modification. They however chose to neglect the maintenance requirement.

2.4.3 Threshold Concentrations

The threshold concentration of a substrate, S_t, represents a concentration of that substrate below which there is insufficient energy available for cell growth. In order to model a system with threshold concentrations, these concentrations must be subtracted from the total concentration of that substrate:

$$\mu = \frac{\mu^{max}}{1 + \frac{K_S}{(S - S_t)}} \tag{2.16}$$

Braddock *et al.* (1984) examined the iron-limited growth of an arsenic tolerant strain of *Acidithiobacillus ferrooxidans*, in both batch and continuous culture, and the growth kinetics of

the culture could be described using a modified Monod model, in which a threshold concentration of ferrous-iron, $[Fe^{2+}]_t$ = 0.25 mM Fe^{2+}, was included.

Boon (1996) investigated the continuous bacterial ferrous-iron oxidation of *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* and determined a threshold ferrous-iron concentration of 7×10^{-3} mM Fe^{2+}.

Breed *et al.* (1999) and Breed and Hansford (1999[a]) investigated the continuous ferrous-iron oxidation by *Leptospirillum ferrooxidans* and found that the threshold ferrous-iron concentrations ranged from 0.054 to 0.268 mM. They determined that the threshold ferrous-iron was very low and could be neglected when modelling the bacterial ferrous-iron oxidation.

Liu *et al.* (1988) found that a Monod based model could be used to describe the growth kinetics of *Acidithiobacillus ferrooxidans* under conditions in which the dissolved oxygen concentration was limiting and included a threshold concentration of oxygen, $[O_2]_t$ = 0.2mg.ℓ^{-1},

2.4.4 Inhibition

At high concentrations of substrate or product and in the presence of inhibitory substances in the medium, growth rate becomes inhibited and growth rate is dependent on the concentration of the inhibitor. The mathematical inclusion is accomplished by incorporating an inhibition term containing the inhibition constant, K_I, and the concentration of the inhibitory species, C_I, in the denominator of the Monod expression (Equation 2.14). The form of the inhibition term depends on the type of inhibition. Inhibition that is important to this investigation can be divided into three categories: substrate inhibition, competitive- and non-competitive product inhibition.

At high substrate concentrations, microbial growth may be inhibited by the substrate. For substrate inhibition, the following form of the Monod model is used:

$$\mu = \frac{\mu^{max}}{1 + \frac{K_S}{S} + \frac{S}{K_{SI}}} \qquad (2.17)$$

High concentrations of product can be inhibitory to microbial growth. Product inhibition may be competitive or non-competitive. For competitive inhibition, the following expression is obtained:

$$\mu = \frac{\mu^{max}}{1 + \frac{K_S}{S} + \frac{K_S}{K_I}\frac{C_I}{S}} \qquad (2.18)$$

For non-competitive inhibition:

$$\mu = \frac{\mu^{max}}{1 + \frac{K_S}{S} + \frac{C_I}{K_I} + \frac{K_S}{K_I}\frac{C_I}{S}} \qquad (2.19)$$

Kelly and Jones (1978) investigated the ferrous-iron oxidation kinetics of *Acidithiobacillus ferrooxidans* in batch and continuous culture. They found the ferrous-iron oxidation kinetics to be subject to competitive inhibition by ferric-iron and non-competitive inhibition by uranyl ions and protons (i.e. decreased pH). Further work on the ferrous-iron oxidation kinetics of *Acidithiobacillus ferrooxidans* in continuous flow bioreactors indicated that both iron oxidation and growth were subject to competitive and non-competitive product inhibition by ferric-iron, and under certain conditions, substrate inhibition by ferrous-iron (Jones and Kelly, 1983).

Nikolov and Karamanev (1992) found that the kinetics of resuspended cells of *Acidithiobacillus ferrooxidans* could be accurately described using a Monod model modified to account for substrate (ferrous-iron) and product (ferric-iron) inhibition.

In their extensive investigation, Nemati and Webb (1997) used initial ferrous-iron oxidation rates to determine the kinetics of bacterial ferrous-iron oxidation by *Acidithiobacillus ferrooxidans*. The initial ferrous-iron oxidation rates were determined from redox potential measurements. Their approach was based on Michaelis-Menten kinetics and the model developed included terms for the effects of temperature, ferrous-iron and bacterial concentration. It also included terms to account for inhibition by both substrate and cells. The resulting model was successfully used to simulate the full range of experimental data obtained. In contrast to most research on the kinetics of bacterial ferrous-iron oxidation performed, the model proposed by Nemati and Webb (1997) does not include parameters to account for inhibition by ferric-iron. Furthermore, the use of artificially high cell concentrations (as a result of cells having been harvested from batch culture) and the use of high initial ferrous-iron oxidation concentrations (relative to those observed in chemostat culture) may have contributed to the ferrous-iron oxidation kinetics being inhibited by these factors.

It was suggested that the ferrous-iron oxidising ability of a cell may be competitively inhibited by other cells, *i.e.* the cells and ferrous-iron compete for the ferrous-iron binding sites of the cells (Suzuki *et al.*, 1989). The experimental results obtained for the strains found to be subject to this form of inhibition could be described using Michaelis-Menten kinetics modified to account for competitive inhibition by cells. Nemati and Webb (1997) also found that at high cell concentrations the bacteria could cause competitive inhibition.

Boon (1996) investigated the ferrous-iron oxidation by *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* in continuous and batch culture and modelled the kinetics using a modified form of the Monod model that included a competitive ferric-iron inhibition term and a threshold ferrous-iron concentration term.

Boon *et al.* (1995) showed that the ferrous-iron saturation term and the threshold ferrous-iron concentration could be neglected and developed the following model:

$$q_{Fe^{2+}} = \frac{-r_{Fe^{2+}}}{c_x} = \frac{q_{Fe^{2+}}^{max}}{1 + K\frac{[Fe^{3+}]}{[Fe^{2+}]}} \qquad (2.20)$$

Braddock *et al.* (1984) found that neither ferric-iron nor the addition of 0.2g.ℓ^{-1} of arsenite had any noticeable inhibitory effect on the ferrous-iron oxidation kinetics of *Acidithiobacillus ferrooxidans* in continuous culture.

Harvey and Crundwell (1997) investigated the ferrous-iron oxidation of *Acidithiobacillus ferrooxidans* in the presence and absence of arsenite using redox controlled experiments and batch experiments. The experimental results obtained by the authors could be accurately described by the Monod equation modified to account for competitive inhibition by ferric-iron and arsenite. The arsenite was found to inhibit at concentrations ranging from 0 to 7 $g.\ell^{-1}$.

2.4.5 Kinetic Control by more than one Substrate

The Monod equation (Equation 2.14) was originally derived for growth on only one limiting substrate and is not applicable for growth on more than one limiting substrate. The Monod expression can, however be modified to account for more than one limiting substrate by multiplying Monod expressions for all the possible substrates in the following way:

$$\mu = \mu^{max}\left(\frac{1}{1+\frac{K_1}{S_1}}\right)\left(\frac{1}{1+\frac{K_2}{S_2}}\right)\cdots \qquad (2.21)$$

Huberts (1994) used Michaelis-Menten kinetics to describe the ferrous-iron oxidation kinetics of *Acidithiobacillus ferrooxidans* with ferrous-iron and oxygen limitation. The proposed rate equation incorporated competitive inhibition of ferric-iron.

2.4.6 Effect of pH

According to enzyme kinetics, pH should affect K_m in the Michaelis-Menten model according to (Shuler and Kargi, 1992):

$$V = \frac{V_{max}}{1+\frac{K_m'\left[1+\frac{K_{H2}}{[H^+]}+\frac{[H^+]}{K_{H1}}\right]}{[Fe^{2+}]}} \qquad (2.22)$$

Breed and Hansford (1999[a]) investigated the effect of pH on the ferrous-iron oxidation kinetics of *Leptospirillum ferrooxidans* in continuous culture. They used the form of the Michaelis-Menten model proposed by Boon (1996) and found that K varied linearly with pH.

2.4.7 Effect of Temperature

The Monod model can be modified to take temperature into account by assuming that the change in μ^{max} (q^{max}) with temperature obeys the Arrhenius equation:

$$\mu^{max} = Ae^{-\frac{E_a}{RT}} \qquad (2.23)$$

Lacey and Lawson (1970) investigated the effect of temperature on bacterial ferrous-iron oxidation using *Acidithiobacillus ferrooxidans* in batch culture. They incorporated the Arrhenius equation to account for the effect of temperature. The activation calculated is 33.5 $kJ.mol^{-1}$.

Nemati and Webb (1997) investigated the effect of temperature on bacterial ferrous-iron oxidation by *Acidithiobacillus ferrooxidans* using an initial rates method. The effect of temperature on the maximum reaction rate was modelled by the Arrhenius equation and the activation energy calculated is 68.4 $kJ.mol^{-1}$.

Breed *et al.* (1999) investigated the effect of temperature on ferrous-iron oxidation of *Leptospirillum ferrooxidans* in continuous culture and used the form of the Michaelis-Menten model proposed by Boon *et al.* (1995) (Equation 2.20) to model their results. The effect of temperature on the bacterial specific ferrous-iron utilisation rate was modelled by the Arrhenius equation. The activation energy calculated is 35.63 $kJ.mol^{-1}$.

The values of the activation energies calculated by Breed *et al.* (1999) and Lacey and Lawson (1970) are very similar. The value of the activation energy calculated by Nemati and Webb (1997), however is approximately double their values. A possible reason for this is that Nemati and Webb (1997) used an initial rates method to obtain their data, while Breed *et al.* (1999) and Lacey and Lawson (1970) used continuous experiments to obtain their data. Other possible reasons to account for the differences are different temperature ranges used and various bacterial species or strains employed.

Table 2.6: Table of Bacterial Ferrous-iron Oxidation Models

Reference	Equation	Conditions	Parameters*
Schnaitman, Korzynski and Lundgren (1969)	$V = \frac{V_{max}}{1 + \frac{K_m}{[Fe^{2+}]}}$	**Batch** T = 30°C pH = 1.8 - 3.6	K_m=5.4×10^{-3}M Fe^{2+}, V_{max} changes with pH
Lacey and Lawson (1970)	$q = \frac{\mu^{max}}{Y_{sx}\left(1 + \frac{K_m}{[Fe^{2+}]}\right)}$	**Batch** T = 20 - 31°C pH = 2 - 2.3	20°:μ^{max}=0,15 h^{-1}, K_m=1.10g$Fe^{2+}.\ell^{-1}$ 25°:μ^{max}=0.20 h^{-1}, K_m=1.05g$Fe^{2+}.\ell^{-1}$ 31°:μ^{max}=0.25 h^{-1}, K_m=1.01g$Fe^{2+}.\ell^{-1}$
Macdonald and Clark (1970)	$\mu = \frac{\mu^{max}}{1 + \frac{K_S}{[Fe^{2+}]}}$	**Batch:** T=32°C pH=1.9 **Continuous:** T=28°C pH=2.2	**Batch:**μ^{max}=0.145 h^{-1}, K_S=0.402g$Fe^{2+}.\ell^{-1}$ **Continuous:** μ^{max} = 0.161 h^{-1}, K_S=0.215 g$Fe^{2+}.\ell^{-1}$
Guay, Silver and Torma (1977)	$V = \frac{V_{max}D}{K + D}$	**Continuous** T = 32°C pH = 2.3	5g.ℓ^{-1}:V_{max}=46.51mmol$Fe^{2+}.\ell^{-1}.h^{-1}$, K=0.526 h^{-1} 7g.ℓ^{-1}:V_{max}=47.85mmol$Fe^{2+}.\ell^{-1}.h^{-1}$, K=0.417 h^{-1} 9g.ℓ^{-1}:V_{max}=52.08mmol$Fe^{2+}.\ell^{-1}.h^{-1}$, K=0.283 h^{-1}
Braddock, Luong and Brown (1983)	$\mu = \frac{\mu^{max}}{1 + \frac{K_S}{[Fe^{2+}] - [Fe^{2+}]_t}}$	**Continuous** T = 22 - 23°C pH = 1.85 - 1.95	μ^{max} = 0.07 h^{-1}, K_S = 0.78 mM Fe^{2+}, $[Fe^{2+}]_t$ = 0.25 mM Fe^{2+}
Jones and Kelly (1983)	$\mu = \frac{\mu^{max}}{1 + \frac{K_S}{[Fe^{2+}]} + \frac{K_S}{K_p}\frac{[Fe^{3+}]}{[Fe^{2+}]}}$	**Continuous** T = 30°C pH=1.6 ± 0.05	**Competitive:** μ^{max}=1.25 h^{-1}, K_s = 0.8 mM Fe^{2+}, K_p = 1.0 mM Fe^{3+}
Jones and Kelly (1983)	$\mu = \frac{\mu^{max}}{1 + \frac{K_S}{[Fe^{2+}]} + \frac{K_S}{K_p}\frac{[Fe^{3+}]}{[Fe^{2+}]} + \frac{[Fe^{3+}]}{K_p}}$	**Continuous** T = 30°C pH=1.6 ± 0.05	**Non-competitive:** μ^{max}=1.33 h^{-1}, K_s = 2.4 mM Fe^{2+}, K_p = 2.5 mM Fe^{3+}
Liu, Branion and Duncan (1988)	$\mu = \frac{\mu^{max}}{1 + \frac{K_S}{[Fe^{2+}]} + \frac{K_S}{K_p}\frac{[Fe^{3+}]}{[Fe^{2+}]}}$	T = 35°C pH = 1.8	**Batch:** μ^{max} = 0.11 h^{-1}, K_s = 0.048 g$Fe^{2+}.\ell^{-1}$, K_p = 0.441g$Fe^{3+}.\ell^{-1}$ **Continuous:** μ^{max} = 0.12 h^{-1}, Ks = 0.089 g$Fe^{2+}.\ell^{-1}$, K_p = 2.326 g$Fe^{3+}.\ell^{-1}$
Liu, Branion and Duncan (1988)	$\mu = \frac{\mu^{max}}{1 + \frac{K_S}{[O_2] - [O_2]_t}}$	**Batch** T = 35°C pH = 1.8	μ^{max} = 0.11 h^{-1}, K_s = 0.056 mg $O_2.\ell^{-1}$, $[O_2]_t$ = 0.2 mg $O_2.\ell^{-1}$,
Lizama and Suzuki (1989)	$q_{O_2} = \frac{q_{O_2}^{max}}{1 + \frac{K_m}{[Fe^{2+}]} + \frac{K_m}{K_p}\frac{[Fe^{3+}]}{[Fe^{2+}]} + \frac{K_m}{K_{ci}}\frac{c_X}{[Fe^{2+}]} + \frac{K_m}{\chi K_{ci} K_i}\frac{[Fe^{3+}]}{[Fe^{2+}]}}$	**Batch** T = 29°C pH = 1.8 - 2.0	$q_{O_2}^{max}$ =3.33 nmol$\ell O_2.s^{-1}$.(mg cells)$^{-1}$, K_m=0.070 mM Fe^{2+}, K_{ci}=135 µg wet cells.mℓ^{-1}, K_p=0.640 mM Fe^{3+}, χ = 5.
Nikolov and Karamanev (1992)	$\mu = \frac{\mu^{max}}{1 + \frac{K_S}{[Fe^{2+}]} + \frac{K_S}{K_p}\frac{[Fe^{3+}]}{[Fe^{2+}]} + \frac{[Fe^{2+}]}{K_{Si}}}$	**Continuous** T = 29°C pH = 1.8 - 2.0	μ^{max} = 0.227 h^{-1}, $\frac{K_S}{K_p}$ = 0.939, K_{si} = 12.0 g$Fe^{2+}.\ell^{-1}$

* The bacteria used are *Acidithiobacillus ferrooxidans* unless otherwise stated.

Table 2.6: continued...

Reference	Equation	Conditions	Parameters
Huberts (1994)	$q_{Fe^{2+}} = \frac{q^{max}_{Fe^{2+}}}{\left(1+\frac{K_S}{[Fe^{2+}]}+\frac{K_S}{K_p}\frac{[Fe^{3+}]}{[Fe^{2+}]}\right)\left(1+\frac{K_{O_2}}{p_{O_2}}\right)}$	**Continuous** T = 30°C pH = 2.0	$q^{max}_{Fe^{2+}}$ =11Fe^{2+}ions.(μs)$^{-1}$.cell^{-1}, K_{O_2} = 0.022atm, K_S = 0.021 mM Fe^{2+}, K_p=10.18 mM Fe^{3+}
Nagpal, Dahlstrom and Oolman (1994)	$q_{Fe^{2+}} = \frac{q^{max}_{Fe^{2+}}}{1+\frac{K_S}{[Fe^{2+}]}+\frac{K_S}{[Fe^{2+}]}\left[\frac{[As]}{K_{As}}\right]^2}$	**Continuous** T = 35°C D = 20 - 110 h^{-1} Feed solids concentration: 18%	$q^{max}_{Fe^{2+}}$ =417 mmolFe^{2+}.h^{-1}.$(g_{protein})^{-1}$, K_S=0.09 mM Fe^{2+}, K_{As}=50 mmolAs.ℓ^{-1}
Harvey and Crundwell (1997)	$\mu = \frac{\mu^{max}}{1+\frac{K_S}{[Fe^{2+}]}+\frac{K_S}{K_p}\frac{[Fe^{3+}]}{[Fe^{2+}]}+\frac{[As^{3+}]}{K_{As^{3+}}}}$	**Batch** T = 30°C pH = 2.0 $[As^{3+}]$= 4 - 9 g.ℓ^{-1}	μ^{max}=0.161 h^{-1}, K_s=1.30 mM Fe^{2+}, K_p = 14.05 mM Fe^{3+}, $K_{As^{3+}}$ = 0.48 - 0.6 gAs^{3+}.ℓ^{-1}
Nemati and Webb (1996)	$q_{Fe^{2+}} = \frac{K_0 e^{\frac{E_a}{RT}}}{1+\frac{K_S}{[Fe^{2+}]}+\frac{K_S}{K_{ci}}\frac{c_X}{[Fe^{2+}]}+\frac{[Fe^{2+}]}{\alpha}+\frac{c_X[Fe^{2+}]}{\alpha\beta}}$	**Batch (Initial rates)** T = 20 - 35 °C pH = 2.0	K_0=115.0kmol.m^{-3}.h^{-1}.(cells.mℓ^{-1})$^{-1}$, Ea=68.4kJ.mol^{-1}, K_S=1.2×10^{-3}kmol.m^{-3}, K_{ci}=2.68×10^7, α=0.466kmol.m^{-3}, β=7.8×10^8cells.mℓ^{-1}
Boon (1996)	$\mu = \frac{\mu^{max}+m_O Y^{max}_{OX}}{1+\frac{K_S}{([Fe^{2+}]-[Fe^{2+}]_t)}+\frac{K_S}{K_p}\frac{[Fe^{3+}]}{([Fe^{2+}]-[Fe^{2+}]_t)}} - m_O Y^m_O$	**Continuous** T = 30°C pH = 1.5-1.6	***L. ferrooxidans*** μ^{max} = 0.078 h^{-1}, K_s = ignored, K_s/K_i=5×10^{-4}, $[Fe^{2+}]_t$=7×10^{-3}mM, Y^{max}_{OX} =0.05±0.005 molC.$(molO_2)^{-1}$, m_0=0.1±0.05molO_2.(molC)$^{-1}$.h^{-1}
Boon (1996)	$\mu = \frac{\mu^{max}+m_O Y^{max}_{OX}}{1+\frac{K_S}{([Fe^{2+}]-[Fe^{2+}]_t)}+\frac{K_S}{K_p}\frac{[Fe^{3+}]}{([Fe^{2+}]-[Fe^{2+}]_t)}} - m_O Y^m_O$	**Continuous** T = 30°C pH = 1.8-1.9	***T. ferrooxidans*** μ^{max} = 0.096 h^{-1}, K_s=0.25 mM Fe^{2+}, K_s/K_i=0.05 ± 0.01, $[Fe^{2+}]_t$=7×10^{-3}mM Fe^{2+}, Y^{max}_{OX} =0.05±0.003 molC.$(molO_2)^{-1}$, m_0=0.1±0.05molO_2.(molC)$^{-1}$.h^{-1}
van Scherpen-zeel *et al.* (1998)	$q_{Fe^{2+}} = \frac{q^{max}_{Fe^{2+}}}{1+K\frac{[Fe^{3+}]}{[Fe^{2+}]}}$	**Continuous** T = 30°C pH = 1.5 - 1.6	***L. ferrooxidans*** $q^{max}_{Fe^{2+}}$ = 6.8 molFe^{2+}.(molC)$^{-1}$.h^{-1}, K = 0.0005
Breed *et al.* (1999)	$q_{Fe^{2+}} = \frac{K_0 e^{\frac{E_a}{RT}}}{1+K\frac{[Fe^{3+}]}{[Fe^{2+}]}}$	**Continuous** T = 30 - 40°C pH = 1.7	***L. ferrooxidans*** K_0 = 1.2×10^7 molFe^{2+}.(molC)$^{-1}$.h^{-1}, E_a = 35.63 kJ.mol^{-1}, K = 0.0002T - 0.0453
Breed and Hansford (1999[a])	$q_{Fe^{2+}} = \frac{q^{max}_{Fe^{2+}}}{1+K\frac{[Fe^{3+}]}{[Fe^{2+}]}}$	**Continuous** T = 40°C pH = 1.1 - 1.7	***L. ferrooxidans*** $q^{max}_{Fe^{2+}}$ = 15.53 molFe^{2+}.(molC)$^{-1}$.h^{-1}, K = 0.0048pH - 0.043

Chapter 3

Theoretical Aspects

The theoretical aspects described in this thesis are based on the methodology developed by Boon (1996) which was based on Roels (1983).

Bacteria obtain energy for growth and maintenance from ferrous-iron oxidation that takes place according to the following stoichiometric formula:

$$4Fe^{2+} + O_2 + 4H^+ \leftrightarrow 4Fe^{3+} + 2H_2O \qquad (1.3)$$

Ferrous-iron oxidation is accompanied by growth of bacteria that are assumed to have the stoichiometric formula $CH_{1.8}O_{0.5}N_{0.2}$ (Jones and Kelly, 1983; Roels, 1983). If the carbon and nitrogen sources are limited to CO_2 and NH_4^+ the formation of biomass occurs according to;

$$CO_2, NH_4^+, H_2O \rightarrow CH_{1.8}O_{0.5}N_{0.2} \qquad (3.1)$$

The carbon dioxide utilisation rate can be used to estimate the growth rate if it is assumed that carbon dioxide is the only carbon source:

$$r_X = -r_{CO_2} \qquad (3.2)$$

The cell concentration in continuous culture can be calculated from the steady state carbon dioxide utilisation rate and the dilution rate:

$$c_x = \frac{r_X}{\mu} = \frac{-r_{CO_2}}{D} \qquad (3.3)$$

The cell concentration in batch culture can be obtained from the initial bacterial concentration and the carbon dioxide utilisation rate:

$$c_X = c_{X0} + \int_{t=0}^{t=t} -r_{CO_2} dt \tag{3.4}$$

By performing an elemental and charge balance, or a "degree of reduction" balance it is possible to relate the rate of ferrous-iron oxidation to the rates of oxygen and carbon dioxide utilisation:

$$-r_{Fe^{2+}} = -4r_{O_2} - 4.2\,r_{CO_2} \tag{3.5}$$

Substitution and simplification yields the overall stoichiometry for the bio-oxidation of ferrous-iron:

$$CO_2 + 0.2NH_4^+ + \left[\frac{1-4.2\,Y_{Fe^{2+}X}}{4\,Y_{Fe^{2+}X}}\right]O_2 + \frac{1}{Y_{Fe^{2+}X}}Fe^{2+} + \left[\frac{1}{Y_{Fe^{2+}X}} - 0.2\right]H^+ \rightarrow CH_{1.8}O_{0.5}N_{0.2} + \frac{1}{Y_{Fe^{2+}X}}Fe^{3+} + \left[\frac{1}{2\,Y_{Fe^{2+}X}} - 0.6\right]H_2O \tag{3.6}$$

In Equation 3.6 the bacterial yield on ferrous-iron, $Y_{Fe^{2+}X}$, is defined as the amount of biomass, as moles carbon, produced per mole of ferrous-iron oxidised, *i.e.*:

$$Y_{Fe^{2+}X} = \frac{r_X}{-r_{Fe^{2+}}} \tag{3.7}$$

Similarly the bacterial yield on oxygen, Y_{O_2X}, is defined:

$$Y_{O_2X} = \frac{r_X}{-r_{O_2}} \tag{3.8}$$

The bacterial specific ferrous-iron utilisation rate, $q_{Fe^{2+}}$, is defined as the rate of ferrous-iron oxidation per mole of biomass (*i.e.* per mole of carbon):

$$q_{Fe^{2+}} = \frac{-r_{Fe^{2+}}}{c_X} \tag{3.9}$$

The bacterial specific oxygen utilisation rate, q_{O_2}, is similarly defined:

$$q_{O_2} = \frac{-r_{O_2}}{c_X} \tag{3.10}$$

The relationship between the amount of substrate consumed by the biomass for bacterial growth and maintenance can be described by means of the Pirt Equation (Pirt, 1965). This relationship assumes that the maintenance energy is independent of the growth rate:

$$q_{Fe^{2+}} = \frac{\mu}{Y^{max}_{Fe^{2+}X}} + m_{Fe^{2+}} \tag{3.11}$$

Equation 3.11 can also be written in terms of the bacterial specific oxygen utilisation rate:

$$q_{O_2} = \frac{\mu}{Y^{max}_{O_2X}} + m_{O_2} \tag{3.12}$$

Therefore, plotting the values of the bacterial specific ferrous-iron or oxygen utilisation rate measured during batch operation versus the specific growth rate, the values of $Y^{max}_{Fe^{2+}X}$ and $m_{Fe^{2+}}$ or $Y^{max}_{O_2X}$ and m_{O_2} can be calculated.

Dividing Equation 3.11 by the specific growth rate, and combining the result with Equations 3.9 and 3.7 gives:

$$\frac{1}{Y_{Fe^{2+}X}} = \frac{1}{Y^{max}_{Fe^{2+}X}} + \frac{m_{Fe^{2+}}}{\mu} \tag{3.13}$$

Equation 3.13 can also be written in terms of the bacterial yield on oxygen:

$$\frac{1}{Y_{O_2X}} = \frac{1}{Y^{max}_{O_2X}} + \frac{m_{O_2}}{\mu} \tag{3.14}$$

Therefore, from steady-state continuous culture data, plotting the values of $Y_{Fe^{2+}X}$ or Y_{O_2X} against $1/\mu$ can be used to determine the values of $Y^{max}_{Fe^{2+}X}$ and $m_{Fe^{2+}}$ or $Y^{max}_{O_2X}$ and m_{O_2}. The validity of the values determined in this manner can be checked using the degree of reduction balance, Equation 3.5:

$$Y^{max}_{O_2X} = \frac{4Y^{max}_{Fe^{2+}X}}{1 - 4.2Y^{max}_{Fe^{2+}X}} \tag{3.15}$$

$$m_{O_2} = \frac{m_{Fe^{2+}}}{4} \tag{3.16}$$

Equation 3.7 assumes that maintenance is independent of the growth rate. Pirt (1982) developed a model that used a maintenance energy term that was dependent on the specific growth rate (see Section 2.2.1):

$$q_{Fe^{2+}} = \left(\frac{1}{Y^{max}_{Fe^{2+}X}} - \frac{m^{v}_{Fe^{2+}}}{\mu^{max}} \right) \mu + m_{Fe^{2+}} + m^{v}_{Fe^{2+}} \qquad (3.17)$$

Equation 3.17 can also be written in terms of the bacterial specific oxygen utilisation rate:

$$q_{O_2} = \left(\frac{1}{Y^{max}_{O_2X}} - \frac{m^{v}_{O_2}}{\mu^{max}} \right) \mu + m_{O_2} + m^{v}_{O_2} \qquad (3.18)$$

Where, $m_{Fe^{2+}}$ and m_{O_2} are the constant maintenance energy and, $m^{v}_{Fe^{2+}}$ and $m^{v}_{O_2}$ are the growth rate dependent maintenance energies at zero growth rate.

The constant maintenance energy and the maximum bacterial yield can be obtained from experiments under energy limited conditions. Under energy limited conditions, the variable maintenance energy falls to zero so Equation 3.17 reduces to Equation 3.11 and the parameters can be obtained from a plot of the specific substrate utilisation rate versus the specific growth rate. The growth rate dependent maintenance energy at zero growth rate can be obtained from experiments under energy sufficient conditions combined with experiments under energy limited conditions. The growth rate dependent maintenance energy at zero growth rate can be obtained from the intercept. The maximum bacterial specific growth rate can be obtained from the intercept of the two lines if it assumed that the growth rate dependent maintenance energy falls to zero at the maximum bacterial specific growth rate (Pirt, 1982)(see Figure 2.2).

Boon *et al.* (1995) suggested that the ferrous-iron oxidation kinetics could be described using a simplified form of the Michaelis-Menten model in which the bacterial specific ferrous-iron utilisation rate, $q_{Fe^{2+}}$, is proportional to the ferric/ferrous-iron ratio:

$$q_{Fe^{2+}} = \frac{q^{max}_{Fe^{2+}}}{1 + K_{Fe^{2+}} \dfrac{[Fe^{3+}]}{[Fe^{2+}]}} \qquad (2.20)$$

The values of the maximum bacterial specific ferrous-iron utilisation rate and the kinetic constant in bacterial ferrous-iron oxidation in Equation 2.20 can be determined by means of a Lineweaver-Burke plot:

$$\frac{1}{q_{Fe^{2+}}} = \frac{1}{q^{max}_{Fe^{2+}}} + \frac{K_{Fe^{2+}}}{q^{max}_{Fe^{2+}}} \frac{[Fe^{3+}]}{[Fe^{2+}]} \qquad (3.19)$$

Hence, from batch or continuous data, plotting the values of $1/q_{Fe^{2+}}$ against $[Fe^{3+}]/[Fe^{2+}]$ can be used to determine the values of $q^{max}_{Fe^{2+}}$ and $K_{Fe^{2+}}$. Furthermore, because the ferrous-iron, oxygen and carbon dioxide utilisation rates are related via the degree of reduction balance,

viz. Equation 3.5, the value of $q_{Fe^{2+}}^{max}$ can be used to calculate the value of $q_{O_2}^{max}$. Alternatively, the equations described above can be defined and written in terms of the bacterial specific oxygen utilisation rate:

$$q_{O_2} = \frac{q_{O_2}^{max}}{1 + K_{O_2}\frac{[Fe^{3+}]}{[Fe^{2+}]}} \tag{3.20}$$

The oxygen based kinetic constants can then be determined by means of a Lineweaver-Burke plot:

$$\frac{1}{q_{O_2}} = \frac{1}{q_O^{max}} + \frac{K_{O_2}}{q_{O_2}^{max}}\frac{[Fe^{3+}]}{[Fe^{2+}]} \tag{3.21}$$

Based on the assumption that the growth rate dependent maintenance energy falls to zero at the maximum bacterial specific growth rate and the Pirt Equation with constant maintenance energy applies, Equations 3.11 and 3.17 may also be written in terms of $q_{Fe^{2+}}^{max}$:

$$q_{Fe^{2+}}^{max} = \frac{\mu^{max}}{Y_{Fe^{2+}X}^{max}} + m_{Fe^{2+}} \tag{3.22}$$

and Equations 3.12 and 3.18 may also be written in terms of $q_{O_2}^{max}$:

$$q_{O_2}^{max} = \frac{\mu^{max}}{Y_{O_2X}^{max}} + m_{O_2} \tag{3.23}$$

The values of $q_{Fe^{2+}}^{max}$, $Y_{Fe^{2+}X}^{max}$ and $m_{Fe^{2+}}$, or $q_{O_2}^{max}$, $Y_{O_2X}^{max}$ and m_{O_2}, determined as described above, may be used to determine the maximum bacterial specific growth rate, μ^{max}.

The ferric- to ferrous-iron ratio in the preceding equations can be obtained from the solution redox potential using the Nernst Equation:

$$E = E_0 + \frac{RT}{zF}\ln\left(\frac{a_{Fe^{3+}}}{a_{Fe^{2+}}}\right) \tag{3.24}$$

This equation can be rewritten as:

$$E = E_0' + \frac{RT}{zF}\ln\left(\frac{[Fe^{3+}]}{[Fe^{2+}]}\right) \tag{3.25}$$

E_0' and $\frac{RT}{zF}$ can be calculated from a calibration graph (see Appendix 1).

The Michaelis-Menten based model is based on the assumption that the bacterial culture is limited by the substrate concentration. During the exponential growth phase the growth is not limited by the substrate concentration and is usually growth rate limited. The exponential growth rate is first order:

$$\frac{dc_X}{dt} = \mu c_X \tag{3.26}$$

Integration of this equation, assuming that $c_X = c_{x0}$ at $t = 0$, yields:

$$\ln\frac{c_X}{c_{X0}} = \mu t \tag{3.27}$$

or:

$$\ln c_X = \mu t + \ln c_{X0} \tag{3.28}$$

(Shuler and Kargi, 1992)

Therefore, if plotting $\ln(c_X)$ vs. t produces a straight line, the growth is restricted to the maximum rate . The growth rate under energy sufficient (growth rate limited) conditions and the initial bacterial concentration can be calculated from the slope and the intercept of the line.

Chapter 4

Materials and Methods

4.1 Bioreactors

The batch ferrous-iron oxidation experiments were carried out in 2 ℓ jacketed Z61104CT04 Applikon® autoclavable bioreactors made of borosilicate glass. The bioreactors had a h/d ≈ 1.32 and a working volume of 1 ℓ. The head plates were constructed of stainless steel and contained a number of ports for baffles, probes and other auxiliaries. Three baffles, 10 mm wide, 220 mm long and constructed of stainless steel were located at 120 degrees to one another. A diagrammatic representation of a single bioreactor is shown in Figure 4.1.

The bioreactors were maintained at the required temperature by circulating water from Grant Y6 constant temperature baths through the bioreactor jackets. The pH of the solution in the bioreactors was monitored continuously by a Mettler Toledo® combination pH electrode. The reactor liquor was maintained at the required pH by the addition of concentrated sulphuric acid (98%) manually by pump. During the experiments to determine the effect of temperature, the pH was maintained at 1.7 while the temperature was set at 30, 35 and 40 °C. In order to determine the effect of pH, the experiments were maintained at 40 °C and the pH was set at 1.1, 1.3, 1.5 and 1.7.

Mixing and gas dispersion were achieved by a pitched (45°) six-blade 316 stainless steel turbine impeller located 2 cm from the base of the reactor. It was rotated at 400 rev.min^{-1} via a flexible coupling linked to an Applikon® P100 motor and an Applikon® 1012 stand alone speed controller.

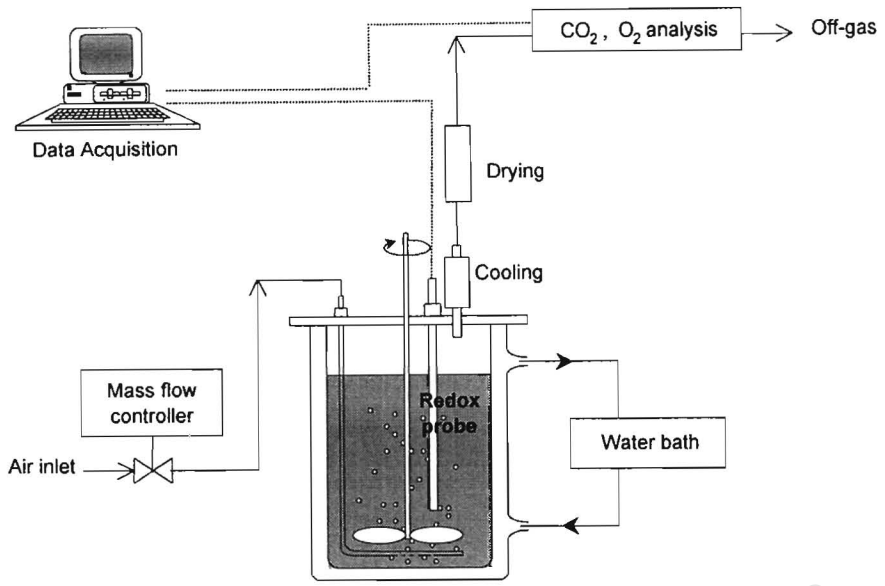


Figure 4.1: Schematic representation of a single bioreactor (after Breed *et al.* 1999).

Inlet gas was supplied by a Peak Scientific OAG2000DA Oil-less Air Generator. The air exiting the generator was filtered by a Walker® A30X1 filter then dried by a Denco® SN0.75 refrigerated compressed air dryer and finally filtered by Walker® A30XA and A30AC filters to produce dry, oil-free compressed air. The flow rate to the bioreactors was controlled at the required rate using Brooks Mass Flow Controllers (Model 5850S) and a Brooks Microprocessor Control and Read Out Unit (Model 0154). Air was introduced via an air sparger located under the impeller. The holes in the sparger were located at the bottom to minimise blockages.

The off-gas was dried using a reflux condenser through which an anti-freeze/water mixture from a Grant LTD6G low temperature bath was circulated. The low temperature bath maintained the coolant at 0 - 1°C. Before entering the gas analysers, the off-gas was also passed through a filter and a Hartmann & Braun CGEK Sample Gas Conditioner fitted with a CGKA 1 Automatic Condensate Outlet.

The carbon dioxide and oxygen concentrations in the bioreactor off-gas were determined by means of a Hartmann & Braun Uras 4 NDIR industrial photometer and a Magnos 6 G paramagnetic oxygen analyser, respectively. A computer alternately logged the carbon dioxide and oxygen concentrations of the off-gas from each bioreactor and the inlet air. A six-minute sampling period was used for each bioreactor. A maximum of two reactors were logged at the same time so that the air was sampled every 18 minutes for each reactor. The switching and data logging hardware and software used were designed, built, and written, within the Department of Chemical Engineering at the University of Cape Town.

The ferrous-iron oxidation kinetics were investigated in batch culture. The reactors were loaded with 500 mℓ inoculum and 500 mℓ ferrous-iron medium. The batches were run until the redox potential in the reactor reached a maximum value and remained constant, usually 8 to 10 hours.

4.2 Bacterial Culture

The bacterial culture used was initially obtained from a vat-type two-stage (2×20 ℓ) continuous bioleaching mini-plant treating an arsenopyrite/pyrite concentrate from Fairview Gold Mine in Barberton, South Africa (Breed *et al.*, 1997). Although it was originally reported to consist primarily of *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans* (Rawlings, 1995), more recent work has shown that it consists primarily of *Acidithiobacillus caldus* and *Leptospirillum ferrooxidans* (Rawlings *et al.*, 1999).

The inoculum for the batches was obtained from steady state continuous cultures of predominantly *Leptospirillum ferrooxidans* grown on ferrous-iron medium at a dilution rate of 0.04 h^{-1}. The bacterial concentrations in the continuous cultures were obtained using Equation 3.3, thus the initial cell concentration for the batches can be calculated. The continuous cultures were grown at the conditions (temperature, pH and arsenic concentration) at which the batches were operated.

4.3 Growth Medium

In addition to ferrous-iron substrate, oxygen and carbon dioxide, the bacteria used in bioleaching require nitrogen, potassium, phosphorous and a number of trace metals. Nitrogen, potassium and phosphorous are usually supplied as ammonium ion, NH_4^+, K_2SO_4 and $(NH_4)_2HPO_4$, respectively. In the case of mineral bioleaching, the required trace elements are usually present in the mineral in sufficient quantity, however during ferrous-iron oxidation trace elements need to be supplied.

The composition of the ferrous-iron medium used in this investigation is listed in Table 4.1. It was adjusted to the required pH using concentrated sulfuric acid (98%).

Table 4.1: Ferrous-iron medium composition (Breed *et al.*, 1999)

Compound	Concentration
$(NH_4)_2SO_4$	1.83 g.ℓ^{-1}
$(NH_4)_2HPO_4$	1.11 g.ℓ^{-1}
K_2SO_4	0.53 g.ℓ^{-1}
$FeSO_4.7H_2O$	59.74 g.ℓ^{-1}
trace metal solution*	10 mℓ.ℓ^{-1}

No attempt was made to maintain sterile conditions.

4.4 Ferric/Ferrous-Iron Ratio Determination

The redox potential in the bioreactors was measured using Mettler Toledo® redox electrodes (Pt-Ag/AgCl) and logged by computer. The total iron concentration in solution was

* Ethylenediamine tetra-acetic acid 50.0 g.ℓ^{-1}, $ZnSO_4.7H_2O$ 22.0 g.ℓ^{-1}, $CaCl_2$ 5.54 g.ℓ^{-1}, $MnCl_2.4H_2O$ 5.06 g.ℓ^{-1}, $FeSO_4.7H_2O$ 4.99 g.ℓ^{-1}, $(NH_4)_6Mo_7O_{24}.4H_2O$ 1.10 g.ℓ^{-1}, $CuSO_4.5H_2O$ 1.57 g.ℓ^{-1}, $CoCl_2.6H_2O$ 1.61 g.ℓ^{-1} adjusted to pH 6.0 using KOH (Vishniac and Santer, 1957).

determined by both Flame Atomic Absorption Spectroscopy and titration with potassium dichromate (Vogel, 1989). This enabled the ferric/ferrous-iron ratio and the ferrous- and ferric-iron concentrations to be determined using a calibration curve for the specific electrode and the Nernst equation (see Appendix 1). The ferrous-iron concentration was also determined by titration with cerium(IV) sulfate (Broadhurst, 1993).

Chapter 5

Re-evaluation of Previously Published Data

Deviations in the maximum bacterial yield and maintenance coefficients published previously, for *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* in batch and continuous culture, have been found (Boon, 1996; van Scherpenzeel, 1996; Jones and Kelly, 1983). Due to these deviations it is proposed that the maintenance coefficients are dependant on the specific growth rate and therefore it may be possible to represent the data using the equation developed by Neijssel and Tempest (1976) and modified by Pirt (1982) (Equation 2.11) which incorporates variable maintenance. The objectives of this chapter are to attempt to represent the data obtained from Jones and Kelly (1983), Boon (1996) and van Scherpenzeel (1996) using the variable maintenance equation and to asses the validity of the variable maintenance equation.

Jones and Kelly (1983) presented two sets of data with different types of ferric inhibition, competitive and non-competitive (Table 5.1). It is apparent from the lower maintenance energy requirements for the experiments with competitive inhibition that they were limited by the energy source (ferrous-iron), but the experiments with non-competitive inhibition were not.

Table 5.1: The maximum bacterial yield on ferrous-iron and the maintenance coefficients obtained by Jones and Kelly (1983)

	$Y^{max}_{Fe^{2+}X}$ (g dry weight.(g Fe^{2+})$^{-1}$)	$m_{Fe^{2+}}$ (g Fe^{2+}.(g dry weight)$^{-1}$.h^{-1})
Competitive Inhibition	0.36 - 0.38	≤0.04
Non-competitive Inhibition	1 - 1.33	0.38 - 0.43

It was then attempted to model the bioenergetics using variable maintenance model (Equation 2.11) and the average parameters obtained from Table 5.1. Straight lines of q vs. μ were plotted using the data in Table 5.1 with slopes of $\frac{1}{Y^{max}_{Fe^{2+}X}}$ and intercepts of $m_{Fe^{2+}}$. The graph of the model is displayed in Figure 5.1 and the parameters calculated are given in Table 5.2.

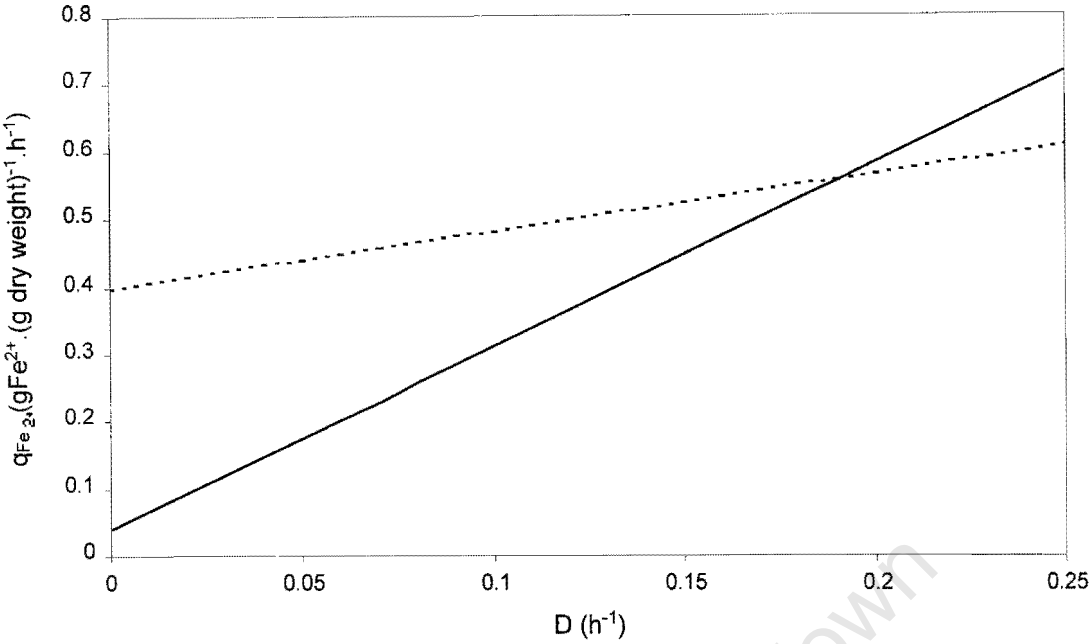


Figure 5.1: Relationship between the specific ferrous-iron utilisation rate and the dilution rate using the parameters of Jones and Kelly (1983). (—) Competitive inhibition, (---) non-competitive inhibition.

Table 5.2: Bioenergetic parameters calculated for the variable maintenance model (Equation 2.11) using the data obtained by Jones and Kelly (1983)

$m_{Fe^{2+}}$ (g Fe^{2+}.(g dry weight)$^{-1}$.h^{-1})	0.04
$m^{v}_{Fe^{2+}}$ (g Fe^{2+}.(g dry weight)$^{-1}$.h^{-1})	0.36
μ^{max} (h^{-1})	0.19
$Y^{max}_{Fe^{2+}X}$ (g dry weight.(g Fe^{2+})$^{-1}$)	0.37
$q^{max}_{Fe^{2+}}$ (g Fe^{2+}.(g dry weight)$^{-1}$.h^{-1})	0.56

It can be seen from the results that the data obtained by Jones and Kelly (1983) can be modelled using the variable maintenance model (Equation 2.11). The μ^{max} calculated using Equation 2.11, however is much smaller than the value published by the authors (μ^{max} = 1.33 h^{-1}). The μ^{max} calculated, however, is very similar to the values published by other authors, Lacey and Lawson (1970): 0.14 - 0.22 h^{-1}, Macdonald and Clark (1970): 0.145 h^{-1} and 0.161 h^{-1}, Liu *et al.* (1988): 0.11 h^{-1} and 0.12 h^{-1} and Shrihari *et al.* (1990): 0.183 h^{-1}.

The ferrous-iron oxidation by *Acidithiobacillus ferrooxidans* was investigated by Boon (1996) in batch and continuous culture. The maximum growth yields and maintenance coefficients are given in Table 2.4. The maintenance coefficient for the initial section of the batches is much higher than that obtained in the final section of the batches or in continuous experiments. The maximum growth yield obtained from the initial section of the batches is lower than that calculated from the final section of the batches and from continuous culture data. The reason for this is that, at the beginning of a batch, the ferrous-iron concentration is very high and therefore the culture is not limited by ferrous-iron, while at the end of a batch

and in continuous experiments, ferrous-iron is the only limiting factor. The batch data for the ferrous-iron oxidation with *Acidithiobacillus ferrooxidans* had two distinct regions, an initial and a final region. Boon (1996) modelled the bioenergetics for each section separately using the Pirt Equation with constant maintenance. It is proposed that both of the regions together can be modelled using the variable maintenance model. The initial and final bioenergetic parameters are given in Table 5.3.

Table 5.3: Values of the average maximum bacterial yield on ferrous-iron, $Y^{max}_{Fe^{2+}X}$, and the maintenance coefficient on ferrous-iron, $m_{Fe^{2+}}$ using *Acidithiobacillus ferrooxidans* at 30°C and a pH of 1.8 in batch culture (Boon, 1996)

Conditions	$Y^{max}_{Fe^{2+}X}$ ($molC.(molFe^{2+})^{-1}$)	$m_{Fe^{2+}}$ ($molFe^{2+}.molC^{-1}.h^{-1}$)
Initial	0.032	8
Final	0.0123	0.8

The bioenergetic parameters in Table 5.3 were used to plot a graph of the bacterial specific ferrous-iron utilisation rate vs. the dilution rate (bacterial specific growth rate), Figure 5.2. It was attempted to model the batch by the variable maintenance model (Equation 2.11). The parameters calculated are given in Table 5.4.

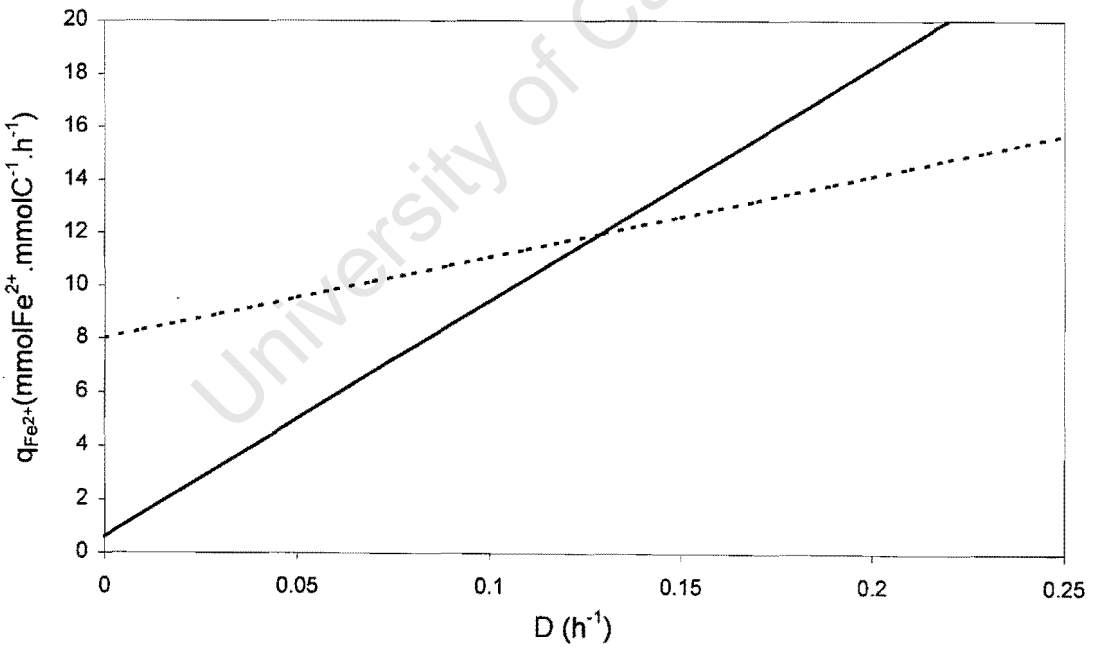


Figure 5.2: Relationship of the specific ferrous-iron utilisation rate and the dilution rate for the batch data obtained from Boon (1996) using *Acidithiobacillus ferrooxidans*. (—) Substrate limited, (---) substrate sufficient.

Table 5.4: Bioenergetic parameters calculated for Equation 2.11 using the data obtained by Boon (1996) in batch culture using *Acidithiobacillus ferrooxidans*

$m_{Fe^{2+}}$ ($mmolFe^{2+}.mmolC^{-1}.h^{-1}$)	0.6
$m^{v}_{Fe^{2+}}$ ($mmolFe^{2+}.mmolC^{-1}.h^{-1}$)	7.4
μ^{max} (h^{-1})	0.129
$Y^{max}_{Fe^{2+}X}$ ($mmolC.(mmolFe^{2+})^{-1}$)	0.011
$q^{max}_{Fe^{2+}}$ ($mmolFe^{2+}.mmolC^{-1}.h^{-1}$)	11.98

From the results it is evident that the variable maintenance model can be used effectively to model the batch bacterial ferrous-iron oxidation of *Acidithiobacillus ferrooxidans*. All the parameters calculated are very similar to the parameters calculated for *Acidithiobacillus ferrooxidans* by Boon (1996) in continuous culture (μ^{max} = 0.096 h^{-1}, $q^{max}_{Fe^{2+}}$ = 8.8 $molFe^{2+}.molC^{-1}.h^{-1}$).

The data published by van Scherpenzeel *et al.* (1998) and van Scherpenzeel (1996) for the continuous bacterial ferrous-iron oxidation using *Leptospirillum ferrooxidans* does not fit the Pirt equation (Equation 2.7). The data appears to have a much higher maintenance coefficient at high dilution rates than at low dilution rates. It was assumed that at low dilution rates the experiment was limited by ferrous-iron, while at high dilution rates the culture was not. Due to the fact that the continuous experiments have two different sections limited by different factors it was decided that the data should be modelled using the variable maintenance model (Equation 2.11). van Scherpenzeel (1996) reported that a dilution rate of 0.03 h^{-1} was the border between the two sections at different growth rates and fitted the Pirt equation to each of the sections separately. All the dilution rates lower than 0.03 h^{-1} were limited by ferrous-iron, while all the higher dilution rates were not. The bioenergetic parameters for the low and high dilution rates are given in Table 5.5. The graph using the variable maintenance model is displayed in Figure 5.3 and the parameters calculated for this model are given in Table 5.6.

Table 5.5: Values of the average maximum bacterial yield on ferrous-iron, $Y^{max}_{Fe^{2+}X}$, and the maintenance coefficient on ferrous-iron, $m_{Fe^{2+}}$ using *Leptospirillum ferrooxidans* at 30°C and a pH of 1.5 - 1.6 in continuous culture (van Scherpenzeel, 1996)

Conditions	**$Y^{max}_{Fe^{2+}X}$ ($molC.(molFe^{2+})^{-1}$)**	**$m_{Fe^{2+}}$ ($molFe^{2+}.molC^{-1}.h^{-1}$)**
Low Dilution Rate (D<0.03 h^{-1})	0.0109	0.0125
High Dilution Rate (D>0.03 h^{-1})	0.0125	0.888

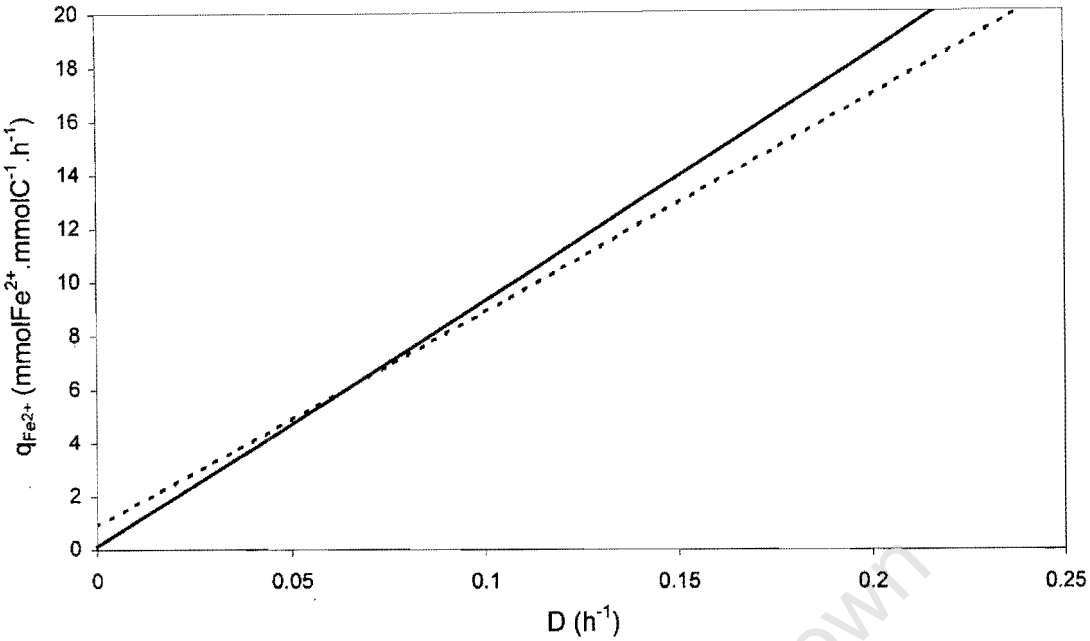


Figure 5.3: Relationship between the specific ferrous-iron utilisation rate and the dilution rate for the continuous data obtained from van Scherpenzeel (1996) using *Leptospirillum ferrooxidans*. (—) Substrate limited, (---) substrate sufficient.

Table 5.6: Bioenergetic parameters calculated for Equation 2.11 using the data obtained by van Scherpenzeel (1996) in continuous culture using *Leptospirillum ferrooxidans**

$m_{Fe^{2+}}$ (mmolFe^{2+}.mmolC^{-1}.h^{-1})	0.116
$m^{v}_{Fe^{2+}}$ (mmolFe^{2+}.mmolC^{-1}.h^{-1})	0.772
μ^{max} (h^{-1})	0.066
$Y^{max}_{Fe^{2+}X}$ (mmolC.(mmolFe^{2+})$^{-1}$)	0.0109
$q^{max}_{Fe^{2+}}$ (mmolFe^{2+}.mmolC^{-1}.h^{-1})	6.15

It can be seen from Figure 5.3 and Table 5.6 that the continuous data using *Leptospirillum ferrooxidans* obtained by van Scherpenzeel (1996) can be modelled using the variable maintenance equation. The specific growth rate and bacterial specific ferrous iron utilisation rate calculated are very similar to the those calculated for *Leptospirillum ferrooxidans* by van Scherpenzeel *et al.* (1998) (μ^{max} = 0.069 h^{-1}, $q^{max}_{Fe^{2+}}$ = 7.09 molFe^{2+}.molC^{-1}.h^{-1} *) and Breed *et al.* (1999) (μ^{max} = 0.046 h^{-1}, $q^{max}_{Fe^{2+}}$ = 8.65 molFe^{2+}.molC^{-1}.h^{-1} *) at 30 °C.

From the data of Jones and Kelly (1983), Boon (1996) and van Scherpenzeel (1996) that was re-worked it is apparent that the bio-oxidation of ferrous-iron, by *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* in batch and continuous culture, can be modelled using the variable maintenance model. It is also apparent that using either continuous or batch experiments that have been limited by more than one factor, e.g. phosphate, sulfate,

* Calculated from oxygen based parameters by "degree of reduction" balance i.e. Equation 3.5

ammonia, heavy metals, growth rate, one can obtain an estimation of the maximum bacterial specific growth rate and the bacterial specific ferrous-iron utilisation rate.

Chapter 6

Results and Discussion

Experiments were carried out in to ascertain the effect of temperature and pH on the batch ferrous-iron oxidation kinetics of *Leptospirillum ferrooxidans*. The results of the batch experiments were compared to the results published by Breed *et al.* (1999) and Breed and Hansford (1999[a]) for the ferrous-iron oxidation by *Leptospirillum ferrooxidans* in continuous culture at the same conditions. During the experiments to determine the effect of temperature, the pH was maintained at 1.7 while temperature was varied from 30 to 40 °C and in order to determine the effect of pH, the experiments were maintained at 40 °C and the pH was varied from 1.1 to 1.7. Replicates of the experiments were performed at all of the conditions.

The material in this chapter is divided into the following topics:

- Validation of the raw data
- Kinetics in batch culture
- Yield and maintenance coefficients
- Kinetic modelling

6.1 Raw Data

Prior to performing any kinetic analysis the validity of the data was checked by comparing trends in the oxygen, and carbon dioxide utilisation rates, redox potential, substrate concentration and cell concentration with time, with the expected trends. The applicability of the "degree of reduction" balance was investigated. Finally the reproducibility of the experimental data was demonstrated.

6.1.1 Oxygen and Carbon Dioxide Utilisation Rates

Plots of the variation in the oxygen and carbon dioxide utilisation rates versus time at 40 °C and a pH of 1.7 for continuous and batch experiments are displayed in Figures 6.1 and 6.2 respectively. These graphs are representative samples and similar trends were noticed at the other conditions.

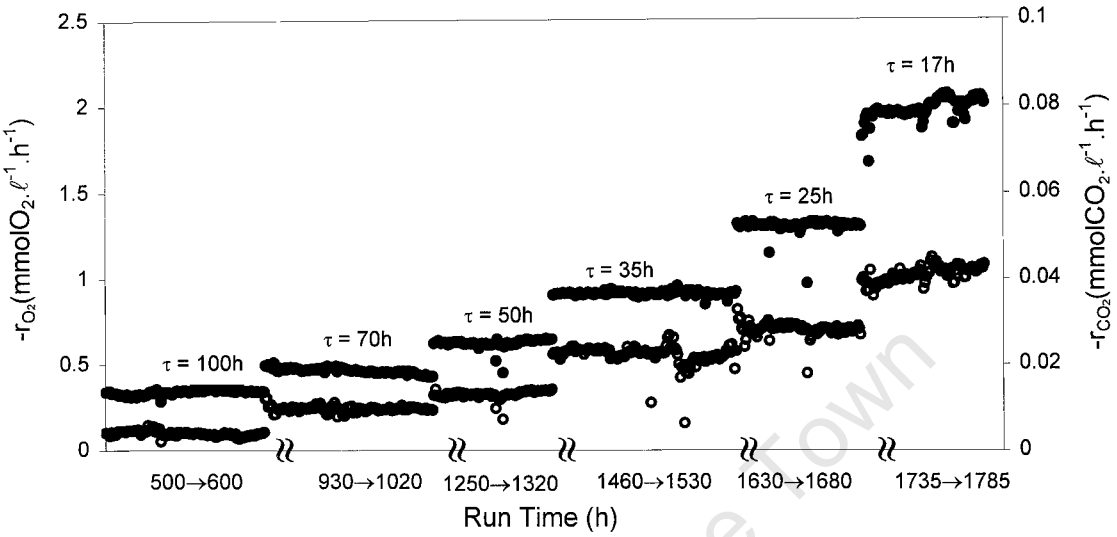


Figure 6.1: Steady state $-r_{O_2}$(●) and $-r_{CO_2}$(○) at each residence time for the continuous experiments at 40 °C, and pH = 1.7 (Dempers and Searby, unpublished data).

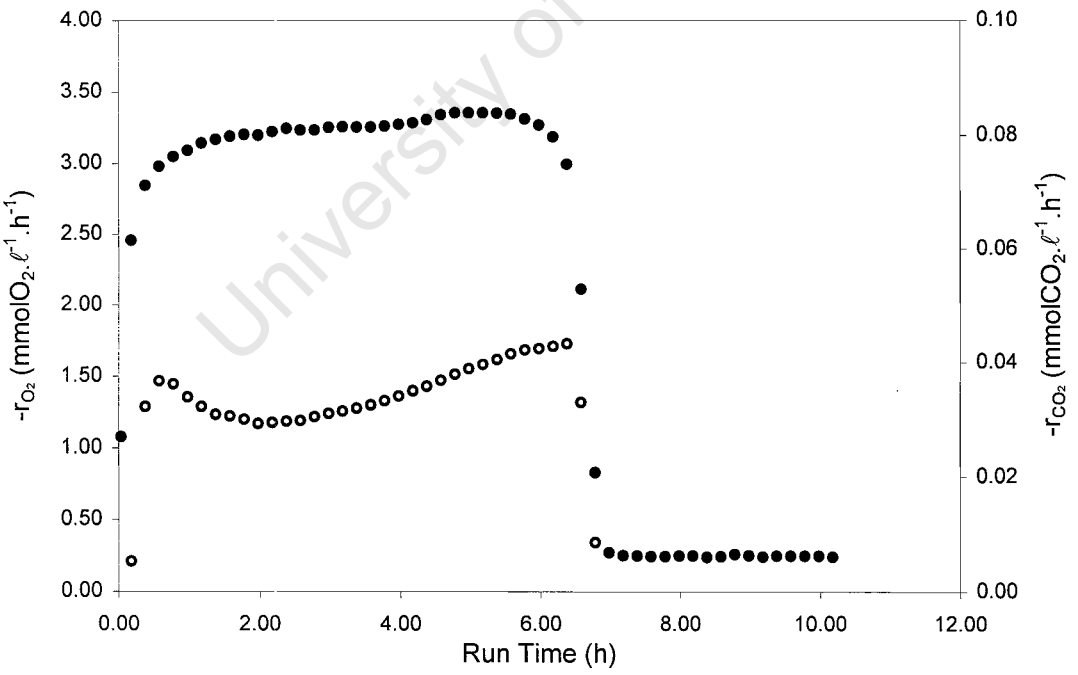


Figure 6.2: Dynamic $-r_{O_2}$(●) and $-r_{CO_2}$(○) vs. run time for a batch experiment at 40 °C and pH = 1.7.

It is evident that the batch experiments are over a much shorter time scale, ±10 h, as opposed to the continuous experiments, ±74 days. The batch experiments are dynamic and there are large changes in the utilisation rates only during approximately one hour at the end

of the batch. This occurs when there is a corresponding drop in the redox potential (see Figure 6.4).

The continuous experiments show a constant incremental increase in the oxygen and carbon dioxide utilisation rates with time, while the batch experiments show dynamic variations in the utilisation rates. The inoculum was taken from a continuous reactor at a residence time of 25 hours, so it would be expected that the oxygen and carbon dioxide utilisation rates in the batch would start off at the same value as the rates attained in continuous culture at a 25 hours residence time. Both the rates start at approximately the same values and display a rapid initial increase. The oxygen utilisation rates in the batch experiments increases to much higher values than those attained in the continuous experiments. The carbon dioxide utilisation rate, however stays low, but increases slightly until it reaches a maximum. The maximum is approximately the same as the steady state value achieved at a residence time of 17 hours. At the end of the batch both of the utilisation rates decrease rapidly to approximately zero.

6.1.2 Redox Potential and Substrate Concentration

Figures containing the variation in the redox potential with time at 40 °C and a pH of 1.7 in continuous and batch experiments are displayed in Figures 6.3 and 6.4 respectively. The variation in the substrate concentration is only displayed for the batch in Figure 6.4. These graphs are representative samples and similar trends were noticed at the other conditions.

The redox potentials for most of the batch experiments are much lower than those attained in continuous experiments. High redox potentials are only attained in the last short section of the batch experiments and the final redox potential attained is much higher in the batch experiments than in the continuous experiments.

The ferrous-iron concentration in the batch experiments starts at a concentration of approximately 100 mmol.ℓ^{-1} then drops to zero. The ferrous-iron concentration for continuous experiments is predominantly below 2.5 mmol.ℓ^{-1} and reaches a maximum of 10 mmol.ℓ^{-1} (Breed, 2000).

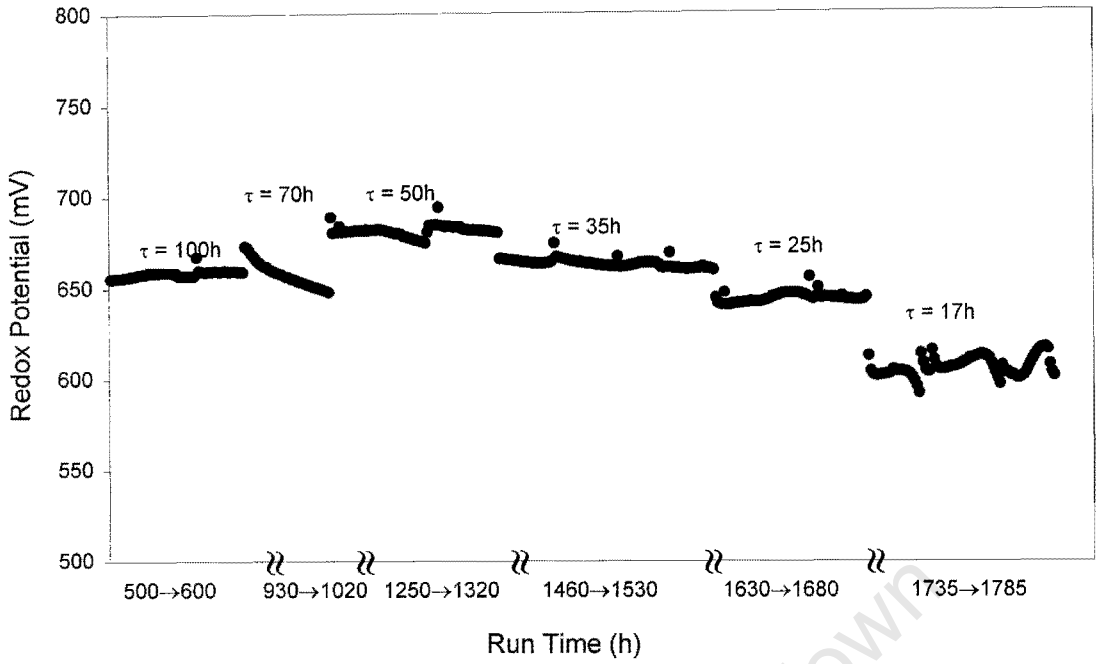


Figure 6.3: Steady state redox potential at each residence time for the continuous experiments at 40 °C, and pH = 1.7 (Dempers and Searby, unpublished data).

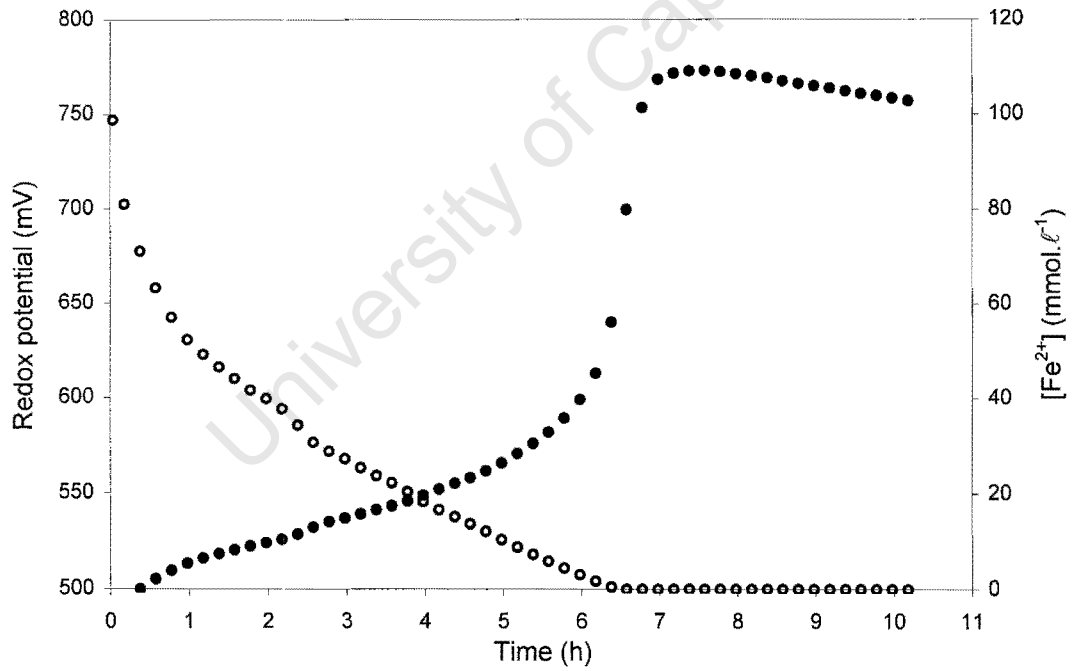


Figure 6.4: Dynamic redox potential (●) and ferrous-iron concentration (○) vs. run time for a batch experiment at 40 °C and pH = 1.7.

6.1.3 Degree of Reduction Balance

The oxygen and carbon dioxide utilisation rates are related to the ferrous-iron oxidation rate by means of the "degree of reduction" balance (Equation 3.5). A comparison between the ferrous-iron utilisation rate determined from the "degree of reduction" balance and from the measured redox potential is demonstrated in Figure 6.5.

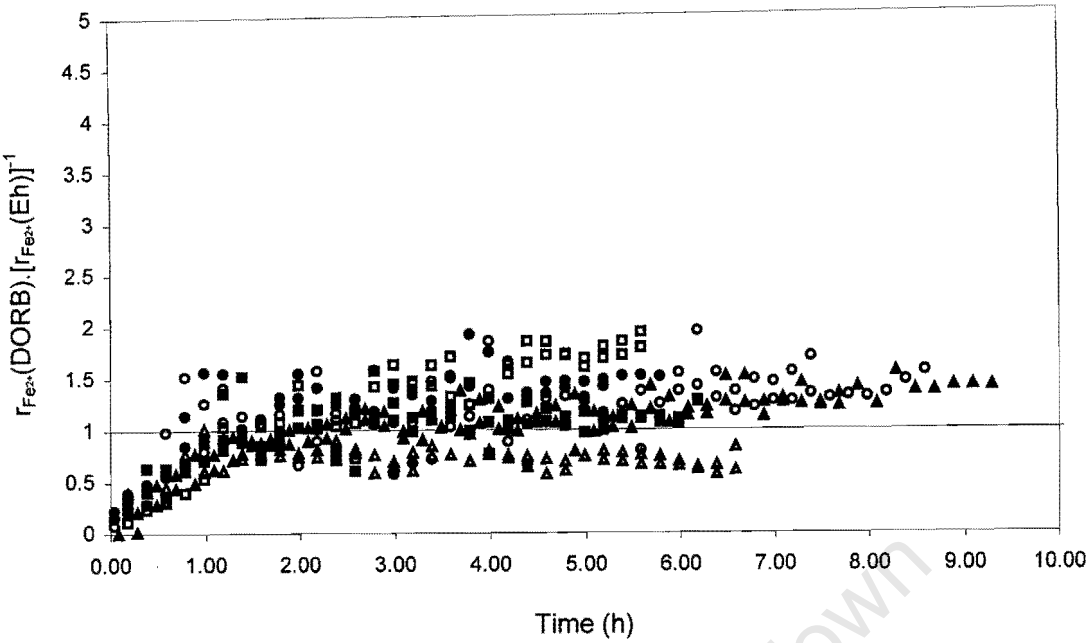


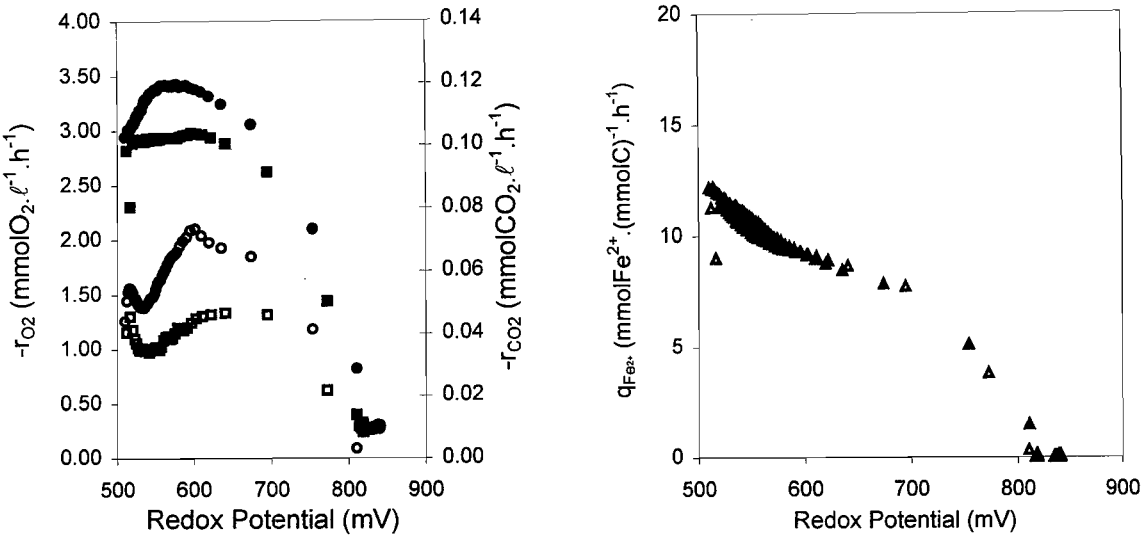
Figure 6.5: Comparison between the ferrous-iron utilisation rate calculated from the off-gas analysis and from the redox potential. ○)30 °C, pH = 1.7, (●) 35 °C, pH = 1.7, (□) 40 °C, pH = 1.7, (■) 40 °C, pH = 1.5, (△) 40 °C, pH = 1.3, (▲) 40 °C, pH = 1.1.

Figure 6.5 provides an indication that the measurement of the ferrous-iron utilisation rate by the "degree of reduction" balance and the redox potential are valid. The ferrous-iron utilisation determined from the redox potential however is larger than that determined from the gas utilisation rates at the beginning of the batch, i.e. less than one hour. This can be explained by the fact that the redox potential is measured in the bulk conditions and not the micro-environment. At the beginning of the batch, the ferrous ions may be migrating from the bulk environment into the extracellular polysaccharide layer but not being oxidised by the bacteria. The bulk redox potential is therefore changing, but the ferrous-iron is not being used by the bacteria, hence the discrepancy between the ferrous-iron utilisation rate measured by the redox potential and the off-gas utilisation.

The oxygen and carbon dioxide utilisation rates are therefore a better method of determining the ferrous-iron utilisation rate than the redox potential.

6.1.4 Reproducibility

In order to determine the reproducibility of the batch experiments replicates of all the experiments were performed. A comparison of the oxygen, carbon dioxide and ferrous-iron utilisation rates versus the redox potential for two replicates performed at 40 °C and pH = 1.1 are displayed in Figure 6.6.



a) b)

Figure 6.6: Reproducibility of (a) the off-gas utilisation rates and (b) the bacterial specific ferrous-iron utilisation rates for two runs at 40 °C and a pH of 1.1. (●) $-r_{O_2}$, Run 1, (○)$-r_{CO_2}$, Run 1, (■)$-r_{O_2}$, Run 2, (□) $-r_{CO_2}$, Run 2, (▲) $q_{Fe^{2+}}$, Run 1, (△) $q_{Fe^{2+}}$, Run 1.

Even though the oxygen and carbon dioxide utilisation rates (Figure 6.6a) do not appear to be reproducible, the bacterial specific ferrous-iron utilisation rates (Figure 6.6b) are reproducible. The reason for this difference is that there is a difference in cell concentration that is not accounted for by just the oxygen and carbon dioxide utilisation rates alone. To prove reproducibility it is necessary to perform parallel experiments with the same inoculum. The bacterial specific ferrous-iron utilisation rate is therefore a better basis for the comparison of replicates and analysis of the data.

In order to obtain a quantitative indication of the reproducibility of the experiments the average error between replicates, at each set of conditions, was calculated and are displayed in Table 6.1. The average error was estimated from the standard deviation of the bacterial specific ferrous-iron utilisation rate from the mean. Details of the error analysis are situated in Appendix 2.

Table 6.1: Table of errors for the batch experiments at different conditions

Temperature	pH	Error
30	1.7	3%
35	1.7	10%
40	1.7	12%
40	1.5	4%
40	1.3	3%
40	1.1	3%

From Table 6.1 it is evident that the average error over all the experiments is 6%. This error is satisfactory in bacterial systems.

6.2 Growth Kinetics

A batch growth curve usually exhibits the following phases: 1) lag phase, 2) exponential growth phase, 3) deceleration phase, 4) stationary phase and 5) death phase (Shuler and Kargi, 1992). The properties of each phase are very dependent on factors affecting the batch e.g. inoculum size and activity, type of substrate, etc.

A batch growth curve representative of all the other batches is displayed in Figure 6.7. It is apparent that the batch growth curve follows the expected trend but has very definite features. No lag phase is observed. This is because the inoculum was taken from an active culture under very similar conditions so the culture did not have to adapt to the conditions. There is a very long exponential growth phase and a very short decelerating growth phase. During the exponential growth phase the substrate, ferrous-iron, is in excess, while during the decelerating growth phase the culture is limited by the substrate. The stationary phase is very short, followed by the death phase.

From Figure 6.7 it is apparent that the growth occurs exponentially as predicted by Equation 3.26 where the specific growth rate is not limited by the substrate. Based on Equation 3.28 it is possible to calculate the specific growth rate under energy sufficient conditions and the initial bacterial concentration from the slope and the intercept respectively. The specific growth rates and the initial concentrations calculated are displayed in Table 6.2.

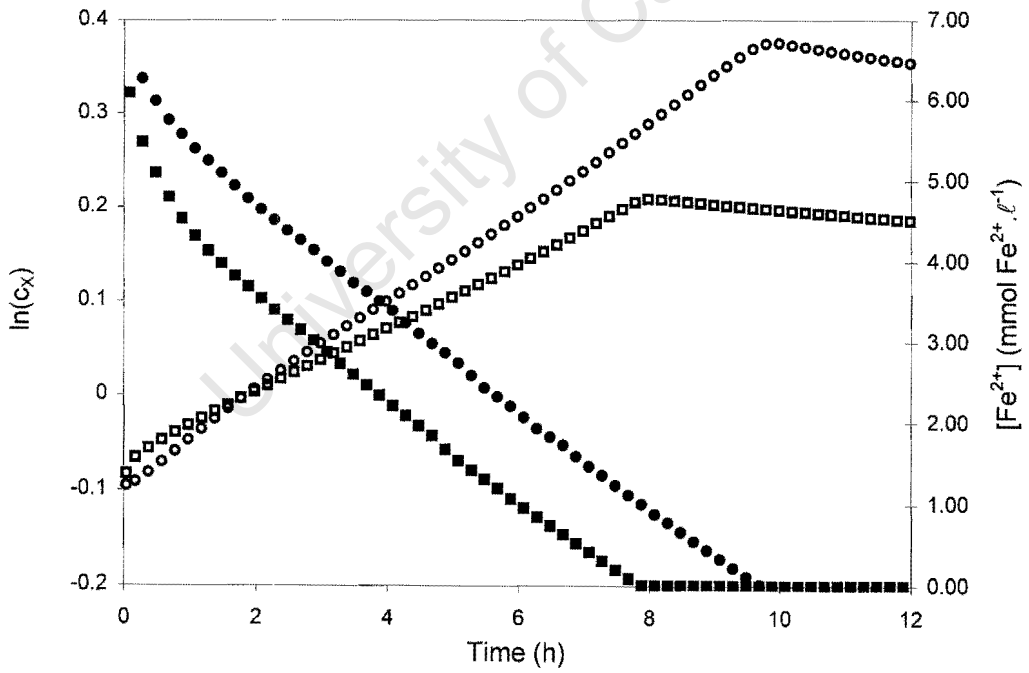


Figure 6.7: Variation in the natural logarithm of the cell concentration and the ferrous-iron concentration with time at 40 °C and a pH of 1.1. (○) $\ln(c_X)$ Run 1, (●) $[Fe^{2+}]$ Run 1, (□) $\ln(c_X)$, Run 2, (■) $[Fe^{2+}]$ Run 2.

Table 6.2: Average specific growth rates and initial bacterial concentrations for the exponential growth phases at all of the experimental conditions calculated from Equation 3.26

Temperature °C	pH	μ (h^{-1})	c_{X0} (mmolC.l^{-1})	R^2
30	1.7	0.0491	0.9018	0.9983
35	1.7	0.0559	1.1278	0.9983
40	1.7	0.0281	1.1461	0.9984
40	1.5	0.0392	1.0437	0.9941
40	1.3	0.0440	0.7752	0.9988
40	1.1	0.0413	0.8510	0.9988

Based on the R^2 value in Table 6.2 (average R^2 = 0.9978) it is apparent that the growth rate during the exponential growth phase follows first order kinetics with respect to the bacterial concentration (Equation 3.26). The implication of this is that the bacterial growth during the exponential phase is not limited by the ferrous-iron or another factor, but is limited by the growth rate attainable by the cells.

The bacterial specific growth rate should vary with temperature according to the Arrhenius equation (Equation 2.23) (Shuler and Kargi, 1992). From Table 6.2 is apparent that over the temperature range 30 to 40 °C the data does not follow the Arrhenius equation, but the bacterial specific growth rate reaches a maximum at 35 °C. A possible reason for the deviation of the data from the Arrhenius equation is that a temperature of 40 °C is above the optimum temperature for mesophilic growth (van Scherpenzeel, 1996; Barrett *et al.*, 1993). In a continuous system, due to a long period of operation, bacteria are adapted to high temperature, or strains with a higher temperature tolerance become dominant, that is why the results of continuous work show a pattern different to that observed in the batch system.

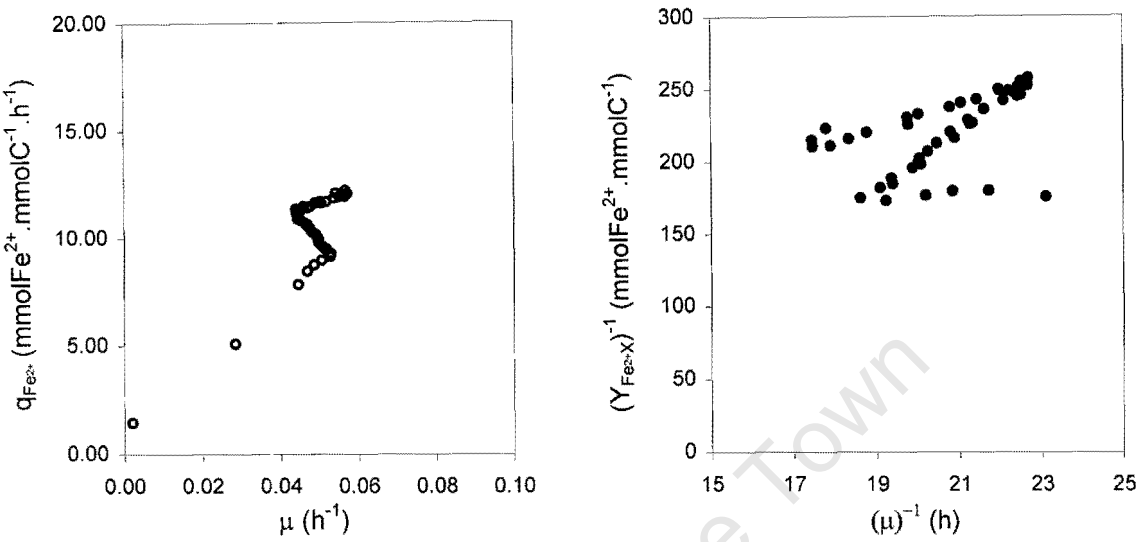
The bacterial specific growth rate increases as the pH is decreased. A maximum rate at a pH of 1.3 is achieved. A possible reason for the optimum at pH 1.3 is that pH 1.1 is below the optimum for acidophilic growth (Breed and Hansford, 1999[a]).

6.3 Yield and Maintenance

An attempt was made to determine the maximum bacterial yield on ferrous-iron, $Y^{max}_{Fe^{2+}X}$, and the maintenance coefficient on ferrous-iron, $m_{Fe^{2+}}$, using Equation 3.11 and 3.13. Figures 6.8 a) and b) contain the data used for one experiment at a temperature of 40 °C and a pH of 1.1. The Figures at the other conditions are very similar to this one and are situated in Appendix 3. From Figure 6.8 it is evident that there are three distinct linear regions. The initial region is at the beginning of the batch at a very high ferrous-iron concentration (low redox potential), while the final region is at the end of a batch at a very low ferrous-iron concentration (high redox potential). Because the three regions do not have the same intercept and slope it is possible to obtain neither the maximum bacterial yield nor the maintenance coefficient using Equations 3.11 and 3.13.

For systems that are limited by more than one factor separately, the maximum bacterial yield and maintenance coefficients can be determined from Equation 3.17. This equation was

therefore used to determine the maximum bacterial yield and the maintenance coefficients in the batch experiments. When implementing this model it was assumed that the final section of a batch was limited by ferrous-iron while the initial section was not. The intermediate section was assumed to be a combination of limitations and was not incorporated into the model.



a) b)

Figure 6.8: Data used in an attempt to determine $Y^{max}_{Fe^{2+}X}$ and $m_{Fe^{2+}}$ at a temperature of 40°C and a pH of 1.1.

The values of the maximum bacterial yield on ferrous-iron, $Y^{max}_{Fe^{2+}X}$, and the constant maintenance coefficient on ferrous-iron, $m_{Fe^{2+}}$, were calculated from the final section of the batches by linear regression using Equation 3.17 and assuming that there is no variable maintenance at the end of a batch. The variable maintenance coefficient on ferrous-iron, $m^{v}_{Fe^{2+}}$, was obtained from the y-intercept of the linear regression through the initial section of the batches. The maximum bacterial specific growth rate, μ^{max}, and the maximum bacterial specific ferrous-iron utilisation rate, $q^{max}_{Fe^{2+}}$, were determined from the intercept of the linear regression of the final and the initial sections (see Figure 2.3).

The values of the maximum yield on oxygen, $Y^{max}_{O_2X}$, the constant maintenance coefficient on oxygen, m_{O_2}, the variable maintenance coefficient on oxygen, $m^{v}_{O_2}$, the maximum bacterial specific growth rate, μ^{max}, and the maximum bacterial specific oxygen utilisation rate, $q^{max}_{O_2}$, were determined in a similar fashion to the ferrous-iron based parameters, however Equation 3.18 was used.

The average oxygen and ferrous-iron based parameters determined at pH 1.70 and 30, 35 and 40 °C are situated in Table 6.3; while the parameters determined at 40 °C and pH 1.1, 1.3, 1.5 and 1.7 are situated in Table 6.4.

Table 6.3: Average bioenergetic parameters based on ferrous-iron and oxygen determined at pH 1.7 and temperatures ranging from 30 to 40 °C

Temperature	$m_{Fe^{2+}}$	$Y^{max}_{Fe^{2+}X}$	$m^{v}_{Fe^{2+}}$	μ^{max}	$q^{max}_{Fe^{2+}}$
30 °C	0.540	0.0056	7.85	0.079	14.65
35 °C	0.855	0.0055	6.50	0.119	22.31
40°C	0.002	0.0036	11.32	0.038	10.65
	m_{O_2}	$Y^{max}_{O_2X}$	$m^{v}_{O_2}$	μ^{max}	$q^{max}_{O_2}$
30 °C	0.131	0.0228	2.32	0.074	3.37
35 °C	0.203	0.0227	1.59	0.123	5.63
40 °C	0.092	0.0158	2.74	0.040	2.61

Table 6.4: Average bioenergetic parameters based on ferrous-iron and oxygen determined at 40 °C and pH ranging from 1.10 to 1.70

pH	$m_{Fe^{2+}}$	$Y^{max}_{Fe^{2+}X}$	$m^{v}_{Fe^{2+}}$	μ^{max}	$q^{max}_{Fe^{2+}}$
1.70	0.00	0.0036	11.32	0.038	10.66
1.50	0.95	0.0058	6.06	0.077	14.32
1.30	2.26	0.0054	10.08	0.087	18.30
1.10	1.00	0.0058	5.95	0.089	16.36
	m_{O_2}	$Y^{max}_{O_2X}$	$m^{v}_{O_2}$	μ^{max}	$q^{max}_{O_2}$
1.70	0.0917	0.0158	2.74	0.040	2.61
1.50	0.2312	0.0236	1.52	0.077	3.50
1.30	0.5638	0.0221	2.51	0.087	4.49
1.10	0.2423	0.0239	1.49	0.090	4.00

Temperature does not appear to affect the maximum bacterial yield on ferrous-iron and oxygen significantly, but a minimum was reached at 40°C. An increase in temperature caused a decrease in the constant maintenance coefficient, but an increase in the variable maintenance coefficient. It is apparent that the maximum bacterial yields on ferrous-iron and oxygen, and the maintenance coefficients on ferrous-iron and oxygen (constant and variable) do not vary significantly with temperature or pH.

The effect of temperature on the maximum bacterial specific growth rate is not obvious, but it appears to reach a maximum at 35 °C. The maximum bacterial specific growth rate decreases as the pH increases. The maximum bacterial specific growth rate follows the same variation with temperature and pH as that observed for the bacterial specific growth rate calculated from the exponential growth phase. The maximum bacterial specific ferrous-iron and oxygen utilisation rates reach a maximum at 35 °C. A trend of decreasing rates was observed for increasing pH at 40 °C, although a local maximum was reached at a pH of 1.3.

The validity of the maximum bacterial yield and the maintenance coefficients in Tables 6.3 and 6.4 were checked by a comparison of the experimental results and the results predicted by the "degree of reduction" balance i.e. Equations 3.15 and 3.16. These comparisons are displayed in Figure 6.9, from which it is apparent that the correlation is good (average $R^2 = 0.9596$).

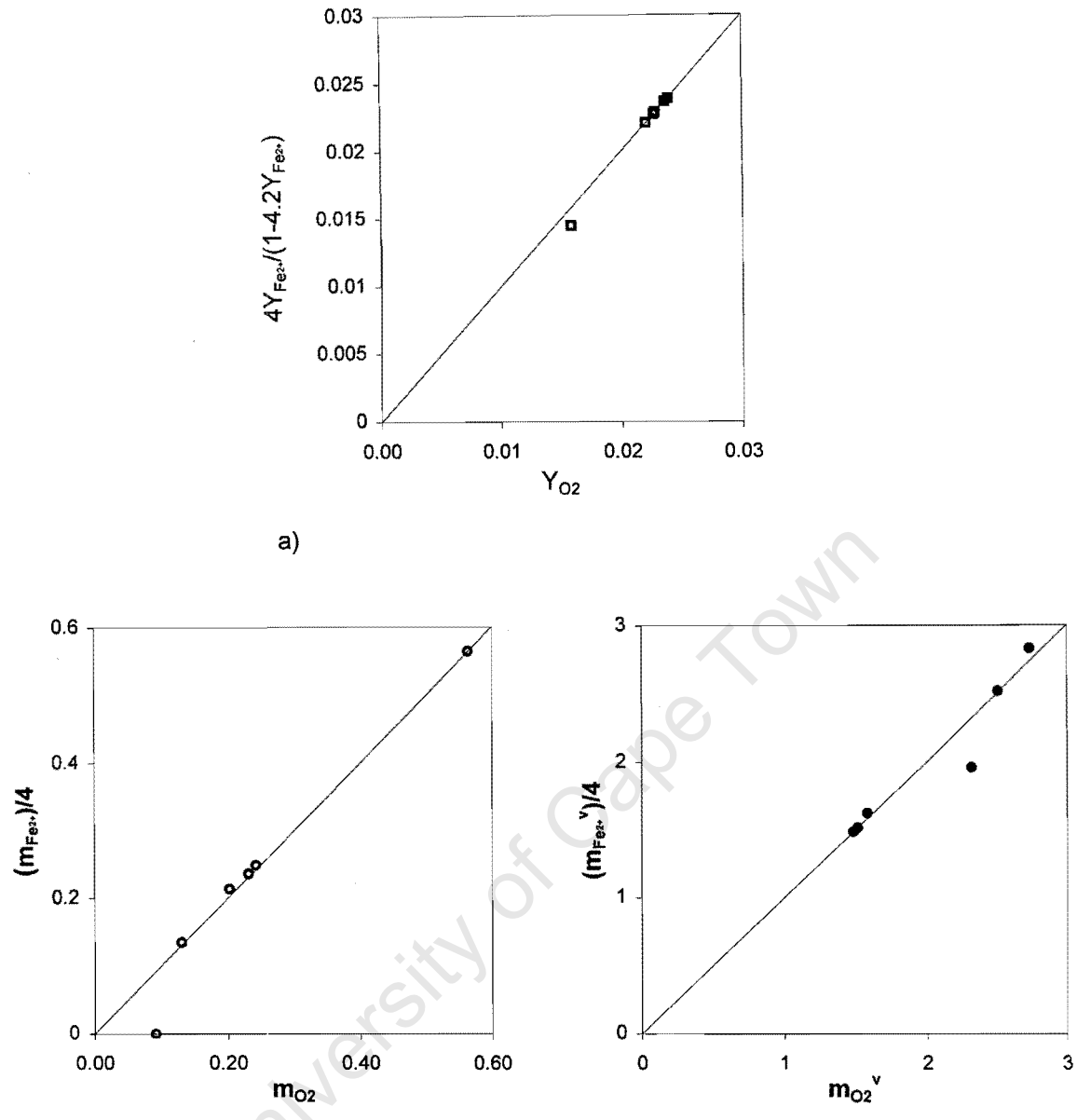
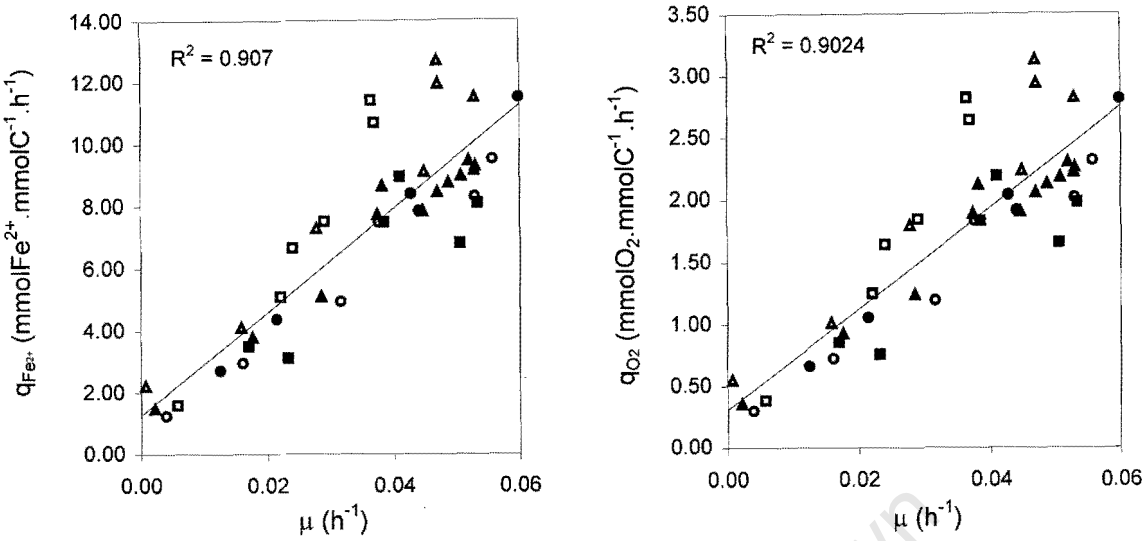


Figure 6.9: Comparison of the predicted and experimental relationships for, a) the maximum bacterial yield (□), b) the constant maintenance coefficient (○) and c) the variable maintenance coefficient (●) for the data listed in Tables 6.3 and 6.4.

The average maximum bacterial yields and the average constant maintenance coefficients on ferrous-iron and oxygen were calculated by linear regression through the final section of the batch data (Figure 6.10). The final section of the batch data was used because the constant maintenance coefficient and the maximum bacterial yield can be calculated directly from the intercept and the slope. The average parameters together with the data published previously are displayed in Tables 6.5 and 6.6.

From Tables 6.5 and 6.6 it is apparent that the average maximum bacterial yields and constant maintenance coefficients for the final section of the batch are very similar to the values previously published. The experimental results are most similar to the results published by Breed (2000). The reason for this is that the same bacterial culture and experimental conditions were used.



a) b)

Figure 6.10: Data from the final regions of the batches used to determine the average maximum bacterial yield and the average constant maintenance coefficient on ferrous-iron (a) and oxygen (b). (○)30 °C, pH = 1.7, (●) 35 °C, pH = 1.7, (□) 40 °C, pH = 1.7, (■) 40 °C, pH = 1.5, (△) 40 °C, pH = 1.3, (▲) 40 °C, pH = 1.1.

Table 6.5: Average values of the maximum bacterial yield and constant maintenance coefficient on ferrous-iron together with previously reported values over the temperature range: 30 - 40 °C and pH range: 1.1 - 1.8

Bacteria	Mode of Operation	$Y^{max}_{Fe^{2+}X}$ ($molC.(molFe^{2+})^{-1}$)	$m_{Fe^{2+}}$ ($molFe^{2+}.molC^{-1}.h^{-1}$)
Predominantly *L. ferrooxidans*	Batch	0.0060	1.261
Predominantly *L ferrooxidans* (Breed, 2000)	Continuous	0.0075	1.196
Leptospirillum - like, (van Scherpenzeel *et al.*, 1998)	Continuous	0.011	0.444
A. ferrooxidans (Boon 1996)	Continuous	0.012	0.32
A. ferrooxidans (Boon, 1996)*	Batch	0.011	0.6

* See Chapter 5. Final section of the batch.

Table 6.6: Average values of the maximum bacterial yield and maintenance coefficient on oxygen together with previously reported values over the temperature range: 30 - 40 °C and pH range: 1.1 - 1.8

Bacteria	Continuous or Batch	$Y_{O_2X}^{max}$ ($molC.(molO_2)^{-1}$)	$m_{Fe^{2+}}$ ($molO_2.molC^{-1}.h^{-1}$)
Predominantly *L. ferrooxidans* (present work)	Batch	0.0247	0.3096
Predominantly *L. ferrooxidans* (Breed, 2000)	Continuous	0.0279	0.2376
Leptospirillum - like, (van Scherpenzeel, 1996)	Continuous	0.046	0.0425
A. ferrooxidans (Boon 1996)	Continuous	0.051	0.1

The bacterial specific ferrous-iron utilisation rate and the bacterial specific growth rate calculated using the variable maintenance model were compared to the parameters published previously. The comparisons are situated in Figures 6.11 and 6.12.

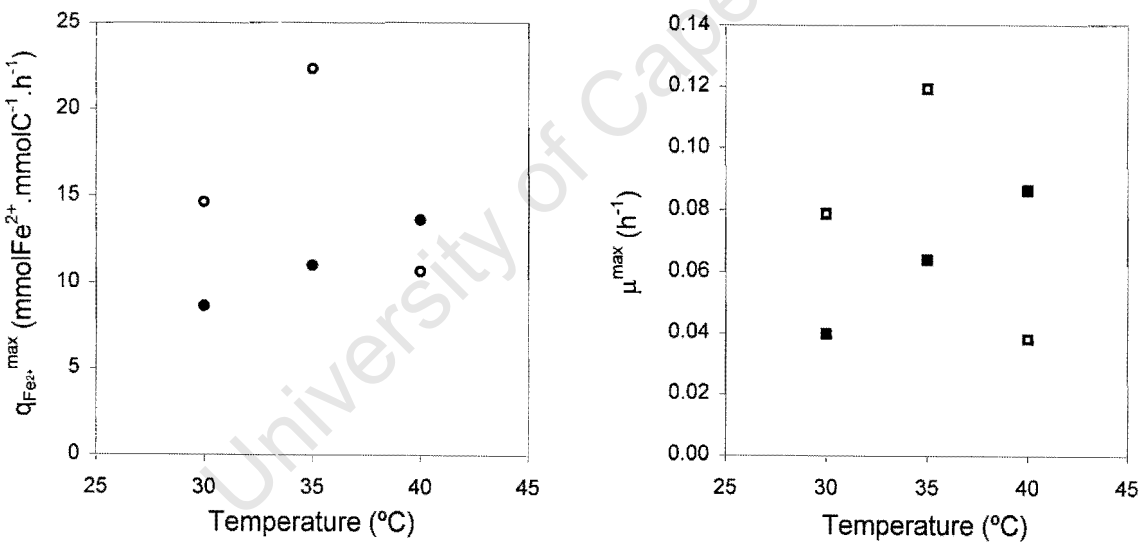


Figure 6.11: A comparison of the maximum bacterial specific ferrous-iron utilisation rate and bacterial specific growth rate calculated, and published by Breed *et al.* (1999) for variation in temperature at a pH of 1.7. (○) $q_{Fe^{2+}}^{max}$ (present work), (●) $q_{Fe^{2+}}^{max}$ (Breed *et al.*, 1999), (□) μ^{max} (present work), (■) μ^{max} (Breed *et al.*, 1999).

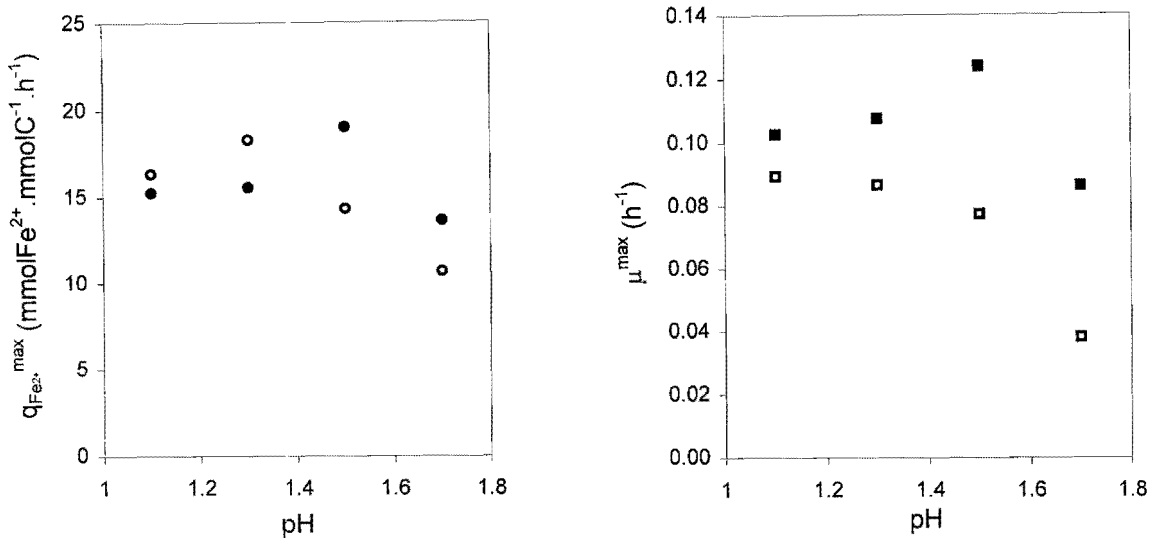


Figure 6.12: A comparison of the maximum bacterial specific ferrous-iron utilisation rate and bacterial specific growth rate calculated, and published by Breed *et al.* (1999) for variation in pH at 40 °C. (○) $q_{Fe^{2+}}^{max}$ (present work), (●) $q_{Fe^{2+}}^{max}$ (Breed and Hansford, 1999), (□) μ^{max} (present work), (■) μ^{max} (Breed and Hansford, 1999).

Based on Figures 6.11 and 6.12 it is apparent that the maximum bacterial specific ferrous-iron utilisation rates and the bacterial specific growth rates for the batch and continuous all appear to fall into the same range. The effect of temperature and pH, however appears to be different in batch and continuous experiments.

An increase in temperature resulted in an increase in the maximum bacterial specific ferrous-iron utilisation rate and the maximum bacterial specific growth rate over the temperature range 30 to 40 °C in continuous experiments using *Leptospirillum ferrooxidans* (Breed *et al.* 1999). They used the Arrhenius equation to represent the effect of temperature. The effect of temperature over the same range in batch experiments, however resulted in a maximum specific ferrous-iron utilisation rate and specific growth rate at 35 °C and hence the Arrhenius equation could not be used to represent the data. In agreement with the batch results however, van Scherpenzeel (1996) reported the optimum temperature for the ferrous-iron oxidation by *Leptospirillum ferrooxidans* in continuous culture to be 35 °C.

The results of Breed and Hansford (1999[a]) showed that maximum bacterial specific ferrous-iron utilisation rate did not vary appreciably with pH. Figure 6.11 shows that pH has the same effect in batch culture i.e. it doesn't vary appreciably with pH. The maximum bacterial specific growth rate in batch culture decreases as the pH is increased in batch culture. In continuous culture, however the maximum specific growth rate does not appear to vary appreciably with pH.

Based on all the data that is presented in this section, it is apparent that the variable maintenance model is valid in the determination of the maximum growth yields and constant- and variable maintenance coefficients in the batch ferrous-iron oxidation by *Leptospirillum ferrooxidans*. The validity of the model was confirmed by the similarity of the maximum

growth yields and constant and variable maintenance coefficients calculated to parameters published previously for the continuous ferrous-iron oxidation by *Leptospirillum ferrooxidans*.

The maximum bacterial specific ferrous-iron utilisation rate, $q^{max}_{Fe^{2+}}$, and the maximum bacterial specific growth rate, μ^{max}, calculated from the variable maintenance model are very similar to those previously published. This affirms the validity of the variable maintenance equation.

6.4 The Kinetics of Bacterial Ferrous-Iron Oxidation

The Michaelis-Menten model is based on the assumption that the bacterial growth is only limited by the substrate. Only the decelerating growth phase in batch culture is limited by ferrous-iron, so this section was modelled using the Michaelis-Menten based model proposed by Boon (1996).

In order to find the constants in the model a Lineweaver-Burke plot was used (Equation 3.20). An example of a Lineweaver-Burke plot at 40°C and pH = 1.1 is shown in Figure 6.13. The Lineweaver-Burke plots for all the batches are very similar to this one.

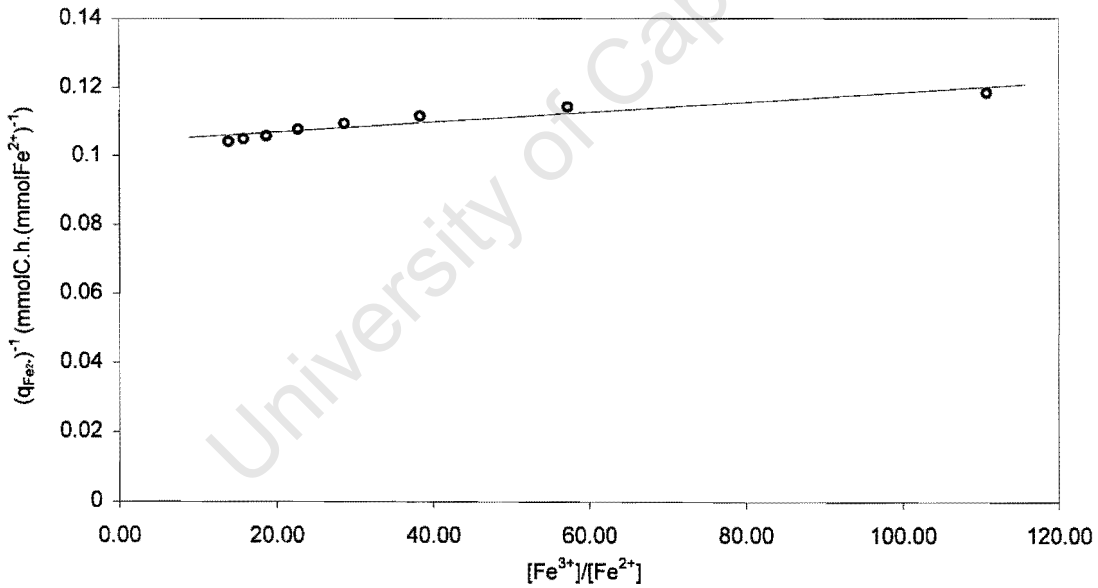


Figure 6.13: Lineweaver-Burke plot for the deceleration phase of a batch experiment at T = 40 °C and pH = 1.1.

Linear regression using Microsoft Excel was used to find the maximum bacterial specific ferrous-iron utilisation rates, $q^{max}_{Fe^{2+}}$, and the kinetic constants, $K_{Fe^{2+}}$. Tables 6.7 and 6.8 contain the Michaelis-Menten parameters for all the batch and continuous (Breed *et al.*, 1999; Breed and Hansford, 1999) experiments. Graphical representations of the changes in the parameters versus the changes in temperature and pH are situated in Figures 6.14 and 6.15 respectively.

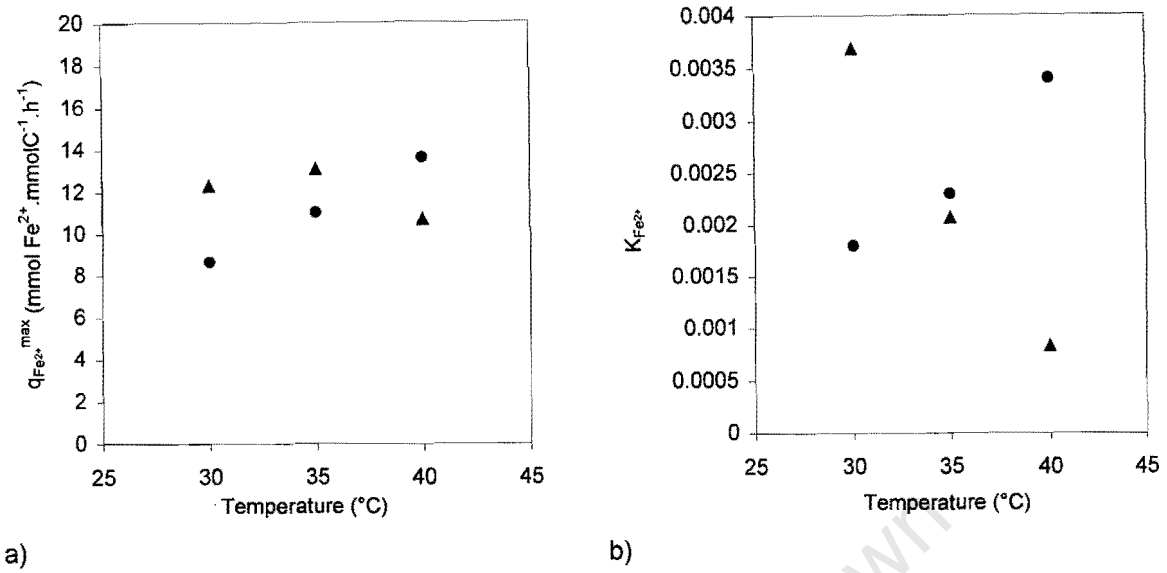


Figure 6.14: Variation in the kinetic parameters with temperature for continuous (Breed *et al.*, 1999) (●) and batch (▲). a) $q_{Fe^{2+}}^{max}$ and b) $K_{Fe^{2+}}$.

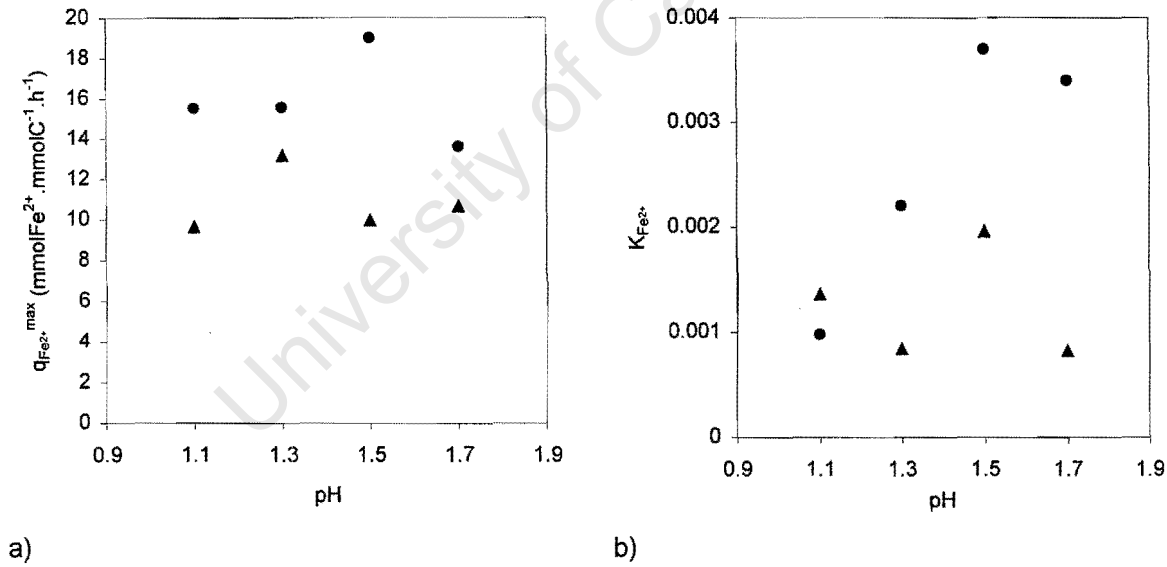


Figure 6.15: Variation in the kinetic parameters with pH for continuous (Breed and Hansford, 1999[a]) (●) and batch (▲). a) $q_{Fe^{2+}}^{max}$ and b) $K_{Fe^{2+}}$.

Table 6.7: Values of the average maximum bacterial specific ferrous-iron utilisation rates, $q^{max}_{Fe^{2+}}$, and their average respective kinetic constants, $K_{Fe^{2+}}$, of *Leptospirillum ferrooxidans* in batch and continuous ferrous-iron oxidation at 30 - 40 °C and a pH of 1.7

Run Type	Temperature (°C)	$q^{max}_{Fe^{2+}}$ ($mmolFe^{2+}.mmolC^{-1}.h^{-1}$)	$K_{Fe^{2+}}$
Batch	30	12.25	0.0037
Batch	35	13.05	0.0021
Batch	40	10.64	0.0008
Continuous†	30	8.65	0.0018
Continuous†	35	11.01	0.0023
Continuous‡	40	13.62	0.0034

Table 6.8: Values of the average maximum bacterial specific ferrous-iron utilisation rates, $q^{max}_{Fe^{2+}}$, and their average respective kinetic constants, $K_{Fe^{2+}}$, of *Leptospirillum ferrooxidans* in batch and continuous ferrous-iron oxidation at 40 °C and a pH of 1.1 - 1.7

Run Type	pH	$q^{max}_{Fe^{2+}}$ ($mmolFe^{2+}.mmolC^{-1}.h^{-1}$)	$K_{Fe^{2+}}$
Batch	1.7	10.64	0.0008
Batch	1.5	9.94	0.0020
Batch	1.3	13.13	0.0008
Batch	1.1	9.62	0.0014
Continuous‡	1.7	13.62	0.0034
Continuous‡	1.5	19.02	0.0037
Continuous‡	1.3	15.57	0.0022
Continuous‡	1.1	15.53	0.00098

All of the kinetic parameters calculated from the batch data are similar to the parameters calculated for continuous experiments, however the effects of temperature and pH appear to be different in the batch experiments and the continuous experiments.

Figures 6.14a) and 6.15a) show that the maximum bacterial specific ferrous-iron utilisation rate in batch culture does not vary with any distinct pattern with a variation in temperature or pH. Breed *et al.* (1999) found that the maximum bacterial specific ferrous-iron utilisation rate increased with an increase in temperature, and could be modelled using the Arrhenius Equation. The effect of pH on the maximum bacterial specific ferrous-iron utilisation rate in continuous culture was similar to the effect in batch culture, however the values in batch culture are consistently lower than those published for continuous culture.

The kinetic constants for the batches appear to decrease linearly with an increase in temperature, while the kinetic constants in continuous experiments increases linearly with an increase in temperature. The kinetic constant appears to be unaffected by pH in batch culture, however in continuous experiments Breed and Hansford (1999[a]) proved that the kinetic constants increase linearly with an increase in pH.

† Continuous data from Breed *et al.* (1999)

‡ Continuous data from Breed and Hansford (1999[a])

Graphs of the change in the bacterial specific ferrous-iron utilisation rate with change in ferric to ferrous-iron ratio were plotted and the plot at T = 40 °C and pH = 1.1 for continuous and for both the batches is shown in Figure 12. The Michaelis-Menten plots for all the conditions are similar to this graph.

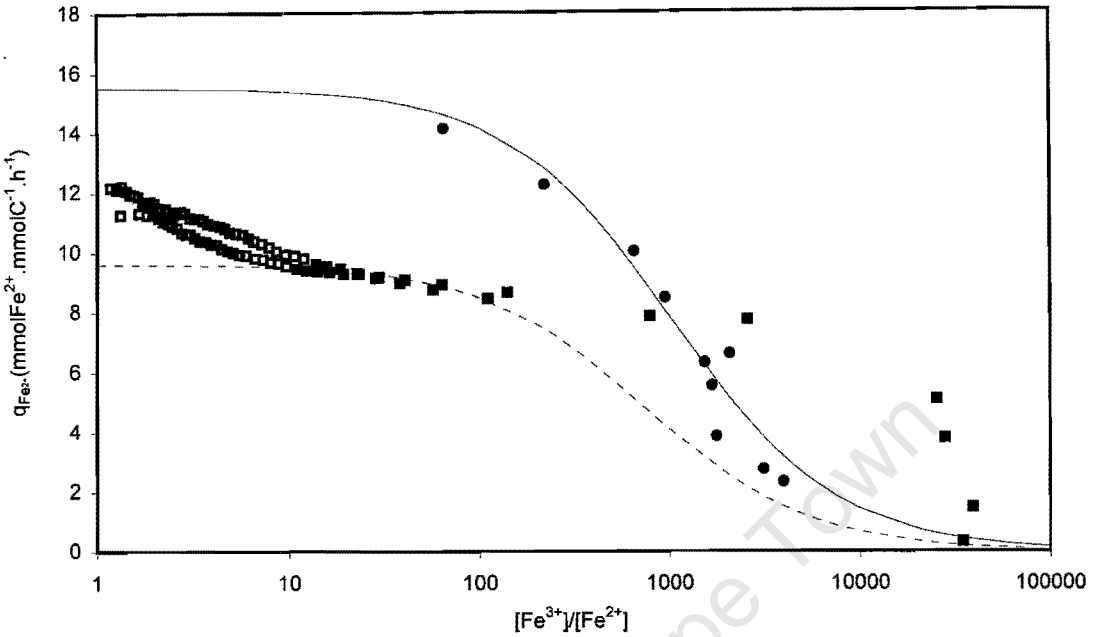


Figure 6.16: Comparison between the experimental variation in the bacterial specific ferrous-iron oxidation rate with changes in the ferric/ferrous-iron ratio at T = 40°C and pH = 1.1 for continuous data (●), continuous equation (—) (Breed and Hansford 1999), batch data (■ high redox potential, □ low redox potential) and batch equation (---).

From Figure 6.16 it can also be seen that even when fitting the Michaelis-Menten based model for the batches at only high redox potentials there is still deviation at very high redox potentials from the model. The deviation at high redox potentials is assumed to be a result of changing growth-limiting conditions.

Based on all the kinetic data presented it is evident that although the batch and continuous experiments produce results that are in the same range, the batch results are different to the continuous results. The effects of temperature and pH on the maximum bacterial specific ferrous-iron utilisation rates and the kinetic constants in continuous experiments are different to the effects in batch experiments.

Continuous experiments are more accurate for modelling bacterial ferrous-iron oxidation kinetics limited by ferrous-iron. The main reason for this is that batch experiments have transient conditions and the limitation by ferrous-iron is only over a very short time so only a limited number of useful data points can be obtained. Continuous experiments however are operated at steady state so there are no transient conditions and any number of steady state conditions can be run, hence no limitation on the number of data points.

Batch experiments, however are useful for initial short-term analysis of a system and an initial indication of the modelling parameters.

The maximum bacterial specific ferrous-iron utilisation rate calculated by the variable maintenance model follows the same trends as that calculated from the Michaelis-Menten model. The values calculated from the variable maintenance model, however are generally higher than those calculated by the Michaelis-Menten model.

Table 6.9: Comparison of the maximum bacterial specific ferrous-iron utilisation rates calculated by the variable maintenance model and the Michaelis-Menten based model in batch culture, and the Michaelis-Menten based model in continuous culture

Temperature (°C)	pH	$q^{max}_{Fe^{2+}}$ ($mmolFe^{2+}.mmolC^{-1}.h^{-1}$)		
		Variable Maintenance Model	Michaelis-Menten Modelling	Continuous
30	1.7	14.66	12.25	8.65
35	1.7	22.32	13.05	11.01
40	1.7	10.66	10.64	13.62
40	1.5	14.32	9.94	19.02
40	1.3	18.30	13.13	15.57
40	1.1	16.36	9.62	15.53

Chapter 7

Conclusions and Recommendations

Deviations in the published values of the maximum bacterial yields and the maintenance coefficients were noticed. These are due to the modelling of the bioenergetics by a constant maintenance energy equation. The use of a constant maintenance energy equation results in a gross overestimate of the maximum bacterial yield when the culture is energy sufficient and not limited by the energy source.

The re-evaluation of previously published data (Jones and Kelly 1983; Boon, 1996) proved that the bio-oxidation of ferrous-iron can be represented using a variable maintenance equation proposed by Neijssel and Tempest (1976) and modified by Pirt (1982). An estimation of the maximum bacterial specific ferrous-iron utilisation rate and the maximum bacterial specific growth rate can be obtained from batch or continuous experiments that have been limited by more than one factor e.g. ferrous-iron, arsenic, ammonia, phosphate, sulfate, growth rate. The maximum bacterial specific ferrous-iron utilisation rates and the maximum bacterial specific growth rates calculated from the variable maintenance model are very similar to those values previously published and affirms the validity of the equation.

The batch growth curves follow expected trends and have a very long exponential phase and a very short deceleration phase. The bacterial growth during the exponential phase follows first order kinetics with respect to the biomass concentration and is independent of the substrate concentration.

It was not possible to obtain the maximum bacterial yields and the maintenance coefficients during the batch bacterial ferrous-iron oxidation using the constant maintenance energy model. The reason for this is that at the beginning of a batch the ferrous-iron concentration is very high and the culture is limited by the growth rate, while at the end of a batch it is limited by ferrous-iron. The variable maintenance energy equation had to be used to calculate the maximum bacterial yields and the constant- and variable maintenance coefficients.

The maximum bacterial yields on ferrous-iron and oxygen, $Y^{max}_{Fe^{2+}X}$ and $Y^{max}_{O_2X}$, and the respective constant, and variable maintenance coefficients, $m_{Fe^{2+}}$, m_{O_2}, $m'_{Fe^{2+}}$ and m'_{O_2} did not vary significantly with temperature or pH. The average maximum yield and constant maintenance coefficient were calculated from the final sections of all the batches assuming that neither temperature nor pH had any effect. The average values calculated were similar to those reported previously for *Leptospirillum ferrooxidans* in continuous culture and for *Acidithiobacillus ferrooxidans* in continuous and batch culture.

The maximum bacterial specific ferrous-iron utilisation rate, $q^{max}_{Fe^{2+}}$, and the maximum bacterial specific growth rate, μ^{max}, calculated from the variable maintenance equation are very similar to those previously published.

Only the effects of two limiting factors on the bacterial ferrous-iron oxidation have been investigated. In order to assess the validity of the variable maintenance equation comprehensively, experiments need to be performed with at least three limiting factors.

The variable maintenance equation is excellent for calculating the maximum bacterial yields and the maintenance coefficients during bacterial ferrous-iron oxidation, because it can account for the variable maintenance due to limiting factors; and if the culture is only limited by the substrate then it can still be used, but the variable maintenance falls to zero. Due to the low redox potentials (high ferrous-iron concentrations) in mesophilic chalcopyrite bioleaching and thermophilic bioleaching of sulfide minerals the cultures may not be limited by ferrous-iron, so the variable maintenance model must be used.

It is apparent that the constant maintenance equation is only valid for bacterial ferrous-iron oxidation if the culture is only limited by the substrate during the whole experiment. If at any stage the culture is limited by another factor then the bioenergetics must be represented using the variable maintenance equation.

The variable maintenance equation should replace the constant maintenance equation when quantifying the bioenergetics of bacterial ferrous-iron oxidation and the bioleaching of sulfide minerals. The variable maintenance equation is especially relevant to the bioleaching of sulfide minerals at low redox potentials (high ferrous-iron concentrations) where the bacterial culture may not be limited by the energy source (ferrous-iron).

The growth rate during the deceleration phase in a batch culture is limited by the substrate, ferrous-iron and follows Michaelis-Menten based kinetics. The kinetic parameters calculated from batch experiments were very similar to those calculated by previous researchers in continuous experiments. The variation of temperature and pH, however produced different results in batch and continuous experiments. The continuous experiments are more accurate for representing bacterial ferrous-iron oxidation kinetics limited by ferrous-iron because batch experiments have transient conditions and the limitation by ferrous-iron is only over a very short time so only a limited number of data points can be obtained. Batch experiments, however are useful for initial short-term analysis of a system and an initial indication of the kinetic modelling parameters.

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APPENDIX 1

REDOX PROBE CALIBRATION

The ferric to ferrous-iron ratio was obtained from the redox potential via the Nernst Equation (Boon, 1996).

$$E = E_0 + \frac{RT}{zF} \ln\left(\frac{a_{Fe^{3+}}}{a_{Fe^{2+}}}\right) \quad (3.25)$$

Using calibration for the probe, this equation was manipulated to give:

$$E = E_0' + \frac{RT}{zF} \ln\left(\frac{[Fe^{3+}]}{[Fe^{2+}]}\right) \quad (3.26)$$

From a calibration graph E_0' and $\frac{RT}{zF}$ can be obtained from the intercept and the slope respectively. The calibration graphs for temperatures ranging from 30 to 40 °C at a pH of 1.7 are shown in Figures A1.1 to A1.3 and a table of the slopes and intercepts is shown in Table A1.1. The calibration curves don't change appreciably with pH, so only the graph at a pH = 1.7 is shown in Figure A1.1. From the results in Table A1.1 it appears that the curves do not change appreciably with temperature either. The data points at high redox potentials were ignored, and the straight line was just plotted through the data points at lower redox potentials. Data points at high redox potentials were ignored until the sum of the squared error was approximately one.

Table A1.1: Table of E_0' and $\frac{RT}{zF}$ from the calibration graphs

pH	Temperature (°C)	E_0' (mV)	$\frac{RT}{zF}$	R^2
1.7	40	506.5	29.4	1.00
1.7	35	507.1	27.1	1.00
1.7	30	507.7	25.7	1.00

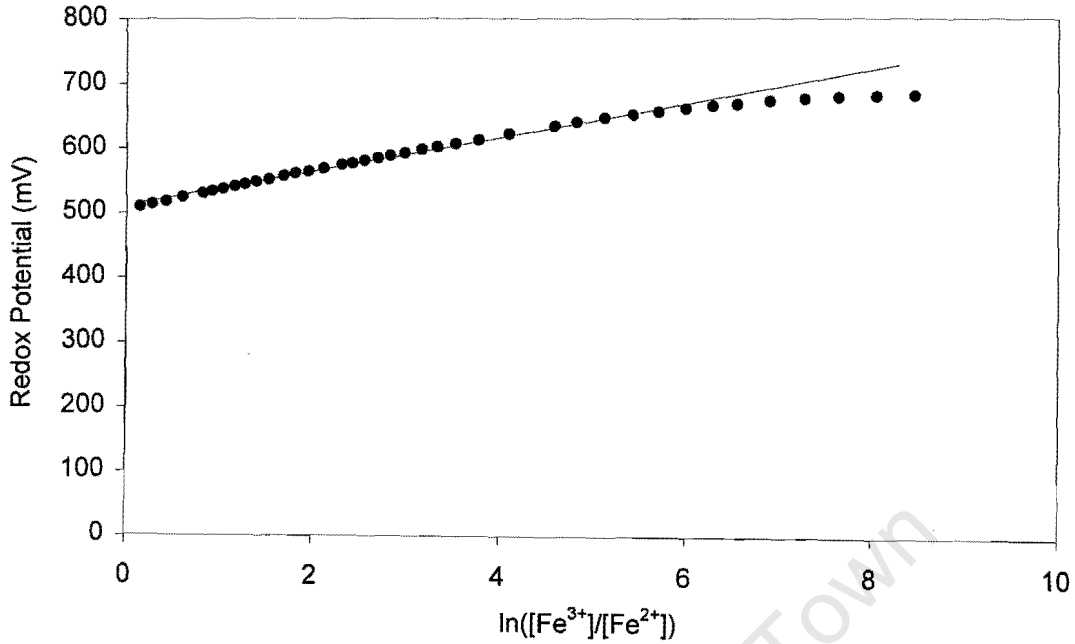


Figure A1.1: Redox probe calibration at a temperature of 40 °C and a pH of 1.7.

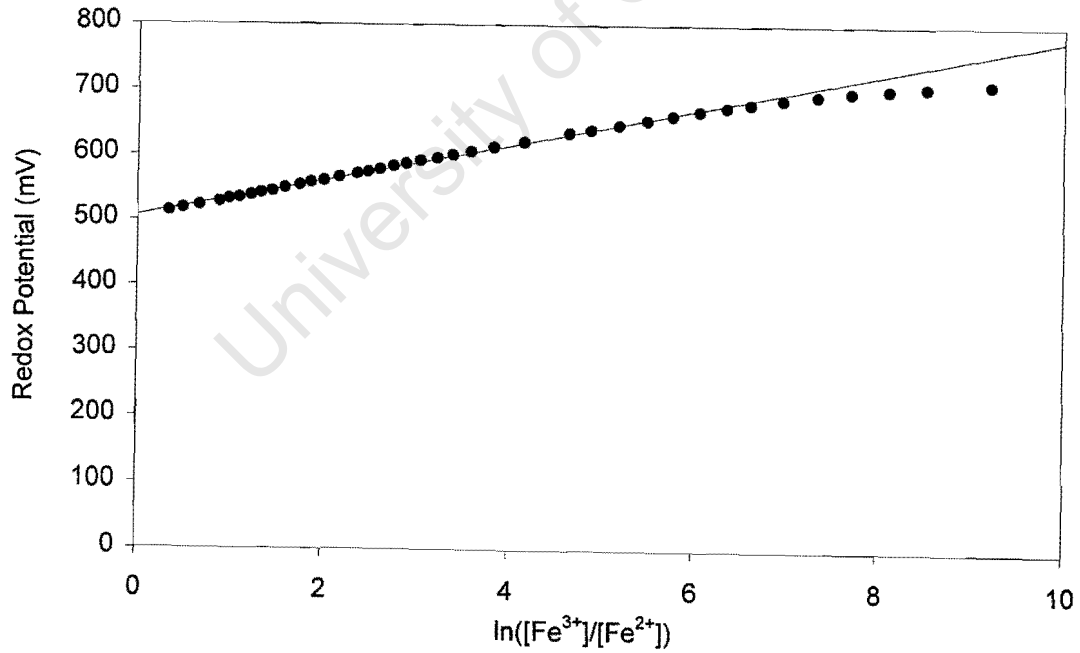


Figure A1.2: Redox probe calibration at a temperature of 35 °C and a pH of 1.7.

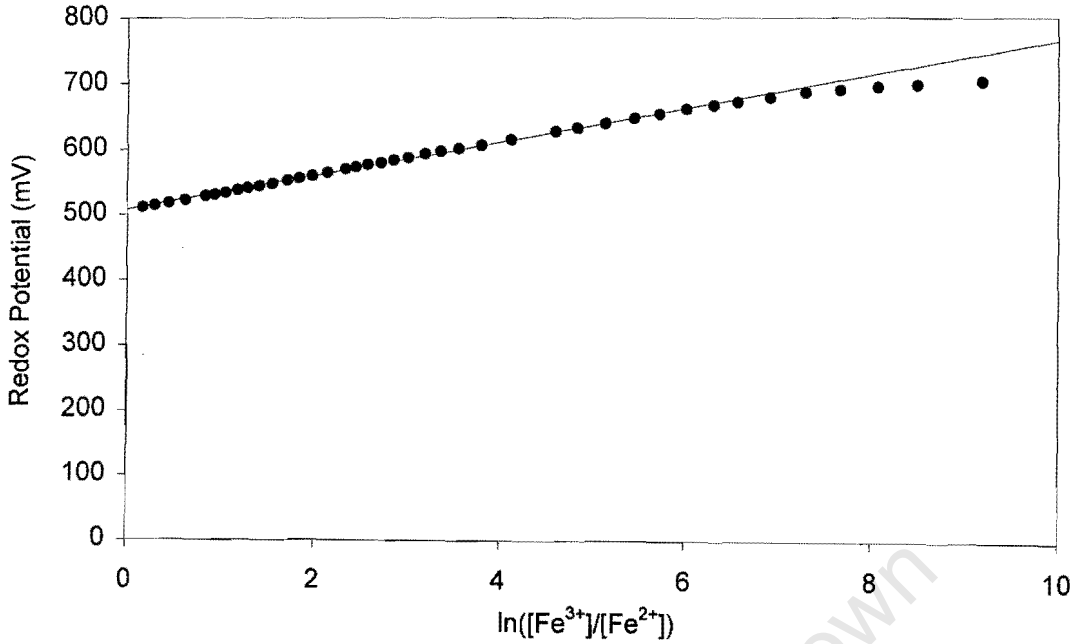


Figure A1.3: Redox probe calibration at a temperature of 30 °C and a pH of 1.7.

APPENDIX 2

ERROR ANALYSIS

An error analysis was performed to obtain an estimation of the magnitude of the error between experiments performed at the same conditions.

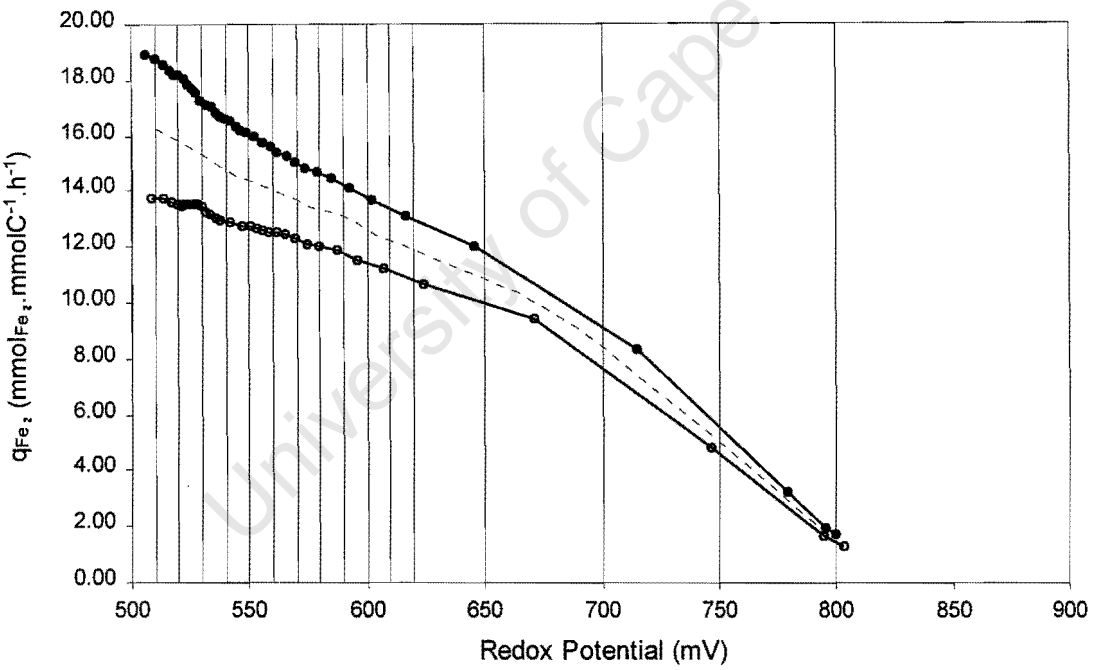


Figure A2.1: Graphical representation of the error analysis. (●) Run 1 data, (○) Run 2 data, (—) linear interpolation, (---) mean.

A graphical representation of the error analysis is situated in Figure A2.1. Firstly the bacterial specific ferrous-iron utilisation rates for each replicate were obtained at discrete redox potentials. The discrete redox potentials were chosen by the number of data points in the region i.e. more discrete redox potentials were chosen at low potentials than at high potentials. If a point was not available at a certain redox potential then the corresponding specific utilisation rate was obtained by linear interpolation between the two closest points.

The standard deviation between the two points was obtained by using the "STDEVP" function in Microsoft Excel®. The "STDEVP" function uses the following formula:

$$\sigma = \sqrt{\frac{n\sum x_2 - \left(\sum x\right)^2}{n^2}} \qquad \text{(A2.1)}$$

In this formula, σ is the standard deviation, n is the number of samples and x is the value of the sample. "STDEVP", and not "STDEV", was used because it assumes that the points are over entire population and not samples of the entire population.

The error at each redox potential was calculated as the error in the standard deviation. This was calculated by:

$$e_{redox} = \frac{\sigma}{\bar{x}} \qquad \text{(A2.2)}$$

Where e_{redox} is the error at the specific redox potential, and $\bar{x}$ is the mean of the replicates at the specific redox potential.

The final error is quoted as the average over all the discrete redox potentials.

APPENDIX 3

YIELD AND MAINTENANCE

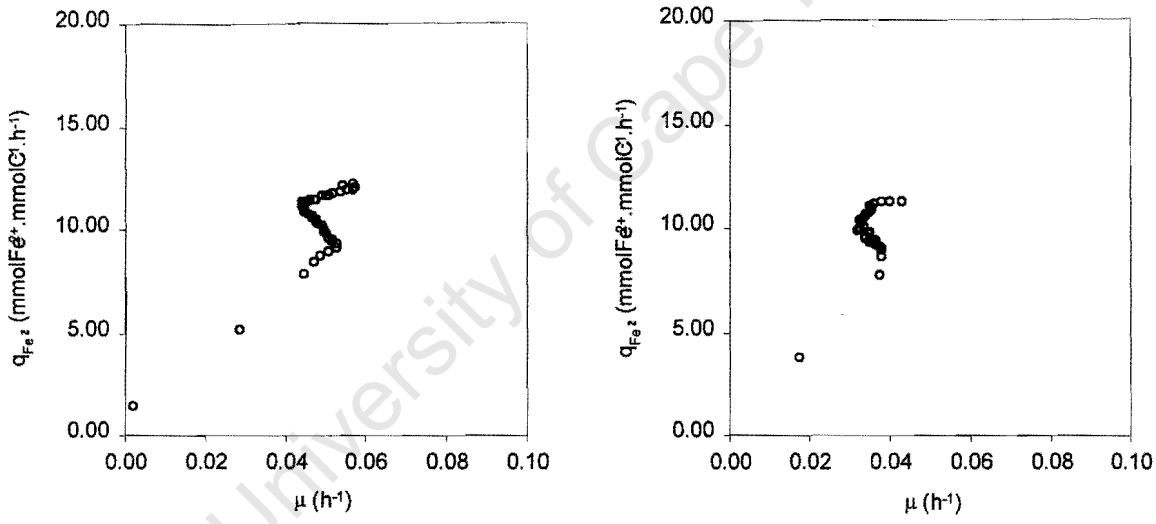
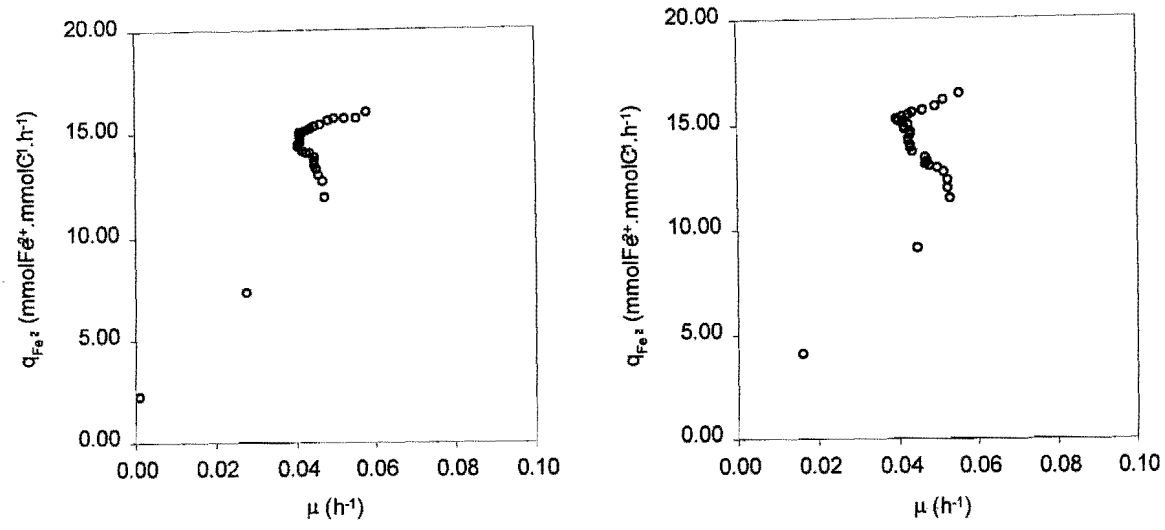
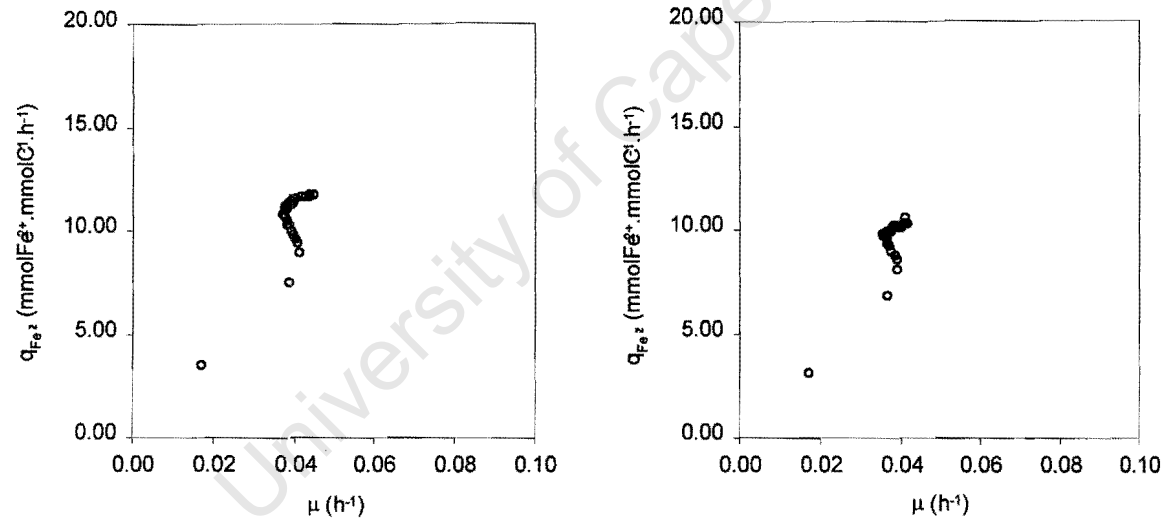


Figure A3.1: Data used to determine $Y^{max}_{Fe^{2+}X}$ and $m_{Fe^{2+}}$ at 40 °C and a pH = 1.1.



Run 1 Run 2

Figure A3.2: Data used to determine $Y^{max}_{Fe^{2+}X}$ and $m_{Fe^{2+}}$ at 40 °C and a pH = 1.3.



Run 1 Run 2

Figure A3.3: Data used to determine $Y^{max}_{Fe^{2+}X}$ and $m_{Fe^{2+}}$ at 40 °C and a pH = 1.5.

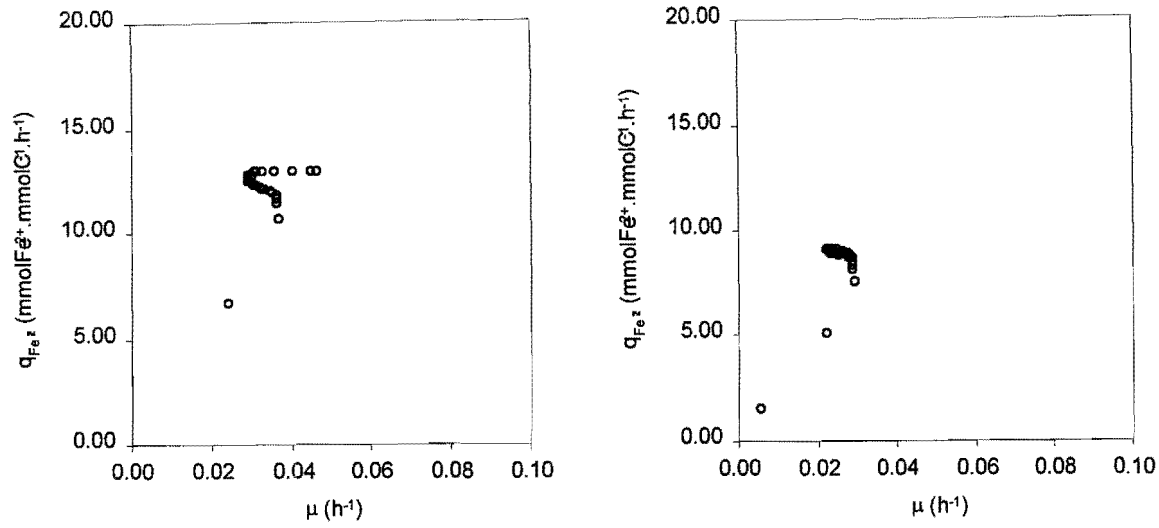


Figure A2.4: Data used to determine $Y^{max}_{Fe^{2+}X}$ and $m_{Fe^{2+}}$ at 40 °C and a pH = 1.7.

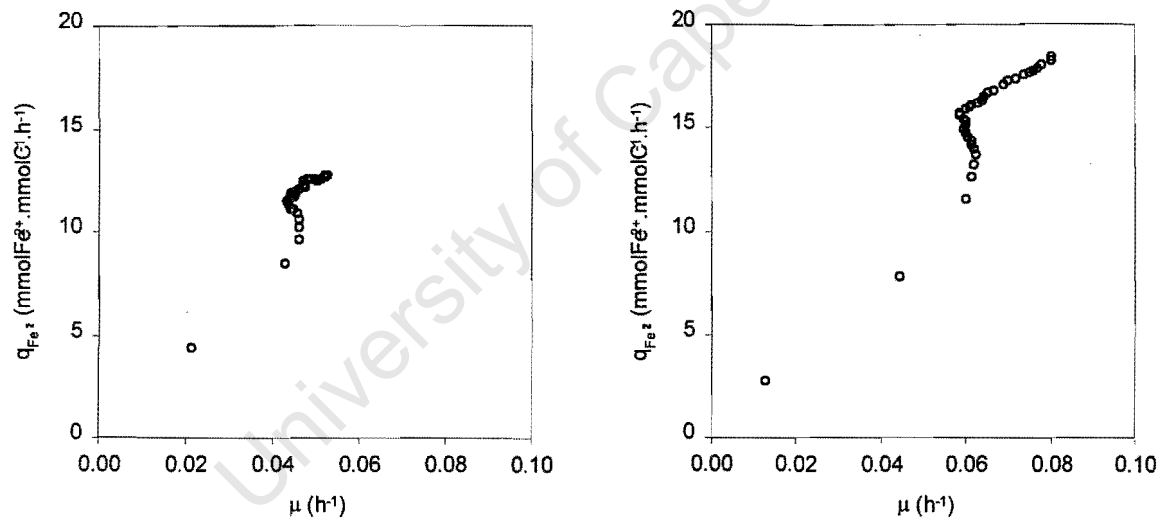


Figure A2.5: Data used to determine $Y^{max}_{Fe^{2+}X}$ and $m_{Fe^{2+}}$ at 35 °C and a pH = 1.7.

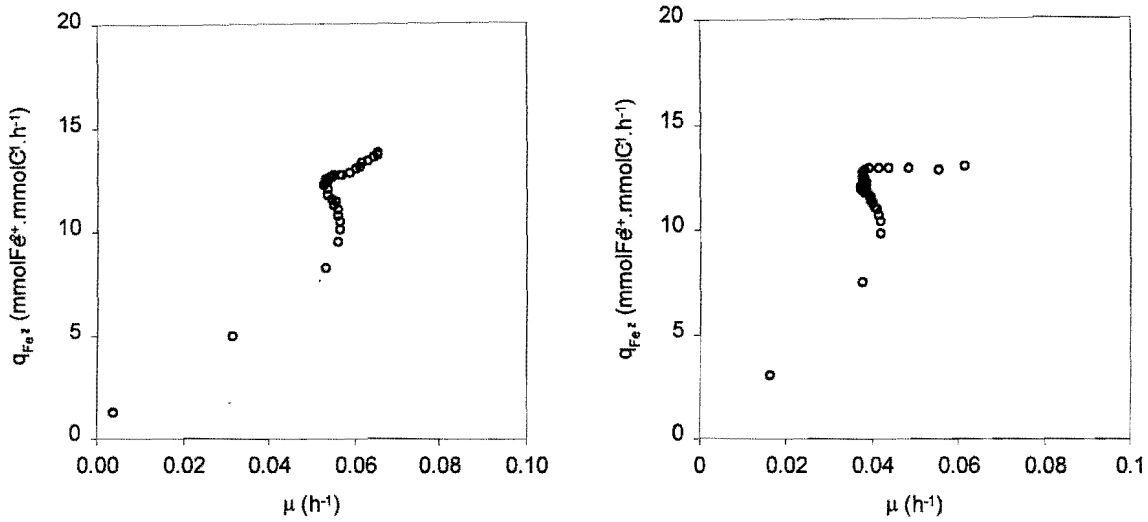


Figure A3.6: Data used to determine $Y^{max}_{Fe^{2+}X}$ and $m_{Fe^{2+}}$ at 30 °C and a pH = 1.7.