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A kinetic study on anaerobic sulphate reduction – Effect of sulphate and temperature

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August 2000

Abstract

The objectives of this work were to provide rigorous kinetic information on the effects of feed sulphate concentration and temperature on the anaerobic sulphate reduction process and to develop a kinetic model to explain this dependency. These objectives were addressed by performing batch and continuous sulphate reduction experiments using a mixed sulphate reducing microbial culture with acetate as the organic carbon and electron donor source. Sulphate concentration, acetate concentration and biomass concentration was used to determine the metabolic activity of the microorganisms and the rate of sulphate conversion.

Trends in the volumetric reduction rate of sulphate and bacterial concentration and their relationship to the feed sulphate concentration indicated that feed sulphate concentration affected the kinetics of microbial sulphate reduction. For feed sulphate concentrations in the range 1.0 to 10.0 kgm⁻³ an enhancement of microbial activity was observed with increasing sulphate concentration. Inhibition of the biological sulphate reduction process was observed when the feed contained 15.0 kgm⁻³ of sulphate. The results obtained indicated that the inhibition could be attributed to the sulphate.

The volumetric sulphate reduction rate and sulphate conversion was found to increase with temperature in the range 20 to 35°C. This increase was attributed to the enzymatic nature of the reduction reaction, which is enhanced with increasing temperature, and to the decrease in unionised hydrogen sulphide relative to the negative hydrogen sulphide ion that results with increasing temperature.

A kinetic model developed using the experimental data proposed that the inlet sulphate concentration, temperature, bacterial concentration and residual sulphate concentration determine the microbial reduction rate of sulphate in a continuous stirred tank reactor. Comparisons between the model prediction and experimental data suggested that the model has the potential for predicting the volumetric sulphate reduction rate of continuous sulphate reduction in a stirred tank reactor.

**A Kinetic Study on Anaerobic Sulphate
Reduction –
Effects of Sulphate and Temperature**

by

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B.Sc. Eng (Chem) M.Sc. Eng. (Chem)

Thesis presented for the degree of

Doctor of Philosophy

in the Department of Chemical Engineering

University of Cape Town

August 2000

Dedication

To my Mother and Father

Without whom none of this would be possible or matter

University of Cape Town

SUMMARY

High sulphate wastewaters originate from various industrial activities. These include the manufacture of pulp and paper, mining and minerals processing, explosive, fertilizer and other petro-chemical industries. An important example of such high sulphate containing wastewater in South Africa is acid mine drainage (AMD). AMD results from the uncontrolled microbial oxidation of sulphidic wastes and is a major problem in terrains affected by acidic streams. Traditionally AMD and other acid sulphate containing wastes are treated by passive methods or lime neutralisation. During lime neutralisation the acidity of waste streams is decreased, with subsequent precipitation of heavy metals as hydroxides. The passive treatment is based on biological and physicochemical processes such as oxidation, reduction, adsorption and precipitation. Passive treatment usually takes place in large reed beds and as such the treatment process cannot be controlled (Kuyucak and St Germain, 1994). Disposal of the sludge or reed mat at the end of the process requires further consideration.

Acid mine drainage and process effluents containing sulphate and heavy metals are amenable to anaerobic digestion with the concomitant removal of the metal pollutants as metal sulphides. The controlled biological treatment of AMD and other acidic sulphate containing wastes, compared with physicochemical

processes and passive treatment techniques offers several advantages. These include the production of clean water and the permanent removal of sulphur and metals. The anaerobic conversion of long chain organic substrates to acetate, carbon dioxide and hydrogen by acidogenic bacteria and microbial reduction of sulphate to sulphide are two major steps in the treatment of sulphate containing wastewaters (Middleton and Lawrence, 1977). Under anaerobic conditions sulphate is used as a terminal electron acceptor by sulphate reducing bacteria. The sulphate reducing bacteria couple the oxidation of the organic substrate to the reduction of sulphate to produce energy for growth and maintenance. The anaerobic reduction of sulphate has been used as a treatment option for a variety of industrial effluents containing sulphate. The simultaneous removal of heavy metals and sulphate from groundwater, treatment of wastewater containing sulphuric acid and mining wastewaters are examples of such processes (Maree and Strydom, 1985; Maree, 1987; Barnes *et al.*, 1992a, b; Stucki *et al.*, 1993; du Preez and Maree, 1994).

Anaerobic sulphate reduction is a complex reaction and as such is influenced by a variety of parameters. These include the availability of electron donor and its structure, pH, temperature, sulphate concentration, as well as the inhibitory effects of heavy metals and sulphide. Against this background the objectives for this study were formulated.

The objectives of this work were to obtain rigorous data to ascertain the effect of physical parameters on the kinetics of anaerobic sulphate reduction. In particular, consideration was given to the effect of feed sulphate concentration and temperature. Furthermore, a kinetic model was developed to describe the effect of feed sulphate concentration and temperature on the sulphate reduction rate of a continuous system.

The above objectives were addressed by:

- i) performing batch experiments at pH ranges of 6.0 to 9.0; temperature ranges of 20 to 35°C and sulphate concentration range of 1.0 to 5.0 kgm⁻³ to ascertain the optimum pH, sulphate and temperature ranges for anaerobic sulphate reduction,
- ii) performing continuous experiments at feed sulphate concentrations of 1.0, 2.5, 5.0, 10.0 and

- 15.0 kgm⁻³ when the temperature and pH were 35°C and 7.8 respectively, to assess the influence of feed sulphate concentration,
- iii) performing continuous experiments at temperatures of 20, 25, 30 and 35°C with a feed sulphate concentration of 5.0 kgm⁻³ and a pH of 7.8, to determine the effect of temperature, and
 - iv) to use the 'stable' state data obtained to develop a kinetic model to describe the rate of sulphate reduction as a function of feed sulphate concentration, temperature, bacterial concentration and residual substrate concentration.

The effect of three physical parameters pH, temperature and initial sulphate concentration were investigated in batch studies using a mixed anaerobic population comprising sulphate reducers, methane producers and acid producers. The results confirmed the importance of reaction pH, temperature and feed sulphate concentration on the sulphate removal and glucose utilising capacity of the microbial culture. According to the results of the batch studies, the environmental conditions for optimum sulphate removal are pH 8.0, temperature 35°C and a feed sulphate concentration in the range 1.0 to 2.5 kgm⁻³. The acid producing bacteria, metabolising the glucose to form the carbon source for the sulphate reducers and methane producers from glucose showed a pH optimum of 7.5 to 8.0, a temperature optimum of 25 to 35°C and sulphate inhibition at 5.0 kgm⁻³.

Continuous one litre bioreactors were operated at five sulphate concentrations viz. 1.0, 2.5, 5.0, 10.0 and 15.0 kgm⁻³ with a mixed microbial population enriched for sulphate reducers, using acetate as the organic source. The kinetics of the anaerobic sulphate reduction process was determined at steady state conditions. The results clearly indicated that initial sulphate concentration influenced the kinetics of the biological sulphate reduction process. As the feed sulphate concentration was increased in the range 1.0 to 10.0 kgm⁻³ an enhancement of the volumetric sulphate reduction rate was observed. When the initial sulphate was further increased to 15.0 kgm⁻³ the volumetric sulphate reduction rate and the sulphate conversion decreased. The enhanced volumetric sulphate reduction rates observed with increasing feed sulphate concentration in the range 1.0 to 10.0 kgm⁻³ was a result of the increase in bacterial concentration observed as the feed sulphate concentration was increased from 1.0 to 10.0 kgm⁻³. At feed sulphate

concentration of 15.0 kgm^{-3} the conversion of sulphate and volumetric sulphate reduction rate decreased indicating that the biological reduction of sulphate to sulphide was inhibited. The bioreactor pH was maintained at 7.8 at which sulphide speciation is biased towards the non-toxic HS^- species. Consequently sulphide inhibition was not implicated for the inhibition of microbial sulphate reduction for the bioreactor receiving 15.0 kgm^{-3} sulphate in the feed. The maximum sodium concentration for these experiments was 7.13 kgm^{-3} corresponding to a feed sulphate concentration of 15.0 kgm^{-3} . Sodium concentrations between 15.0 and 17.5 kgm^{-3} have been reported as being inhibitory to anaerobic digestion (Soto *et al.*, 1993, Visser, 1995; Lens *et al.*, 1998). Subsequently, it was concluded that sodium was not inhibiting the anaerobic reduction of sulphate at an initial concentration of 15.0 kgm^{-3} . It is therefore hypothesised that at a feed sulphate concentration of 15.0 kgm^{-3} , the sulphate had an inhibitory effect on the kinetics of the sulphate conversion process. Substrate inhibition has been observed by Nemati and Webb (1996) studying the biological oxidation of ferrous iron in a packed bed reactor. They too, noted a decrease in the conversion and the reduction rate of ferrous iron as the ferrous iron concentration was increased from 5.0 to 20.0 kgm^{-3} .

Continuous bioreactors with a microbial culture enriched for sulphate reducers were operated at four temperatures (20 , 25 , 30 and 35°C). The steady state data was used to ascertain the effect of temperature on the activity of the sulphate reducers. The results showed that increasing temperature in the range 20 to 35°C resulted in an increase in the sulphate conversion and the volumetric rate of sulphate reduction. A similar trend of increasing volumetric sulphate reduction rate with temperature was observed by Middleton and Lawrence (1977) when studying the temperature effect on batch microbial growth kinetics of a mixed sulphate reducing culture at temperatures of 20 , 25 and 31°C . Furthermore, Barnes *et al.* (1992a) observed a similar response to increased temperature in the range 22 to 31°C in terms of volumetric sulphate reduction rate with increasing volumetric sulphate loading rate to that observed in this study. The trends presented in this thesis in terms of residual sulphate concentration, sulphate conversion, residual acetate concentration, volumetric reduction rate and bacterial concentration were similar for all four temperatures considered. The enhancement of the rate and conversion of sulphate observed with temperature was attributed to the enzymatic nature of the reduction reaction that is enhanced with

increasing temperature in the range 20 to 35°C and to the decrease in unionised hydrogen sulphide concentration with increasing temperature.

Initial sulphate concentration and temperature had a significant effect on the kinetics of anaerobic sulphate reduction and microbial growth. The effect of feed sulphate concentration and reaction temperature on the bacterial growth rate was modelled using a modified Contois model. The maximum specific growth rate remained constant at 0.061 h⁻¹ across the temperature (20 to 35°C) and initial sulphate concentration (1.0 to 10.0 kgm⁻³). The decay coefficient, k_d , was a function of temperature described by the function:

$$k_d = 8.8 \times 10^{11} e^{-78.7/RT}$$

The saturation constant, K_s , displayed a linear increase with increasing inlet sulphate concentration and an Arrhenius decrease with increasing temperature. This is represented by:

$$K_s = 6.52 \times 10^{-36} e^{198/RT} [S_0]$$

Using experimental data a model for the rate of sulphate reduction as a function of inlet sulphate concentration, temperature, bacterial concentration and residual sulphate concentration was developed.

The derived model is:

$$r_s = \left(\frac{0.061[S]}{6.52 \times 10^{-35} e^{198/RT} [S_0][X] + [S]} - 8.8 \times 10^{11} e^{-78.7/RT} \right) \frac{[X]}{0.568}$$

The model was shown to represent the data to within the defined experimental error of 20 and 24 %.

Of the numerous parameters that affect the reduction rate of sulphate in an anaerobic sulphate reducing system (pH, sulphate concentration, temperature and sulphide concentration), feed sulphate concentration and temperature are accounted for in the model developed in this thesis. The model can be extended using the methodology employed for this study to include the effects of pH, sulphide inhibition and sulphate inhibition. The results obtained to date suggest that the model has potential for predicting the reduction rate of sulphate in continuous stirred bioreactors reducing sulphate, and hence finding use as the starting point to a model that will ultimately assist in the design of full scale sulphate reducing reactors.

ACKNOWLEDGEMENTS

There have been times while doing this PhD that I could not see the 'wood for the trees'. Those were not such happy times. However, now that the end is in sight they seem nothing compared to the happy and memorable times that I have had both at work and at home. If I look at it holistically, the past few years have been enjoyable and a learning experience, both educationally and personally. Now is my chance to thank all the wonderful people who have contributed in many ways to make this so. They are too numerous to mention by name, to all of you THANK YOU!

There are some people that I feel deserve a special thank you for putting up with me.

Associate Professor Sue Harrison for being brave enough to supervise me. This project was a sharp learning curve for me and I am most thankful to Sue for never judging and allowing me to make my own mistakes and learn from them. She has kept me motivated by her interest in the work and I have learnt tremendously from her, relating to many aspects of life, Thanks Sue.

Dr Mehdi Nemati for tirelessly assisting me with this work. For all the advice, both academic and personal and for always listening to me, even at times when I did not make sense. These few lines cannot do justice to all that you have done.

Professor Geoff Hansford and Dr Alison Lewis for never ceasing to offer assistance, motivation and encouragement.

Sue Jobson for her warmth and encouragement that illuminated the dark moments which have cropped up all too often over the past few years. Many thanks to you Sue.

For being such great people and for always being nice to me, Pauline Bettison, Meg Winter, Zedre Hartman, Sue Buerger, Debbie Collings, Dee Bradshaw and Jill Stevenson.

For being a friend and for always sorting out all my hassles with the computers and printers a thank you to Craig Balfour. Also to Bill Randall for his assistance with problems of an electronic nature.

For all the photocopying, binding and always smiling, thanks Nellie.

On the experimental side of the work I have to say a big thank you to workshop guys, Joachim Macke, Peter Dobias and David Bramble. Not to mention all the help I received from Granville de la Cruz and Martin Williams.

For being a dear friend and also for proof reading my thesis Brett Cohen. Thanks, I know that it was not an easy task, on both accounts.

I will always remember the warmth, encouragement and friendship that I got from Mary Stewart, Andrew Dustan, Jochen Petersen, Pippa Notten, Ashli Breed and Sashnee Raja. They made me believe that it could be done! Also to Jo-Ann and Gillian Moon, Andrew Robinson, Kriveshini Pillay, Clive Erasmus and Bronwen Butler a huge hug for your support and friendship.

On a personal side I do not know how to thank all the superb people in my life for putting up with me over the last few years. Firstly, my family definitely deserve a medal, in particular: My Mummy and Daddy for instilling in me the desire to learn and believing in myself never mind what the odds. Bhai, Bhen, Faizel and Fazila for being such great brother's and sisters and allowing me to be a bit 'odd' without judgement. To my nieces and nephews (all eight of you) Zaheera, Ahmed Riaz, Tariq, Faheem, Adil, Shehaam, Michael and the latest addition Zahrah you have all kept my spirit going through some trying times... ..Thanks. To my sisters in law, Fatima and Rahima and my brothers in law Ebrahim Bhai and Ni'amatullah for always being there.

A special thanks to Andrea McDonald and Hani du Toit for their special friendship and warmth.

Most of all I want to thank Allie Ebrahim. I really cannot put into words all that you have done for me. Thanks for being with me through the best and worst of times, for the practical day to day things such as providing food when required, for the emotional support, encouragement and inspiration that you have given me.

I would like to acknowledge the financial support received from Mintek.

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NOMENCLATURE

ABBREVIATIONS

ADP	Adenosine diphosphate
APB	Acid producing bacteria
APS	Adenosine phosphosulphate
ATP	Adenosine triphosphate
B	Batch
C ₃	Tetraheme cytochrome C ₃
CL	Carbon limited
CoA	Coenzyme A
CR	Continuous reactor
EGSB	Expanded granular bed reactor
F _d	Ferredoxin
Hase	Hydrogenase
HPLC	High pressure liquid chromatograph
MPB	Methanogens
NAD ⁺	Reduced nicotinamide-adenine-dinucleotide
NADH	Nicotinamide-adenine-dinucleotide
NADPH	Nicotinamide-adenine-dinucleotide-phosphate
PAPS	Phospho-adenosine-phosphosulphate
PBR	Packed bed reactor
PP	Pyrophosphate
RSB	Raked sludge bed reactor
SBR	Sludge bed reactor

SCFA	Short chain fatty acids
SF	Shake flask
SL	Sulphate limited
SRB	Sulphate reducing bacteria
STR	Stirred tank reactor
UASB	Upflow anaerobic sludge blanket
UPB	Upflow packed bed reactor

ROMAN SYMBOLS

e-	Electron	
E_a	Pseudo-activation energy	kJmol^{-1}
F	Feed flowrate	m^3h^{-1}
$f(\text{H}_2\text{S})$	hydrogen sulphide inhibition function	
$f(\text{pH})$	pH inhibition function	
[I]	Concentration of the inhibitory compound	kgm^{-3}
k_d	decay rate	h^{-1}
K_2	concentration of inhibitor at which the growth rate is twice decreased	kgm^{-3}
K_{100}	concentration of inhibitor at which the growth rate is decreased 100 times	kgm^{-3}
K_I	Half saturation constant of the inhibitory compound	kgm^{-3}
$K_{I,\text{acetate}}$	Half saturation constant for acetate as the inhibitory compound	kgm^{-3}
K_o	Constant to describe the Arrhenius dependency of the half saturation constant	
K_s	Half saturation constant	kgm^{-3}
K_s'	Apparent half saturation constant	kgm^{-3}
K_{s0}	Half saturation constant as described in the kinetic model to describe bacterial growth at high substrate concentrations	kgm^{-3}
K_{s1}	Half saturation constant of the first limiting substrate	kgm^{-3}
K_{s2}	Half saturation constant of the second limiting substrate	kgm^{-3}
$(K_s)_1$	Half saturation constant at temperature one	kgm^{-3}
$(K_s)_2$	Half saturation constant at second temperature	kgm^{-3}
K_{sn}	Half saturation constant of the n^{th} microbial groups	kgm^{-3}
K_{sp}	Solubility product	
m_s	maintenance energy	h^{-1}
[P]	Product concentration	kgm^{-3}
r_p	Product formation rate	$\text{kgm}^{-3}\text{h}^{-1}$
r_s	Volumetric sulphate reduction rate	$\text{kgm}^{-3}\text{h}^{-1}$

$[\bar{r}_s]$	Average volumetric sulphate reduction rate	$\text{kgm}^{-3}\text{h}^{-1}$
r_x	Cell growth rate	h^{-1}
$[S]$	Substrate concentration	kgm^{-3}
$[S_0]$	Feed substrate concentration	kgm^{-3}
$[S_1]$	Concentration of first limiting substrate	kgm^{-3}
$[S_2]$	Concentration of second limiting substrate	kgm^{-3}
T	Absolute temperature	K
T_1	Temperature one	K
T_2	Temperature two	K
V	Working volume	m^3
W_n	Weighing function	
$[X]$	Bacterial concentration	gL^{-1}
$[\bar{X}]$	Average bacterial concentration	gL^{-1}
$[X_0]$	Initial bacterial concentration	gL^{-1}
$[X_{\text{max}}]$	Maximum bacterial concentration	gL^{-1}
$Y_{x/s}$	Yield coefficient of bacteria X based on substrate S	g/g
$Y_{x/s(\text{obs})}$	Observed yield coefficient of bacteria X based on substrate S	g/g
$[\bar{Y}_{x/s}]$	Average yield coefficient of bacteria X based on substrate S	g/g

GREEK SYMBOLS

β	Constant found in the Webb model for the microbial growth rate	
$\Delta G^{\circ'}$	Gibbs free energy of formation	kJmol^{-1}
δ	Error	
μ	Specific microbial growth rate	h^{-1}
$\bar{\mu}$	Average specific microbial growth rate	h^{-1}
μ_m	Maximum specific microbial growth rate	h^{-1}

GLOSSARY

Acetyl coenzyme A	A combination of acetic acid and coenzyme A that is energy rich; it is produced by many catabolic pathways and is the substrate for the tricarboxylic acid cycle, fatty acid synthesis and other pathways.
Acidophile	A microorganism that has its growth optimum between about pH 1.0 and 5.5.
Active site	The part of an enzyme that binds the substrate to form an enzyme-substrate complex and catalyse the reaction.
Adenine	A purine derivative, 6-aminopurine, found in nucleosides, nucleotides, coenzymes and nucleic acids.
Adenosine-diphosphate	The nucleoside diphosphate usually formed upon breakdown of ATP when it provides energy for work.
Adenosine-triphosphate	The triphosphate of the nucleoside adenosine, which is a high energy molecule and serves as the cells major form of energy.
Autotroph	An organism that uses carbon dioxide as its sole or principal source of carbon.

Bacillus	A rod shaped bacterium.
Batch culture	A culture of microorganisms produced by inoculating a closed culture vessel containing a single batch of medium.
Chemical Oxygen Demand (COD)	The amount of chemical oxidation required to convert organic matter in water and wastewater to carbon dioxide.
Chemostat	A continuous culture apparatus that feeds medium into the culture vessel at the same rate as medium containing microorganisms is removed.
Citric acid cycle	The cycle that oxidizes acetyl coenzyme A to carbon dioxide and generates NADH and FADH ₂ for oxidation in the electron transport chain.
Coccus	A roughly spherical bacterial cell.
Competitive inhibitor	A molecule that inhibits enzyme activity by competing with the substrate at the enzyme's active site.
Endogenous respiration	The use of nutrients, usually internally stored reserves, to maintain a an organism without growth.
Fatty acid	A monocarboxylic acid that may be unbranched or branched, and saturated or unsaturated; the fatty acids most common in lipids are 16 or 18 carbons long.
Heterotroph	An organism that uses reduced, preformed organic molecules as its principal carbon source.
Mesophile	A microorganism with a growth optimum around 20 to 45°C.
Nicotinamide adenine dinucleotide	An electron carrying coenzyme that most often participates as an electron carrier in biosynthetic metabolism.

Non competitive inhibitor	Inhibition of enzyme activity that results from the inhibitor binding at a site other than the active site and altering the enzyme's shape to make it less active.
Oxidative phosphorylation	The synthesis of ATP from ADP using energy made available during electron transport.
Planktonic	Free-floating, mostly microscopic microorganisms that can be found in almost all waters, a collective name.
Thermophile	A microorganism that can grow at temperatures of 55°C or higher, the minimum is usually around 45°C.

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

High sulphate wastewaters, defined as aqueous streams having a sulphate content greater than 500 mg l^{-1} (Department of Water Affairs and Forestry, 1999), originate from various industrial activities. These include the manufacture of pulp and paper, mining and minerals processing, explosive, fertilizer and other petro-chemical industries. Table 1.1 provides typical chemical compositions in terms of pH, sulphate and metal concentrations for sulphate containing streams from five South African industries, viz., mining, minerals processing, petrochemical industry, gelatine manufacturing and a yeast factory. The mining, minerals processing and petrochemical industry effluent data are confidential and consequently no location can be associated with them. The gelatine manufacturing factory and yeast factory are situated in Krugersdorp, South Africa. Table 1.2 presents the composition of seven acid mine drainage (AMD) streams, four in South Africa, one in the USA, one in Ireland and one in Norway.

Table 1.1. Chemical composition of aqueous effluents from various industries in South Africa.

	Mining ¹	Gelatin manufacturing ²	Yeast factory ³	Minerals processing ⁴	Petrochemical industry ⁵
pH	5.95	9.77	5.74	7.0	-
Total dissolved solids	3788	5100	111100	-	-
Sulphate	1831	747	18000	2480	3000
Chloride	250	717	-	315	7600
Fluoride	1.7	-	-	-	-
Sodium	258	-	-	285	3900
Potassium	18.2	-	-	-	-
Iron	210	-	-	1.5	80
Nickel	-	-	-	6.7	10
Calcium	980	160	-	-	280
Manganese	7	-	-	-	5
Magnesium	720	-	-	-	21

All units in mgL⁻¹ except pH.

¹Personal communication (SRK Consulting Engineers, 1995)

²Lloyd *et al.* (1999)

³Lloyd *et al.* (1999)

⁴Personal communication (SRK, Consulting Engineers, 1995)

⁵Ecoliban group (1999)

Table 1.2. Composition of AMD streams, four from mines in South Africa, one in the USA, one in Ireland and one in Norway.

	South Africa ¹				USA ²	Ireland ³	Norway ⁴
	Grootvlei Gold	West Rand Gold	Klipspruit Coal	Brugspruit Coal	Anaconda Copper	Avoca Copper /Zinc	Wallenburg Copper
pH	6.3	2.4	2.6	2.2	2.5	2.7	5.5
TDS	4200	38448	11512	4490	-	-	-
Sulphate	2200	22556	8122	3947	3510	10579	2940
Sodium	291	77	1893	129	72	-	-
Potassium	12.9	-	-	-	22	-	-
Iron	187	6674	-	-	300	1031	139
Zinc	-	12	-	-	155	362	34
Copper	-	2	-	-	29	243	2
Nickel	3	13	-	-	-	-	-
Aluminum	-	-	-	-	125	-	1
Manganese	-	5	-	-	88	-	350

All units in mgL⁻¹ except pH

¹Data obtained from Department of Mineral and Energy Affairs (1995)

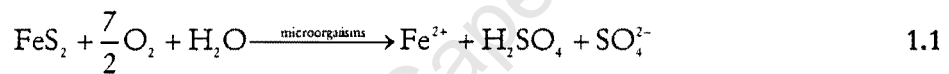
²Data obtained from Jenke and Deibold (1983)

³Data obtained from Gray (1996, 1997)

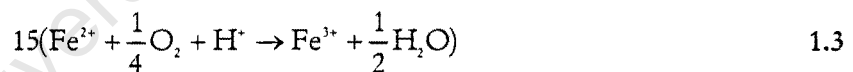
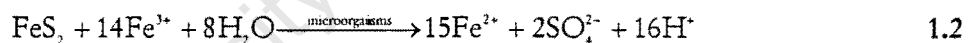
⁴Data obtained from Christensen *et al.* (1996)

An important example of such high sulphate wastewater in South Africa is acid mine drainage (AMD). Acid mine drainage (AMD) is an acidic stream containing sulphate and metal ions and typically occurs as runoff and seepage from waste rock stockpiles and tailings or coal rejects. It may also be discharged from underground workings via shafts, or seep from open pit wells where groundwater is intercepted (Johnson, 2000). Problems associated with AMD include; acidity, high metal content and variable sulphate content. Generation of AMD is predominantly the result of oxidation of the sulphide minerals present in the terrain in which the drainage flows with concomitant leaching of metals. This is a natural process resulting from the exposure of the ores to atmospheric conditions coupled with bacterial activity. An example is the oxidation of pyrite, which is accelerated by the presence of microorganisms such as *Thiobacillus ferrooxidans*, *Thiobacillus thiooxidans*, *Thiobacillus caldus* and *Leptospirillum ferrooxidans* (Garcia *et al.*, 1996). The oxidation of sulphide minerals is illustrated by the following reactions describing pyrite oxidation:

Direct mechanism (attached):



Indirect Mechanisms (spatially removed):



The first reaction is usually considered as a direct mechanism (or attached), while Eqns 1.2 and 1.3 represent the indirect mechanism (or spatially removed) for sulphide mineral oxidation (Gray, 1997; Johnson, 2000).

The formation of AMD has the potential, at least in the South Africa context, to have a significant impact (Thompson, 1980). Two factors contribute to this: South Africa is a semi-arid country in which water resources are particularly valuable. South Africa has a large mineral processing industry. As a consequence a large number of mines are present and working, generating dumps, while further AMD generation from abandoned mine sites require treatment and the planned closure of exhausted mines needs to be achieved.

1.2 IMPACTS OF SULPHATE CONTAINING EFFLUENTS

A typical feature of the sulphate effluent streams is the high and variable load of sulphate and in the case of AMD the low pH and variable metal content. High sulphate wastewater streams discharged into the environment have a number of associated environmental and social penalties. Sulphate contributes to the total dissolved solids (TDS) of water, affecting its usefulness downstream (e.g. as drinking water, irrigation water or industrial water). In the environment sulphate may be biologically reduced, resulting in the formation of hydrogen sulphide with concomitant odour and safety problems. Furthermore hydrogen sulphide corrodes stainless steel and concrete piping (Heitz *et al.*, 1996; Hvitved-Jacobsen and Nielsen, 2000). If the pH of the sulphate containing stream is low, its discharge can result in the complete sterilization of the receiving waters ultimately resulting in permanent ecological damage. With AMD, a particular problem is its high heavy metals content. The low pH of AMD augments leaching of metals from the surrounding rock. Iron, usually present in significant quantities can further mediate leaching in its oxidised state while the presence of other heavy metals is dependent on the nature of the ore.

Very often it is desirable to reuse industrial water streams to reduce water consumption and effluent output. Comparison of the sulphate containing effluents with the industrial water standards for South Africa (Table 1.3) highlights the problem associated with the sulphate containing wastewater. The sulphate content of industrial waste streams range between 700 and 18000 mgL⁻¹ making them unsuitable for industrial use without prior treatment to bring the sulphate levels to below 500 mgL⁻¹. Sulphate containing wastewaters cannot be readily reused due to scaling, biocorrosion and weakening of concrete structures due to calcium leaching.

Table 1.3. Industrial effluent standards for reuse of water for South Africa^a (Department of Water Affairs and Forestry, 1999) and the USA^b (Environmental Protection Agency, 2000).

Constituent	pH		Total dissolved solids		Sulphate		Chlorine		Zinc	
Concentration (mgL ⁻¹)	5.5 - 9.5 ^a	6.5 - 8.5 ^b	15 - 25 ^a	500 ^b	500 ^a	250 ^b	200 ^a	200 ^b	5 ^a	5 ^b

Copper, cadmium and manganese levels all below 1 mgL⁻¹. Where ^a is data for South Africa and ^b is data for the USA.

1.3 OVERVIEW OF EXISTING TREATMENT TECHNOLOGIES

There are various methods for the treatment of acidic streams with a high sulphate content. These range from mature technologies for which a number of full scale plants exist to emerging technologies which may only have been tested on a laboratory or pilot plant scale. The following subprocesses contribute towards the effective technology: oxidation and hydrolysis (Barnes *et al.*, 1992a), adsorption (Gould and Genetelli, 1978; Callander and Barford, 1983; Alibhai *et al.*, 1984; Ginter, 1993), absorption (Ginter, 1993), reduction (Brierley, 1991; Kalin *et al.*, 1991; Lyew and Sheppard, 1997) and neutralisation (Ferguson *et al.*, 1984). Table 1.4 details the bases of these subprocesses.

Table 1.4. The various subprocesses that contribute to active and passive treatment processes and their bases.

Subprocess	Bases
oxidation and hydrolysis	The removal of metals as precipitates. oxidation: $4 \text{Fe}^{2+} + \text{O}_2 + 4 \text{H}^+ \rightarrow 4 \text{Fe}^{3+} + 2 \text{H}_2\text{O}$ hydrolysis: $\text{Fe}^{3+} + 3 \text{H}_2\text{O} \rightarrow \text{Fe}(\text{OH})_{3(\text{solid})} + 3 \text{H}^+$
adsorption	The metals adsorb to microbial surfaces due to ionic attraction of metal cations to negatively charged outer surface of microbial cell.
absorption	The direct uptake of metals by microorganisms.
reduction	The bacterial reduction of sulphate to sulphide by sulphate reducing bacteria using an organic carbon, energy and electron source.
neutralisation	The precipitation of metals with neutralizing agents such as calcium hydroxide, calcium carbonate, magnesium hydroxide, sodium sulphide and calcium sulphide.

Treatment processes for the removal of sulphate and heavy metals can be broadly categorised into passive and active processes. Passive treatment methods provide an alternative to conventional neutralisation techniques with alkaline reagents such as lime and limestone. The passive treatment processes involve the utilisation of naturally occurring geochemical and biological systems to improve the quality of influent waters with minimal operation and maintenance costs (Huntsman *et al.*, 1978; Kalin *et al.*, 1991; Brierley, 1992; Ledin and Pederson, 1996; Cole, 1998). The pH is raised when the water is mixed with alkaline

water or through direct contact with carbonate rocks. The removal of metals occurs through precipitation (Kuyacak and St Germain, 1994; Gazea, *et al.*, 1996). Passive treatment processes have been shown to increase the pH of waste streams from 6.0 to values above 7.0 (Resource Development Consultants, 1998). Furthermore the iron content has been reported to decrease by more than 90 % and the sulphate content by 40 % (Kalin *et al.*, 1991). Because passive treatment is a slow process with low reduction rates the water is pumped into ponds and left for long periods of time before being released into local water systems. As such there is no control on the efficiency of the treatment. The passive treatment methods include the use of aerobic wetlands, compost wetlands, and anoxic limestone drains. Typically passive treatment methods require low energy input, limited reagent addition and minimal manpower and present low operation and maintenance costs. As a result passive treatment processes finds applicability when the rapid treatment of metal and sulphate containing effluent is not required. It is also ideal for the treatment of accumulated mining effluent present at abandoned mine sites. However, various secondary disadvantages may arise from passive treatment processes. These include large land requirements which may be expensive or unavailable, a potential long term build up of heavy metal sludge in the wetland, formation of hydrogen sulphide which if not collected results in an odour and safety problem, and disposal of the metal sludge.

Based on the same fundamental mechanisms as passive treatment methods, active treatment methods can be operated with improved efficiency and control. The active processes, however, require operator intervention to maintain the conditions at a predetermined set point (Cinnani *et al.*, 1996). A number of treatment methods classified as active are given in Table 1.4, along with the components they remove.

Table 1.5. Summary of active treatment processes employed to treat AMD (Barnes *et al.*, 1992a).

Active treatment process	Components removed
neutralisation	metals, trace amounts of sulphate
ion exchange	metals, sulphate
liquid membrane extraction	metals
reverse osmosis	metals, sulphate
solvent extraction	metals
biological sulphate reduction	metals, sulphate

Of the numerous active treatment processes, the biological sulphate reduction process offers good potential. While only a few full scale plants treating high sulphate streams exist (Herlihy and Mills, 1985; Barnes *et al.*, 1992a, b; 1994; Scheeren *et al.*; 1994; Colleran *et al.*, 1994; Paques Environmental Technology, 1999) numerous laboratory and pilot scale studies are reported (Du Preez and Maree., 1994; Colleran *et al.*, 1995; Visser, 1995). These studies focus primarily on the choice of carbon source and reactor configuration for a cost effective process. Limited information is available in the open literature on the kinetics of biological sulphate reduction using mixed sulphate reducing cultures in continuous systems.

Given the magnitude of sulphate effluents generated and the potential for anaerobic sulphate reduction as a treatment for acid mine drainage and other sulphate containing liquid effluents, it is desirable that rigorous kinetic data be available and models developed to ascertain the effect of various parameters on the kinetics of anaerobic sulphate reduction. In this thesis, a kinetic analysis of biological sulphate reduction is presented with particular consideration of feed sulphate concentration and reaction temperature.

1.4 THESIS OUTLINE

In Chapter 2, a comprehensive literature review of the biological sulphate reduction process is presented. Initially operational full-scale plants treating sulphate containing wastewaters are described to highlight the potential of anaerobic sulphate reduction to treat sulphate containing effluents. Thereafter the general anaerobic process is reviewed with particular attention being paid to the sulphate reduction process. This is followed by a discussion of the various sulphate waste streams treated and finally the interactions between sulphate reducers and methane producers are presented. The literature review serves to provide the background on which the experiments were based.

The objectives of this work and research strategy implemented are presented in Chapter 3. To achieve the objectives, one litre stirred tank reactors of standard geometry were used to study the effect of environmental conditions on a mixed anaerobic culture and to ascertain the kinetics of anaerobic sulphate

reduction as a function of feed sulphate concentration and reaction temperature. Details of the experimental apparatus, the experimental protocol and the analytical techniques used are detailed in **Chapter 4**.

Results of batch studies to ascertain the range of pH, temperature and sulphate concentration for optimum sulphate reduction by a mixed anaerobic culture with glucose as the organic carbon source and electron donor is presented in **Chapter 5**. In **Chapter 6** the results of continuous bioreactor experiments to ascertain the effect of feed sulphate concentration on the kinetics of the biological sulphate reduction are presented and discussed. An anaerobic culture enriched for sulphate reducers was used with acetate as the organic source and electron donor. The results of continuous bioreactor experiments to determine the effect of temperature on the kinetics of the anaerobic sulphate reduction process by a mixed sulphate reducing microbial culture using acetate as the organic source and electron donor are given in **Chapter 7**. A discussion of the results is also presented in this chapter.

In **Chapter 8** a kinetic model is developed to describe the rate of biological sulphate reduction as a function of feed sulphate concentration, residual sulphate concentration, biomass concentration and temperature. To place the modelling approach adopted into context a review of the literature on kinetic growth models used to describe the growth of microbial cultures in continuous systems is provided. This review is extended to include existing models used to describe anaerobic sulphate reduction. The approach adopted was to choose an appropriate model to describe the dependency of the microbial growth rate on residual sulphate concentration. Subsequently the microbial growth rate model was incorporated into a model used to describe the rate of sulphate reduction. Using the appropriate microbial growth rate model the microbial kinetic constants were calculated and compared with literature values. Furthermore, the dependency of the calculated microbial kinetic constants on feed sulphate concentration and temperature was determined and incorporated into the reaction rate model. In **Chapter 9**, the conclusions drawn from the study are presented and recommendations are made of areas in which further work may yield fruitful results on the kinetics of the anaerobic reduction of sulphate.

CHAPTER 2

REVIEW OF RELEVANT LITERATURE

The function of this section is to provide the reader with a basic overview of the theory and application of the main themes discussed in this thesis. This takes the form of highlighting the potential of biological sulphate reduction for the treatment of sulphate containing effluents by introducing the reader to the full scale plants presently in operation for the reduction of sulphate from liquid effluents. This then provides the background for the present work with particular attention being given to the anaerobic reduction of sulphate. The sulphate reduction process forms part of a broader anaerobic digestion process. Subsequently, the general anaerobic digestion process (including acidogenesis and methanogenesis) is outlined and the relevant literature regarding the sulphate reduction process is discussed. This is followed by a discussion of the various effluents treated by the anaerobic sulphate reduction process and finally the factors affecting the interactions between sulphate reducers and methane producers are presented.

2.1 THE POTENTIAL OF BIOLOGICAL SULPHATE REDUCTION FOR THE TREATMENT OF SULPHATE CONTAINING WASTEWATERS

The biological sulphate reduction process is applicable as both an active and passive treatment method and has been highlighted as having potential to treat sulphate containing acidic effluents cost effectively (De Walle *et al.*, 1979; Maree and Strydom, 1987; Barnes *et al.*, 1992a, b; Scheeren *et al.*, 1994; Colleran *et al.*, 1995; De Vegt and Buisman, 1995; Christensen *et al.*, 1996). As a passive treatment system the sulphate reduction process takes place in the anaerobic zone of a wetland. As an active treatment method the sulphate reduction process occurs in bioreactors. The sulphate reduction process has been used extensively, both commercial and pilot scale (Colleran *et al.*, 1994). However, the kinetics of the process with regards to various environmental parameters e.g. temperature, pH, feed sulphate concentration, are not fully understood. A few commercial scale sulphate reducing plants are outlined here to highlight the potential of the process as an emerging technology.

During the sulphate reduction process the sulphate reducing bacteria (SRBs) use organic compounds as a carbon and energy source for reducing sulphate to sulphide. Carbon dioxide and additional biomass is formed. When metals are present they precipitate as sulphides (Brüne *et al.* 1982; Bos, 1994;). A representation of the overall process is shown as Figure 2.1.

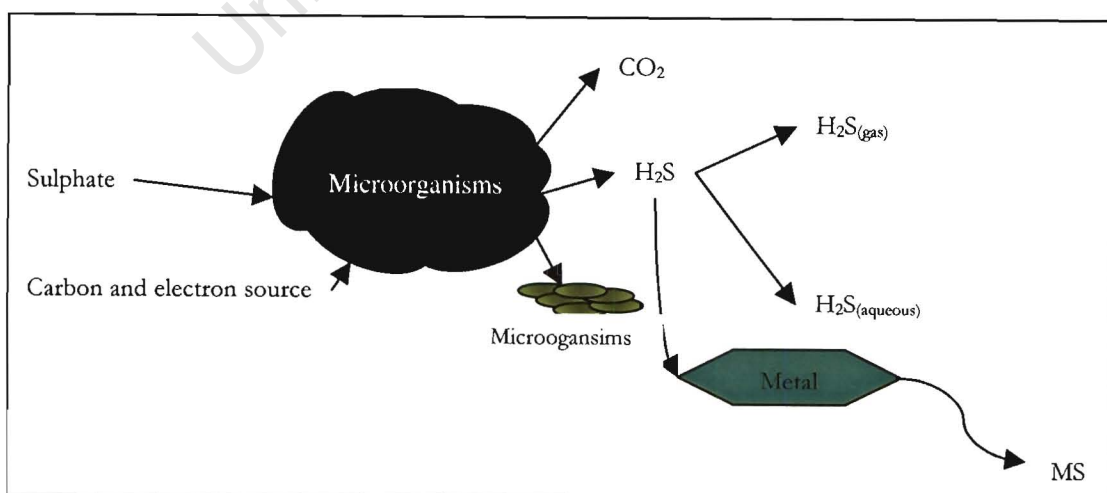


Figure 2.1. Representation of biological sulphate reduction with concomitant metal sulphide precipitation.

One of the most sophisticated sulphate reduction processes employed on a commercial scale is the BIOPAQ process patented by Paques, the Dutch company (Barnes, *et. al.*, 1992 a, b). It has been shown that the process is able to remove both the sulphate and metal content of the waste. A schematic diagram of the process is shown in Figure 2.2.

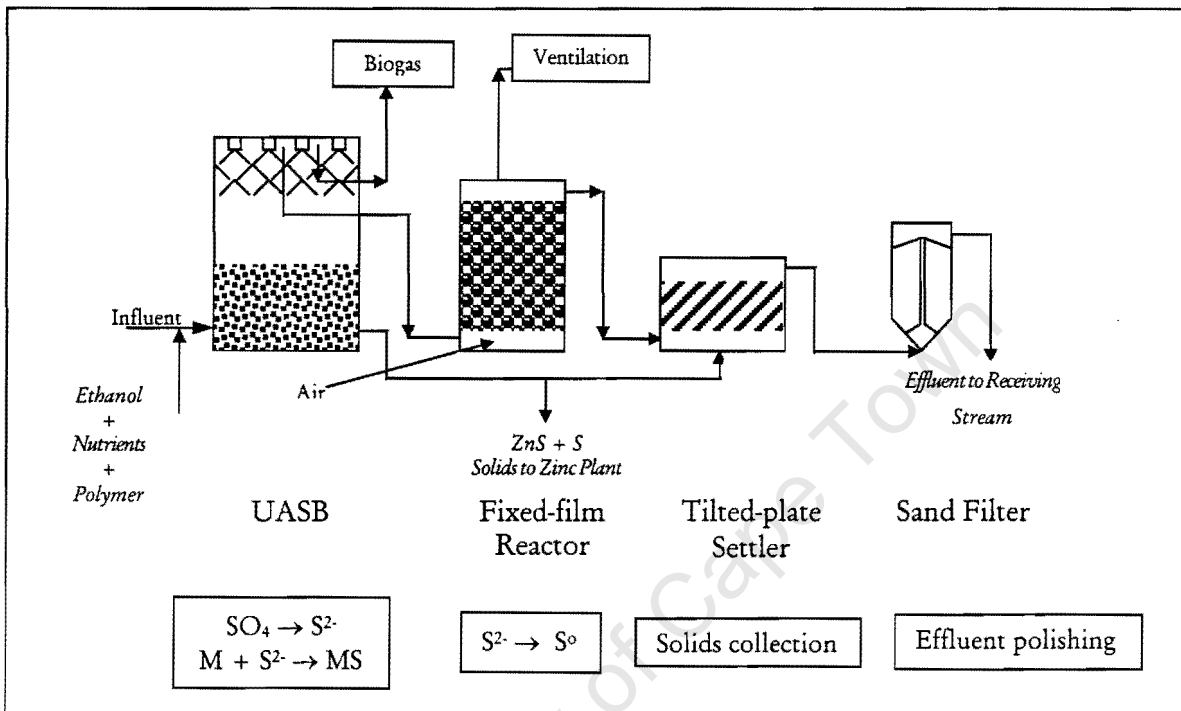


Figure 2.2. Schematic flow diagram of the BIOPAQ process at the Budelco zinc refinery in the Netherlands (Paques Environmental Technology, 1999).

The plant consists of four primary parts:

- an upflow anaerobic sludge blanket (UASB) for the reduction of sulphate to sulphide using ethanol as the organic source and electron donor,
- a submerged fixed film reactor (SFF) for the conversion of sulphide to sulphur,
- a tilted settler plate for the removal of solids, including any metal precipitates, and
- a sand bed filter as a final polishing step prior to discharge of purified water.

Typical water quality data before and after treatment by this process is shown in Table 2.1. The effluent has a relatively low sulphate concentration, which is removed to 83 % efficiency. Zinc, present at concentrations around 95 mgL^{-1} is the major metal present.

Table 2.1. Performance data for the BIOPAQ process at the Budelco zinc refinery in The Netherlands (Paques Environmental Technology, 1999).

Parameter	Value	
size of UASB (m^3)	1800	
residence time (h)	6	
Temperature ($^{\circ}\text{C}$)	35	
	Inlet	Outlet
ethanol (mgL^{-1})	370.0	1.0
sulphate (mgL^{-1})	1200.0	200.0
zinc (mgL^{-1})	95.0	N/D
cadmium (mgL^{-1})	0.6	N/D
copper (mgL^{-1})	0.3	N/D
iron (mgL^{-1})	15.0	N/D
pH	4.9	7.5

Based on the BIOPAQ process at the Budelco zinc refinery a similar plant is being operated in Kennecott's Bingham Canyon at the Utah copper mine. Here the process has been modified to contact the sulphide produced with the untreated waste stream prior to anaerobic reduction in order to precipitate metals as metal sulphide thereby eliminating metal toxicity effects. Furthermore, the electron donor and carbon source are hydrogen and carbon dioxide respectively. A schematic diagram of this process is shown in Figure 2.3. Performance data of the influent and effluent from the Paques plant at Kennecott Utah copper mine is shown in Table 2.2. The high sulphate concentration of the effluent is decreased in the precipitation reactor by dilution with the nutrients and the carbonate and sulphide recycle. The high metal concentrations are removed prior to entering the anaerobic reactors through precipitation with recycled sulphide as metal sulphides to prevent toxic effects.

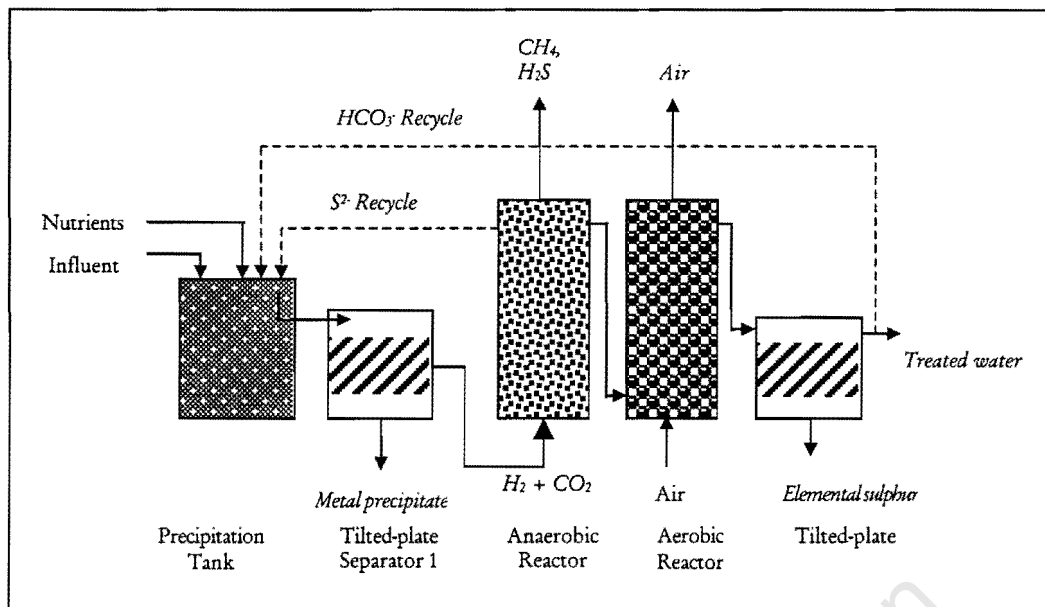


Figure 2.3. Schematic flow diagram of the BIOPAQ process at the Kennecott Utah copper mine (Paques Environmental Systems, 1999).

Table 2.2. Performance data for the BIOPAQ process at Kennecott Utah copper mine (Paques Environmental Systems, 1999).

Parameter	Value	
flowrate (m^3hr^{-1})	0.2	
	Influent	Effluent
pH	3.9	8.5
sulphate (mgL^{-1})	30000	< 500
aluminum (mgL^{-1})	2050	< 2
calcium (mgL^{-1})	480	50
copper (mgL^{-1})	60	< 0.1
iron (mgL^{-1})	675	< 0.3
magnesium (mgL^{-1})	4500	1950
manganese (mgL^{-1})	330	0.3
zinc (mgL^{-1})	65	< 0.1

Pilot plants in operation in South Africa include the Rhodes Biosure Process in operation at the Grootvlei mine (Corbett, 2000). Typical performance data from the pilot plant treating effluent from the Grootvlei mine is shown in Table 2.3.

Table 2.3. Performance data from the Rhodes Biosure Process (Corbett, 2000).

Determinant	Influent (kgm ⁻³)	Effluent (kgm ⁻³)
pH	7.1	-
COD	3.4	1.06
sulphate	1.71	0.65
TDS	3.3	-

Of the active processes that have been mentioned in Section 1.3 the sulphate reduction process is an attractive option for treatment of sulphate containing wastes from an applicability perspective. A drawback of the anaerobic sulphate reduction process is the production of hydrogen sulphide. When there is not sufficient metal present to precipitate the hydrogen sulphide a certain proportion is released as H₂S_(gas). In such cases a further treatment process for treatment of the H₂S_(gas) is required (Buisman, 1996). The most commonly used method for the removal of H₂S_(gas) and carbon dioxide is treatment with an alkanolamine solution. Some of the commonly used amines include monoethanolamine (MEA), diethanolamine (DEA), diglycolamine (DGA) and methyldiethanolamine (MDEA). A review of these methods is presented by Jensen and Webb (1995). Biological oxidation for the treatment of hydrogen sulphide streams is receiving increasing attention (Jansen and Webb, 1995). A summary of their findings is presented as Tables 2.4 and 2.5. Table 2.4 summarises the physicochemical processes and Table 2.5 the biological processes.

Table 2.4. Review of physicochemical processes for treating sour gas (Jensen and Webb, 1995).

Category	Example	Reagents	Products
liquid phase chemical reaction	alkanolamine	amines	H ₂ S and CO ₂
	alkaline salt	potassium carbonate	H ₂ S and CO ₂
liquid phase physical adsorption	sulfinol selexol	sulfolane and diisopropanolamine	H ₂ S and CO ₂
		dimethyl ether of polyethylene glycol	H ₂ S and CO ₂
dry bed	iron sponge molecular sieve	iron oxide	elemental sulphur
		cryastalline alkali-metal aluminosilicates	elemental sulphur
direct conversion	stretford lo-Cat	sodium carbonate, sodium vanadate,	elemental sulphur
		anthrquinone, disulfonic acid Iron ion	elemental sulphur

Table 2.5. Review of efficiencies for biological processes for hydrogen sulphide removal (Jensen and Webb, 1995).

Microorganisms	H ₂ S removal efficiency (%)	Products
<i>Chlorobium thiosulfatophilum</i>	99.9	S, SO ₄ ²⁻
<i>Chlorobium thiosulfatophilum</i>	100	S
<i>Xanthomonas</i>		polysulphide
<i>Thiobacillus denitrificans</i>	91 - 97	SO ₄ ²⁻
<i>Thiobacillus thioparus</i>	95 - 99	SO ₄ ²⁻
<i>Thiobacillus ferrooxidans</i>	93 - 100	S

2.2 FUNDAMENTALS OF THE ANAEROBIC TREATMENT OF SULPHATE WASTEWATERS

The anaerobic sulphate reduction process is an attractive option for the treatment of sulphate containing effluents (Speece, 1996; Stams and Oude Elferink, 1997). The process as well as the microorganisms involved will be discussed in this section.

2.2.1 GENERAL DEGRADATION PROCESS

In anaerobic environments complex organic wastes are broken down into short chain fatty acids, hydrogen, methane and carbon dioxide by fermentative bacteria, acidogens, acetogens and methanogens (MPB) (McCarty, 1964; Gujer, 1983). When sulphate is present sulphate reducing bacteria (SRBs) occur and compete with the MPB for carbon and energy sources (Barton and Tomei, 1995). The following processes occur during the breakdown of insoluble organic compounds in the presence of sulphate:

- Breakdown of insoluble compounds by external hydrolytic enzymes secreted by the bacteria.
- Acidogenesis of sugars and amino acids to hydrogen, CO₂ and short chain fatty acids (SCFAs) such as acetic acid, propionic and lactic acids.
- Beta oxidation of long chain fatty acids to yield acetate, propionate and hydrogen.

- Acetogenesis of propionate and other SCFAs to acetate, hydrogen and carbon dioxide.
- Methanogenesis using hydrogen and acetate as substrates.
- Biological sulphate reduction utilizing hydrogen, acetate, propionate and other SCFAs as substrates.

A representation of the degradation of insoluble compounds in the presence of sulphate is shown as Figure 2.4. Depending on the nature of the organic substrate certain pathways may not be active. For purposes of this study the literature review will focus primarily on the sulphate reduction process. Additionally anaerobic processes directly resulting in substrates for the SRBs will be outlined. Furthermore, methanogenesis will be discussed since the MPB are in direct competition with the SRBs for substrates such as acetate, hydrogen and carbon dioxide.

The occurrence of sulphate reduction during the anaerobic process has been considered by many researchers as undesirable (Winfrey and Zeikus, 1977; Banat, 1981; Smith and Klug, 1981; Banat and Nedwell, 1983; Mulder, 1984; Isa *et al.*, 1986, 1991). There are two reasons for this:

- The SRBs utilise the organic compounds that would otherwise be used by the methanogens.
- SRBs produce sulphide. This is toxic to the methanogens and imposes negative impacts on the environment in which it is produced.

However, recently the utilisation of the sulphate reduction process to reduce the sulphate content of certain effluent streams has been recognised. The removal of sulphates from mine water, groundwater and industrial effluents have been studied extensively (Maree, 1987; Barnes *et al.*, 1992a, b; Dvorak *et al.*, 1992; Du Preez and Maree, 1994; Oude Elferink *et al.*, 1994; Visser, 1995; Colleran *et al.*, 1996; Genschow *et al.*, 1996; Dries *et al.*, 1998; Elliot *et al.*, 1998).

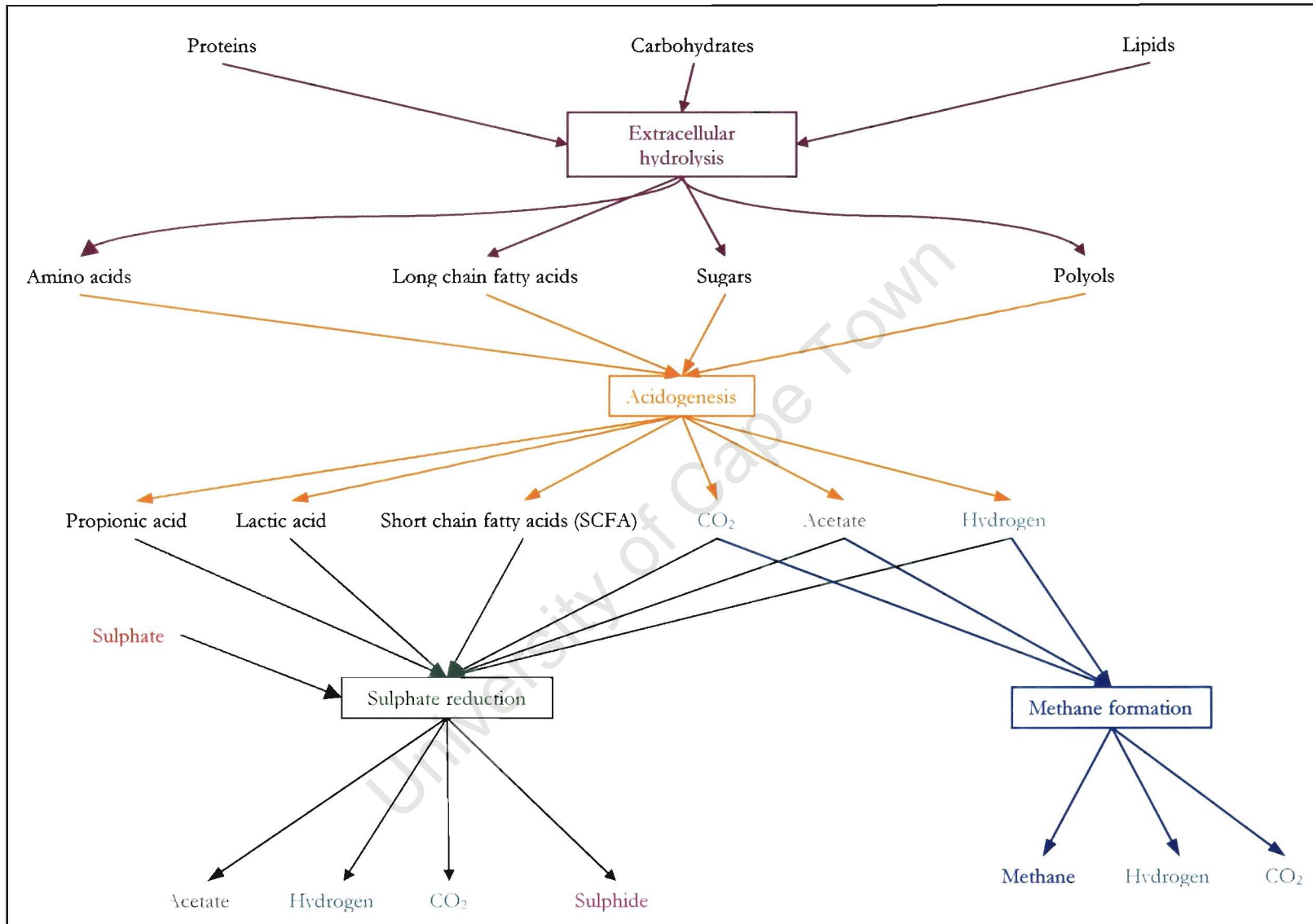


Figure 2.4. Representation of the general anaerobic digestion process. Sub processes include: hydrolysis, acidogenesis, sulphate reduction and methane formation.

The reduction of sulphate is part of the biological sulphur cycle shown in Figure 2.5 (Barton and Tomei, 1995). During the sulphur cycle, sulphate is reduced to sulphide by dissimilatory sulphate-reducing bacteria (*Desulfovibrio*, *Desulfotomaculum*, *Desulfobacter*, etc.) thereby providing substrates for sulphide oxidising bacteria (*Beggiatoa*, some thiobacilli, coloured sulphur bacteria etc.) These convert sulphide, via elemental sulphur, back to sulphate. In assimilatory sulphate reduction, the sulphate sulphur passes through the sulphide level of oxidation and becomes incorporated into amino acids (RSH) before being built into plant or microbial protein. These are consumed by animals and the sulphur is eventually returned to the cycle as sulphide formed during breakdown and putrefaction (anaerobic breakdown of proteins by bacteria) of dead organisms.

As shown in Figure 2.4 the degradation of insoluble organic compounds consists of several steps. These include hydrolysis, acidogenesis, methanogenesis and oxidation (Zehnder, 1988). The products formed during acidogenesis provide the carbon and energy source for the SRBs. Consequently in the presence of long chain organic compounds the acidogenic phase plays an important role in providing substrates for the anaerobic reduction of sulphate (Widdel, 1988). It should be mentioned that the hydrolysis step is important during the degradation of insoluble organic compounds (Eliosov and Argaman, 1995) but is not within the scope of this review and consequently will not be discussed. Methanogenic bacteria compete with SRBs for the carbon and electron source; in most cases this is acetate, hydrogen and carbon dioxide. This has direct consequences for the sulphate reduction process. In order to understand these interactions, acidogenesis, methanogenesis and sulphate reduction are reviewed in subsequent sections.

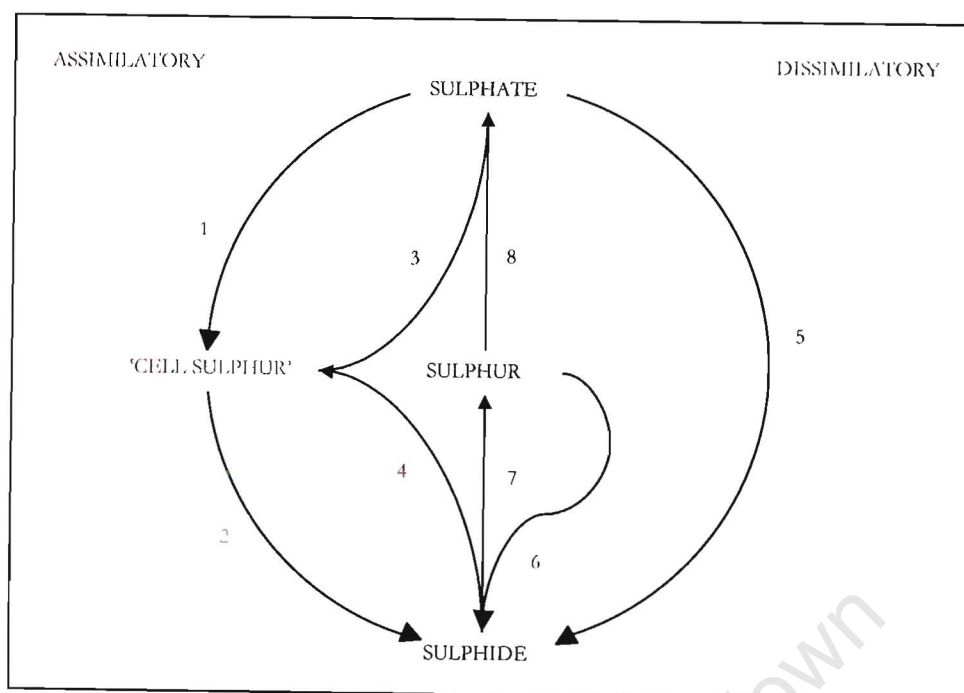


Figure 2.5. Biological sulphur cycle (Barton and Tomei, 1995).

The sulphur cycle consists of the following stages: (1) assimilatory sulphate reduction by bacteria, fungi, plants, (2) death and decomposition of bacteria and fungus, (3) sulphate excretion by animals, (4) sulphide assimilation by bacteria, (5) dissimilatory sulphate reduction, (6) dissimilatory elemental sulphur reduction, (7) sulphide oxidation (chemotrophic and phototrophic), (8) sulphur oxidation (chemotrophic and phototrophic).

2.2.2 ACIDOGENESIS

During acidogenesis acid producing bacteria use the soluble intermediates produced during the hydrolysis stage as the energy source for synthesis and growth, resulting in the formation of acetate, propionate, butyrate, hydrogen and carbon dioxide and cellular material (Eastman and Ferguson, 1981; Dinopoulou *et al*, 1988; Speece, 1983; 1996). A mechanistic representation of the acidogenic stage is shown as Figure 2.6.

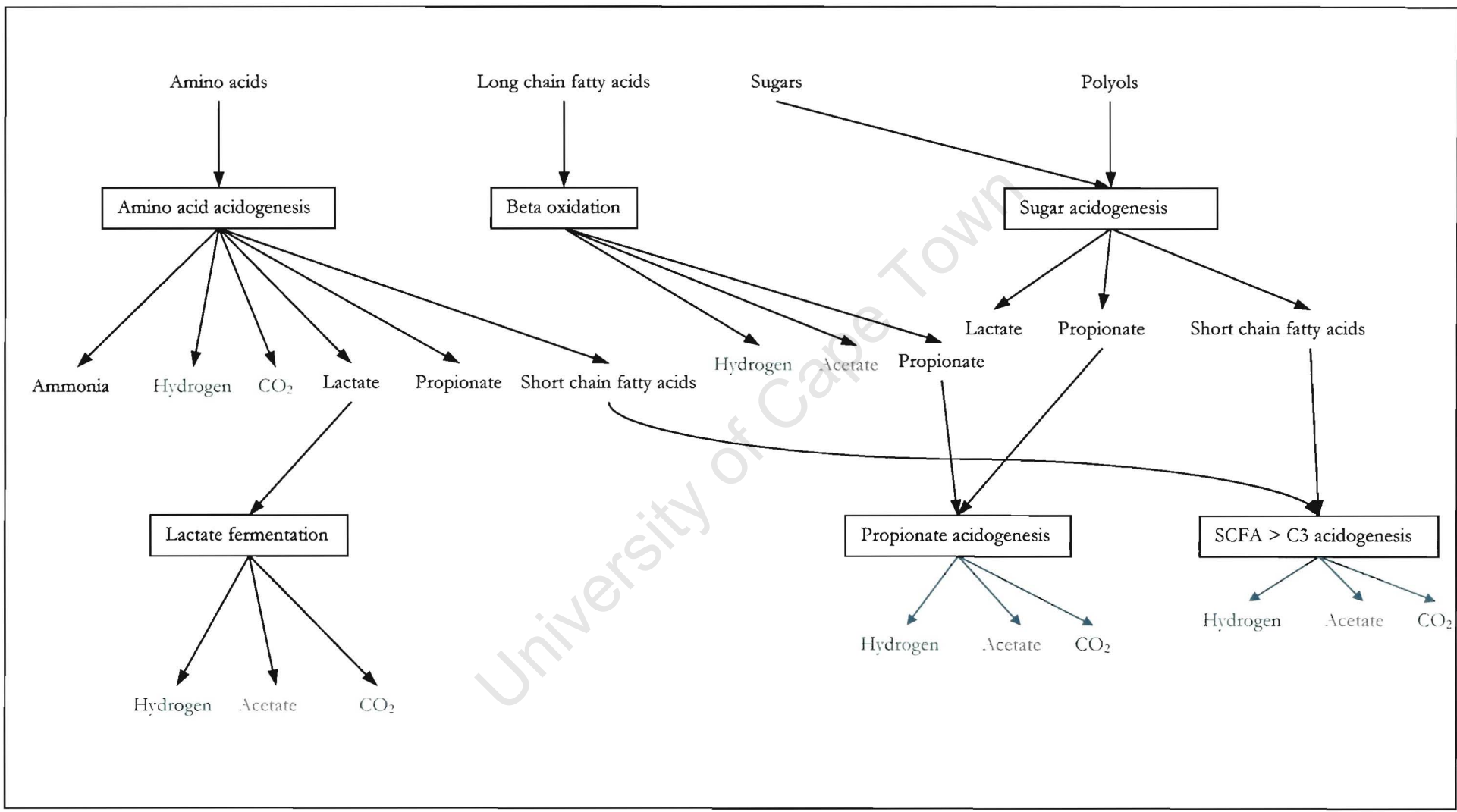
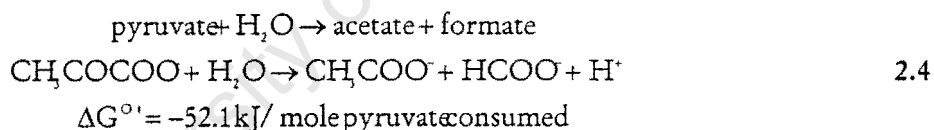
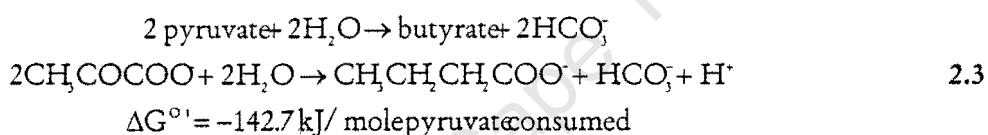
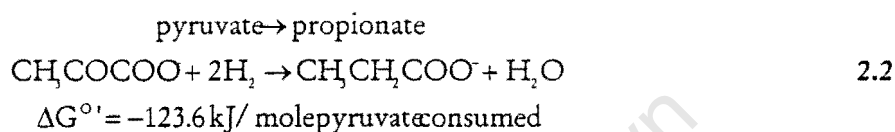
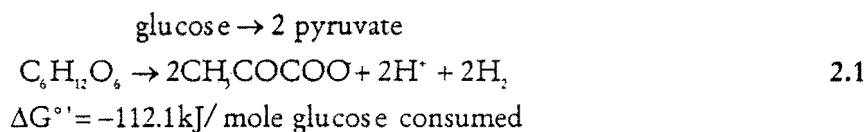


Figure 2.6. Representation of the anaerobic acidogenic phase

Using glucose as a model energy source, the acidogenic phase can be represented by the reactions shown below (Zehnder, 1988). In all cases the conversion of the organic compound is bioenergetically favourable (the value of $\Delta G^{\circ'}$ is negative).



From Figure 2.6 it can be seen that carbohydrates and nitrogen compounds are the only compounds that can be fermented in the acid-producing phase. For further degradation of the acids and hydrogen produced SRBs and methanogens are required. In the absence of SRBs and methanogens the accumulation of short chain fatty acids and hydrogen may be toxic to the acidogenic bacteria (Zoetermeyer *et al.*, 1982b)

2.2.2.1 HEAVY METAL TOXICITY

Work on the inhibition of the acidogenic bacteria by metals has been reported by Lin (1993) and Yenigun *et al.* (1996). Lin (1993) showed the following toxicity of metals in the production of acetic acid and butanol respectively: copper > zinc > chrome > cadmium > lead > nickel, and copper > zinc >

chrome > cadmium > nickel > lead. The experiments were carried out in batch at 35°C using sewage sludge. A copper concentration of 0.0018 kgm⁻³ caused a 50 % decrease in the formation of acetic acid. The inhibitory concentrations of zinc, cadmium and nickel for a 50 % decrease in formation of acetic acid were 0.0042 kgm⁻³, 0.03 kgm⁻³ and 0.6 kgm⁻³ respectively. Yenigun *et al.* (1996) confirmed these results for cadmium and nickel. Using batch tests with an anaerobic culture obtained from the acidogenic section of an industrial plant they observed a 50 % reduction in acetic acid production at a cadmium and nickel concentration of 0.056 kgm⁻³ and 0.78 kgm⁻³ respectively.

2.2.2.2 SULPHIDE TOXICITY

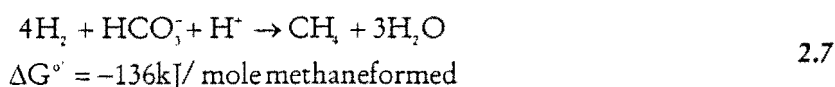
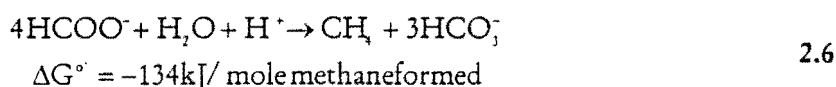
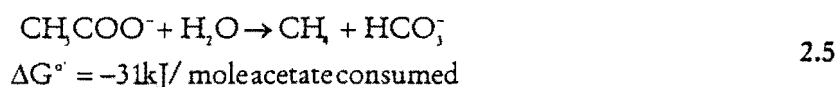
Hilton and Oleskiewicz (1988) investigated the inhibitory effect of sulphide on acetogenesis. Lactose uptake was examined over a pH range of 6.0 – 8.0 and at total sulphide concentrations of 0.10 kgm⁻³ and 1.00 kgm⁻³. For both sulphide concentrations they observed that when the pH was 8.0 lactate utilisation was more rapid than when the pH was 7.0 or 6.0. They concluded that unionised hydrogen sulphide, which is present at the pH values below 8.0, was inhibitory to the acetogenic microorganisms.

2.2.2.3 PH

Zoetermeyer *et al.* (1992a) measured the maximum specific growth rate of acidogenic bacteria on glucose over a pH range of 4.5 to 8.0. An optimum pH for glucose utilisation was observed at 8.0. The maximum specific growth rate rapidly decreased to 50 % at pH 5 and gradually decreased to 25 % at pH 8.0. Hilton and Oleszkiewicz (1988) report a similar trend over a pH range of 6.0 to 8.0 for the utilization of lactose and attribute the result to the absence of unionised hydrogen sulphide at a pH of 8.0.

2.2.3 METHANOGENESIS

In the anaerobic system carbon is finally reduced to methane. Methanogenic bacteria use acetic acid, formic acid or carbon dioxide and hydrogen as substrates, through the following reactions (Zehnder *et al.*, 1982):



The conversion of acetic acid to methane (Eqn 2.5) can be catalysed by two bacterial species viz. *Methanosaeta* (previously known as *Methanonotrix*) and *Methanosarcina*. *Methanosaeta soehngenii* grows slowly with a doubling time of 4 d (days). A high affinity for acetate is reported, $K_s = 30 \text{ mgL}^{-1}$ at a pH of 7. In contrast *Methanosarcina barkeri* grows faster (doubling time = 1.5 d (Zinder, 1984) but is a poor scavenger for acetate ($K_s = 0.40 \text{ kgm}^{-1}$)

The methane produced leaves the system as a gas, because of its low solubility. The carbon dioxide is either converted to bicarbonate or leaves the system as a gas. Zinder (1984) showed that in the absence of sulphate reduction, approximately 70 % of the biogas produced by methanogenic bacteria is due to conversion of acetic acid by a group of methanogens known as acetoclastic methanogens. The bulk of the remaining 30 % of the biogas is produced from the reduction of carbon dioxide by hydrogen, detailed in Eqn 2.7.

The free energy of formation drives the reaction towards the right and therefore methanogens have a high affinity for hydrogen. According to Mosey (1983), the utilization of hydrogen by the methanogens is only possible for hydrogen partial pressures below 10^{-4} atm. Above this partial pressure, hydrogen is toxic to the methanogens. The methanogens in a mixed culture perform an important function for the pH homeostasis of the ecosystem by removing the acetic acid (Zeikus and Henning, 1975)

The methanogens are a broad group of bacteria comprising various species that have in common the metabolic capability of producing methane (Zeikus and Henning, 1975). The morphology and the types of the substrates utilised by the various species are shown in Table 2.6.

Table 2.6. Methanogens, their morphology and organic substrates utilised by them (Sahm, 1984; Zeikus and Henning, 1975).

Species	Morphology & Size	Substrates
<i>Methanobacterium formicicum</i>	rods (1.2µm)	H ₂ /CO ₂ , formate
<i>Methanobacterium bryantii</i>	rods, filaments	H ₂ /CO ₂ ,
<i>Methanobrevibacter ruminantium</i>	Cocci	H ₂ /CO ₂ , formate
<i>Methanobrevibacter arboriphilius</i>	rods (0.5µm)	H ₂ /CO ₂ ,
<i>Methanobrevibacter smithii</i>	Cocci	H ₂ /CO ₂ , formate
<i>Methanococcus vannielii</i>	motile cocci (0.5µm)	H ₂ /CO ₂ , formate
<i>Methanococcus voltae</i>	motile cocci (0.5µm)	H ₂ /CO ₂ , formate
<i>Methanococcus mazei</i>	psuedo-sarcina	H ₂ /CO ₂ , acetate
<i>Methanogenium cariaci</i>	motile irregular cocci	H ₂ /CO ₂ , formate
<i>Methanogenium marisnigri</i>	motile irregular cocci	H ₂ /CO ₂ , formate
<i>Methanomicrobium mobile</i>	motile irregular cocci	H ₂ /CO ₂ , formate
<i>Methanosarcina barkeri</i>	irregular cocci ((1.0µm)	H ₂ /CO ₂ , formate, acetate
<i>Methanospirillum hungatei</i>	motile curved rods	H ₂ /CO ₂ , formate
<i>Methanosaeta soehngenii</i>	Filaments	acetate

Methanogens are strict anaerobes, as they lack the enzymes catalase and superoxide dismutase (Schlegel, 1997). They are very sensitive to any change in the environmental conditions. The influential parameters on the activity of methanogens include: pH, temperature, nutrient requirements and toxicity or inhibition by organic and inorganic substances.

2.2.3.1 pH

In contrast to acidogenic bacteria, which have an optimal growth at pH 6.0, methanogenic microorganisms are inhibited below 6.5 (Speece, 1996). The formation of methane is confined to the very narrow range of pH values between 6.8 to 7.4 (McCarty, 1964). This has been confirmed by studies on

pure cultures carried out by Zeikus and Henning (1975) and Zehnder *et al.* (1982). In the anaerobic system the pH is controlled by the carbon dioxide – bicarbonate buffer system. The methanogens thus become inactive when insufficient buffering is accompanied with the presence of high concentrations of volatile fatty acids (Zehnder, 1988).

2.2.3.2 TEMPERATURE

Traditionally, anaerobic reactors are run at temperatures ranging from 35 to 40°C using mesophilic methanogens (Speece, 1996). However thermophilic methanogens functioning at temperatures between 50 and 60°C offer the advantage of a significantly shorter generation time than mesophilic methanogens (Parkin and Owen, 1986). According to Zinder (1984), thermophilic *Methanosarcina* have a generation time of 12 h while mesophilic *Methanosarcina* have a generation time of 24 h (Speece, 1996). The population of thermophilic *Methanobrix* cultures doubles in 24 to 36 h whereas mesophilic *Methanosaeta* has a doubling time of 4 to 9 d.

2.2.3.3 NUTRIENT REQUIREMENTS

The nutrient requirements of bacteria involved in anaerobic digestion are relatively simple (Grobicki and Stuckey, 1992). Organic carbon, nitrogen and phosphorous compounds form the macronutrients. In addition magnesium, manganese, potassium, calcium, iron, nickel and cobalt are also required in trace amounts. Parkin and Owen (1986) reported that the phosphorous requirement for bacterial growth and maintenance is approximately $\frac{1}{7}$ to $\frac{1}{5}$ of the nitrogen requirement. Some 4 to 10 % of the organic compound is converted to cell mass (McCarty, 1964).

2.2.3.4 TOXICITY / INHIBITION

Of the entire consortium of anaerobes the methanogens are commonly considered to be the most sensitive group to toxicity (Kugelman and McCarty, 1964; Kugelman and Chin, 1971; Speece, 1983). It has been shown that toxic substances influence or inhibit the kinetics of organic decomposition during

anaerobic digestion (McCarty, 1964). The extent of the effect is dependent on the nature and concentration of the toxic substance (Parkin and Owen, 1986).

Salts

Sodium, potassium, calcium and magnesium at concentrations of 8.0, 12.0, 8.0 and 3.0 kgm⁻³ respectively are shown to be toxic to the activity of methanogens (McCarty, 1964). They did not define or quantify the degree of toxicity. Kugelman and Chin (1971) ran continuous experiments using digested sewage sludge to determine the toxicity levels of various cations to methanogens. The toxicity was determined in terms of the utilisation rate of the organic source relative to a control, containing no toxic substances. The utilisation rate of the organic compound was reduced by 50 % in the presence of sodium, ammonium, potassium, calcium, and magnesium at concentrations of 7.32, 4.50, 5.85, 4.40 and 1.94 kgm⁻³ respectively. Direct comparison of the work done by McCarty (1964) and Kugelman and Chin (1971) is not possible since McCarty (1964) did not mention the nature of the toxicity measurements whereas Kugelman and Chin (1971) determined toxicity relative to a control. However, the concentrations implicated in toxic responses by Kugelman and Chin (1971) are the same order of magnitude as that found by McCarty (1964).

Metals

Lawrence and McCarty (1965) and Lawrence *et al.* (1966) demonstrated that the toxicity of metals could be overcome by their precipitation as metal sulphides. This was shown by adding sodium sulphide to the media, as a precipitating agent. To investigate the effect of soluble metals on the process, sodium sulphide was excluded from the medium and the metals were added as chlorides. The results were compared to a control, which contained no metals. At a copper concentration of 0.397 kgm⁻³ there was 76 % decrease in gas production in the absence of sodium sulphide. For zinc and nickel at concentrations of 0.409 kgm⁻³ and 0.367 kgm⁻³ respectively in the absence of sulphide, the decrease in gas production was 95 % and 78 % respectively. In the presence of sodium sulphide the metals precipitated and had no effect on the production of gas observed. Hence metals in solution are toxic to methanogens but do not impose any toxic effect in the precipitated form.

Batch tests were carried out by Mosey *et al.* (1971) and Mosey and Hughes (1975) to ascertain which factors affect the toxicity of metals. They defined toxicity as a 20 % drop in gas production over 24 h relative to a control system. pH and the availability of soluble sulphide were reported to affect the extent of metal toxicity. By increasing the pH from 6.1 to 8.0 the soluble sulphide concentration increased and an increase in metal tolerance was observed due to metal precipitation. The copper tolerated by the bacterial system increased by 73 %, while the zinc, nickel and cadmium tolerated increased by 31, 59 and 82 % respectively. According to their results the order of toxicity was copper > lead > cadmium > zinc.

The distribution of metals in a continuous anaerobic bacterial system at a ten day retention time was studied by Hayes and Theis (1978). Nickel, copper, chromium, lead and zinc were investigated. Less than 1 % of the metals were extracellular and in solution, 30 and 60 % was intracellular and the rest precipitated outside of the cell. The toxic limits (defined as the concentration at which total gas production was reduced to 70% of that in the control system) determined were: nickel: 0.030 kgm⁻³, copper: 0.070 kgm⁻³, lead: 0.34 kgm⁻³, chromium³⁺: 0.26 kgm⁻³, chromium⁵⁺: 0.42 kgm⁻³ and zinc: 0.60 kgm⁻³. The reported order of toxicity was found to be: nickel > copper > chromium³⁺ > lead > chromium⁵⁺ > zinc. This is consistent with the trend reported by Mosey *et al.* (1971). Subsequent work by Hickey *et al.* (1989) confirmed the same trend. They found the relative toxicity to be: copper > cadmium > zinc. Using a batch system in which toxicity was defined as a 50 % inhibition of gas production after 24 h, copper was toxic at 0.075 kgm⁻³, cadmium at 0.1375 kgm⁻³ and zinc at 0.325 kgm⁻³. A consistent order of toxicity, nickel > copper > zinc, was also reported by Tijero *et al.* (1991), based on a continuous study with a retention time of 40 d in which toxicity was defined as a 50 % decrease in gas production.

Table 2.7 summarises the level of toxicity of metals reported by above. Clearly the order of toxicity of the metals is similar even though the concentrations tolerated vary as a result of the definition of toxicity and experimental configuration. Furthermore it is apparent that the calcium, potassium, magnesium and sodium concentrations tolerated are much higher than the metal tolerance limits.

The specific mechanism of heavy metal toxicity has not been fully understood. Interference in transmembrane potentials, electron transport chains and substrate translocation systems due to disruption of metabolic enzyme activities or changes in ionic gradients have been implicated. Several enzymes such as alcohol dehydrogenase and coenzyme A are susceptible to inactivation by heavy metals, which react with the enzymic sulfhydryl (-SH) groups (Mosey and Hughes., 1975). The reversibility of metal toxicity is indicative of competitive inhibition. Hence it is the dissociated metal in solution that exhibits toxicity. Precipitation of the metals as metal sulphides reduce their toxicity.

Table 2.7. Comparison of toxicity limits of different metals on activity of methanogens as reported in independent works.

Concentration (kgm ⁻³)	McCarty (1964)	Kugelman and Chin (1971)	Lawrence and McCarty (1965)	Hayes and Theis (1978)	Mulder (1984)	Hickey <i>et al.</i> (1989)	Tijero <i>et al.</i> (1991)
Criteria for Toxicity	Not defined	Not defined	Not defined	70%↓ gas*	Not defined	50%↓ gas*	50% ↓ gas*
Calcium	8.00	4.40	-	-	-	-	-
Potassium	12.00	5.85	-	-	-	-	-
Magnesium	3.00	1.94	-	-	-	-	-
Sodium	8.00	7.32	-	-	-	-	-
Sulphide	0.20	-	-	-	0.70	-	-
Cadmium	-	-	-	-	-	0.14	-
Copper	-	-	0.39	0.07	-	0.08	0.04
Chromium	-	-	-	0.42	-	-	-
Nickel	-	-	0.37	0.03	-	-	0.01
Lead	-	-	-	0.34	-	-	-
Zinc	-	-	0.41	0.60	-	0.33	0.45

*70%, 50% ↓ indicates that toxicity was determined by a 70% or 50% decrease in gas production relative to the control

Sulphide

In the absence of metals, unionised sulphide concentrations above 0.20 kgm⁻³ (McCarty, 1964) to 0.70 kgm⁻³ (Mulder, 1984) have an inhibitory effect on the activity of methanogens. Hilton and Oleskiewicz (1986) studied the effect of sulphide on methanogenesis utilising acetate as a substrate over a pH range of 6.8 to 8.0. They concluded that it is the unionised hydrogen sulphide and not total sulphide that is inhibitory. They confirmed that methanogenesis is severely inhibited above 0.20 kgm⁻³ hydrogen sulphide (H₂S). Visser (1995) showed that hydrogen sulphide concentrations in the range 0.05 kgm⁻³ to 0.25 kgm⁻³ result in 50 % inhibition of methanogenesis ranged from.

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2.2.4 ANAEROBIC SULPHATE REDUCTION

The biological sulphate reduction process, shown diagrammatically in Figure 2.7 is discussed in this section. Particular attention is paid to the use of the process for the treatment of sulphate containing effluents.

2.2.4.1 MICROBES

When sulphate is present in wastewater, the sulphate reducing bacteria (SRB), comprising 15 genera exist (Gibson, 1990; Thauer and Kunow, 1995; Briglia and Verstraete, 1995; Hamilton, 1996; Brüser *et al.*, 2000), utilise it as the terminal electron acceptor in the oxidation of hydrogen and other organic compounds available in the wastewater (Harper and Pohland, 1986; Lettinga, 1995; Lens and Hulshoff Pol, 1998; Lens *et al.*, 2000). The organic compounds serve as both the electron donor and the carbon source. This process provides the bacteria with energy for growth and cell maintenance. The end products of the reduction process are sulphides and carbon dioxide. At a pH of 7 most of the sulphide is present in the form of hydrogen sulphide and leaves the system as a gas (Perry and Green, 1984). A representation of the biological sulphate reduction process is shown as Figure 2.7. The reduction of sulphate can be represented by the following reaction:



As well as oxidised sulphur compounds, some species of SRB can utilise other electron acceptors such as nitrate, metal ferrous and manganic ions (Hamilton, 1996).

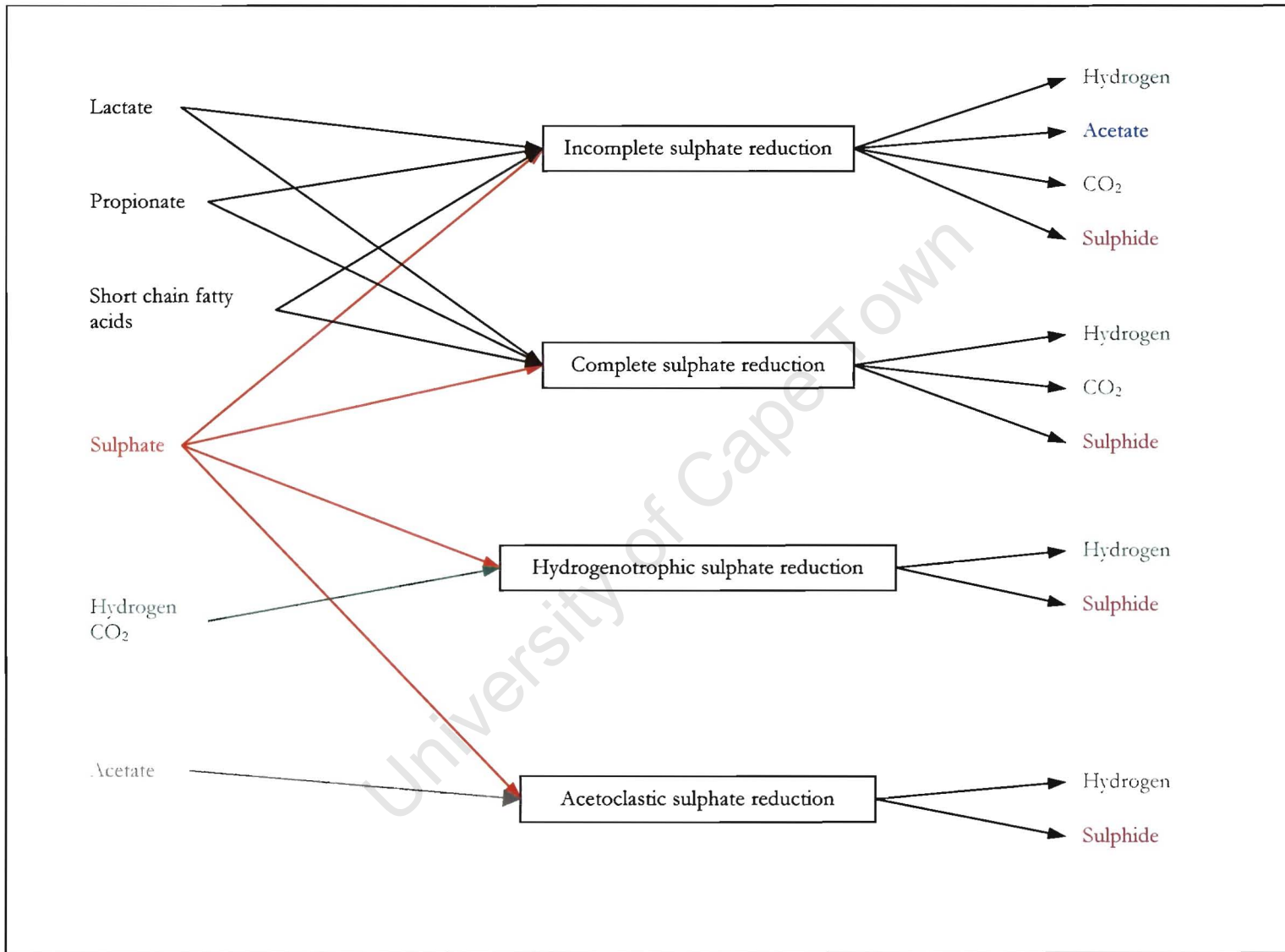
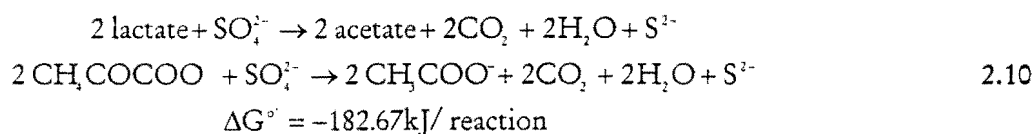
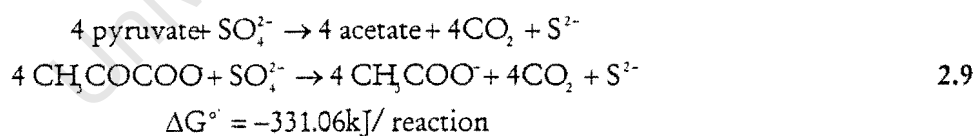


Figure 2.7. Representation of the biological sulphate reduction process. Sulphate is used as the terminal electron acceptor for the reduction of the organic source. The end products include, hydrogen, carbon dioxide and sulphide.

SRB have been isolated from soils, fresh, marine and brackish waters, hot springs, geothermal areas, oil and natural gas wells, sulphur deposits, human intestines, rumina of sheep and guts of insects (Briglia and Verstraete, 1995, Colleran *et al.*, 1994). They comprise a broad taxonomic range of microorganisms belonging to three phylogenetic groups: gram positive bacteria, proteobacteria and archaeobacteria (Barber, 1999; Oude Elferink *et al.*, 1994). Based on functionality, they may be broadly classified into two groups: those that completely oxidise organic compounds to carbon dioxide and hydrogen sulphide and those which carry out incomplete oxidation of the organic compound usually to acetate (Widdel, 1988). The SRB species that oxidise the organic substrate incompletely to acetate include *Desulfovibrio thermophilus*, *Desulfovibrio sapovarans*, *Desulfomas pigra*, *Thermodesulfobacterium commune* and the majority of the species of the genera *Desulfotomaculum*, *Desulfomonas* and *Desulfobulbus*. The incomplete oxidisers do not possess the citric acid cycle, which allows for the oxidation of the acetate unit, acetyl Co-A. These SRB produce organic compounds other than acetate. In general the incomplete oxidisers are nutritionally less versatile than the complete oxidisers (Colleran *et al.*, 1995). They do, however, grow faster than the complete oxidisers. For example, under optimum conditions, *Desulfovibrio* growing on hydrogen, lactate or pyruvate and *Desulfotomaculum* species using pyruvate have doubling times of 3 to 4 h compared to 20 h for complete oxidisers (Widdel 1988).

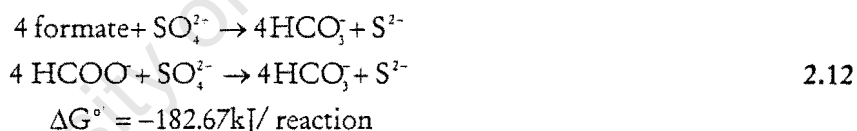
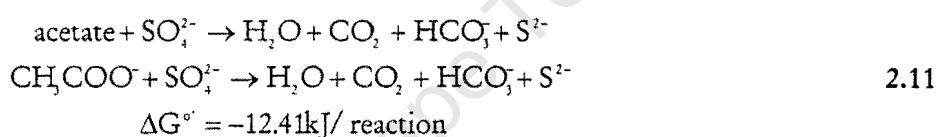
Examples of incomplete oxidation reactions are:



The complete oxidisers convert the substrate to carbon dioxide. Examples include species of the genera *Desulfobacter*, *Desulfosarcina*, *Desulfococcus*, *Desulfobacterium*, *Desulfoarculus*, *Desulfomonile* and *Desulfonema* as well as *Desulfotomaculum acetoxidans*, *Desulfotomaculum sapomandens* and *Desulfovibrio baarsii* (Postgate, 1984; Widdel,

1988; Barton and Tomei, 1995; Collieran *et al.*, 1995). These SRB can use various substrates as electron donors, including hydrogen, formate, acetate, butyrate and higher fatty acids, branched fatty acids, lactate, methanol, ethanol and higher alcohols, fumarate, succinate, malate and aromatic compounds. The affinity for acetate utilisation is not the same for all the complete oxidisers. The *Desulfobacter* species use acetate as their preferred substrate and form high cell yields whereas other complete oxidisers prefer organic acids or alcohols and take up acetate from the medium slowly with low cell yields (Widdel, 1988). Despite their ability to completely oxidise organic compounds, the *Desulfobacter* species growing on ethanol and *Desulfotomaculum acetoxidans* growing on ethanol or butyrate will produce acetate. When the organic source is limited, these species will utilise the acetate produced (Widdel, 1988).

The complete oxidation of organic substrate takes place according to the following reactions:



When hydrogen is present as the electron donor the reduction of sulphate proceeds by the following reaction:



The morphology and environmental conditions tolerated by a few species of sulphate reducers is shown as Table 2.8.

Table 2.8. The morphology, carbon and energy source, growth pH and temperature ranges of mesophilic SRB genera (Widdel 1988).

Species	Morphology and Size	Carbon and Energy Source	pH		Temp (°C) Range
			Range	Optimum	
<i>Desulfobacter</i>	curved (1-2µm)	acetate	6.2 - 8.5	7.3	28 - 32
<i>Desulfobulbus</i>	tapered spheres (1-1.3µm)	propionate, lactate, pyruvate, ethanol, propanol	6.0 - 8.6	7.2	28 - 39
<i>Desulfococcus</i>	spheres, in clusters (1.5-2.2µm)	formate, acetate, lactate, butyrate, pyruvate			30 - 36
<i>Desulfotomaculum</i>	straight	lactate, pyruvate, acetate, ethanol, hydrogen			
<i>Desulfomonas</i>			6.5 - 8.5	7.2	30
<i>Desulfonema</i>	long filaments	acetate, malate, benzoate, pyruvate			
<i>Desulfosarcina</i>	clusters of rod shaped cell (1-1.5µm)	formate, acetate, propionate, butyrate	6.9 - 7.0	7.4	33 - 38
<i>Desulfovibrio</i>	curved (2.5-10µm)	lactate		7.5	25 - 35

2.2.4.2 ELECTRON DONORS AND CARBON SOURCES

The literature contains extensive lists of electron donors utilised by SRB (Postgate, 1984; Fauque, 1995). The comprehensive list provided by Hansen (1988, 1993) is reproduced in Table 2.9. SRB do not degrade polysaccharides, proteins or lipids but depend on the acidogenic bacteria for the supply of electron donors from these compounds. The SRB are unique in their ability to grow with reduced organic compounds that cannot be utilised by other anaerobic bacterial groups. These compounds include propionate, butyrate, higher fatty acids or phenyl substituted organic acids. By using sulphate as an external electron acceptor the SRB can utilise reduced compounds as energy sources (Widdel, 1988). In most instances the SRB use the same compound as the carbon source and electron donor. The exception is when carbon monoxide or carbon dioxide is used as the carbon source. An additional electron donor, usually hydrogen, is then required.

Table 2.9. Electron donors and carbon sources used by SRB (Hansen, 1988).

Class of compound	Type of compounds
inorganic	hydrogen, carbon monoxide
monocarboxylic acids	Formate, acetate, propionate, isobutyrate, 2- and 3- methylbutyrate, higher fatty acids up to C ₁₈ , pyruvate, lactate
dicarboxylic acids	succinate, fumarate, malate, oxalate, maleinate, glutarate, pimelate
alcohols	methanol, ethanol, propanol, butanol, ethylene glycols 1,2- and 1,3-propanediol, glycerol
amino acids	lysine, serine, cysteine, threonine, valine, leucine, isoleucine, aspartate, glutamate, phenolalanine
miscellaneous	choline, furfural, oxamate, fructose, benzoate, 2-, 3- and 4 - OH - benzoate, cyclohexanecarbonate, hippurate, nicotinic acid, indole, anthranilate, quinoline, phenol, p-cresol, catechol, resorcinol, hydroquinine, protococatechuate, phloroglucinol, pyrogallol, 4-OH-phnylacetate, 3-phenylpropionate, 2-aminobenzoate, dihydroxyacetone

2.2.4.3 METABOLISM

A formalised scheme for the metabolism of sulphate coupled to the utilisation of a carbon source is shown in Figure 2.8. The carbon source and electron donors vary from species to species as shown in Table 2.9. In most instances the electron donor and carbon source are the same compound.

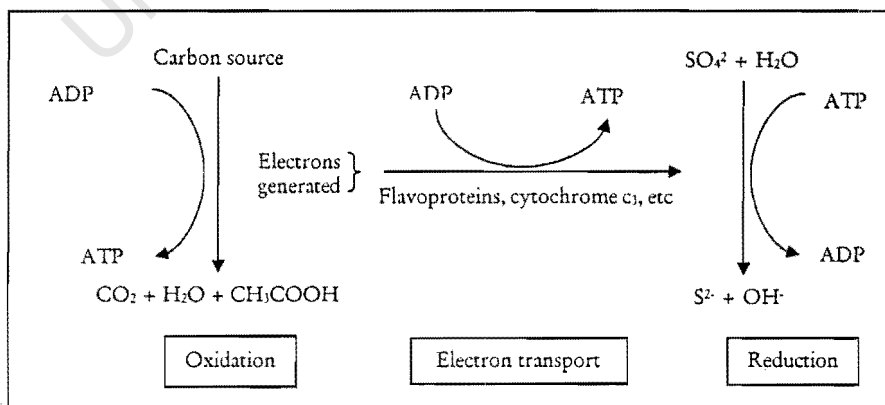


Figure 2.8. Schematic representation of sulphate reduction coupled to organic utilisation (Postgate, 1984).

(ATP: adenosine triphosphate; ADP: adenosine diphosphate)

Sulphur metabolism

Sulphate reduction may be either assimilatory or dissimilatory. During the assimilatory reduction of sulphate, the sulphur moiety is used by the cell for the synthesis of cysteine, methionine, co-enzyme A or other sulphur containing nutrients. During dissimilatory sulphate reduction, the oxygen moiety is cleaved from the sulphur oxyanion for respiration (Postgate, 1984).

Postgate (1984) proposed a cyclic pathway for the reduction of sulphate to sulphide, shown in Figure 2.9. Confirmation of the pathway has been complicated by the fact that most of the intermediates are unstable. The sulphate ion outside the cell is accumulated and enters the cell. In the cytoplasm the sulphate ion reacts with ATP to form adenosine phosphosulphate (APS) and pyrophosphate (PP) activates it. To enter the assimilatory route, the APS is further reduced to phosphoadenosine phosphosulphate (PAPS) with concomitant dephosphorylation of one mole of ATP to ADP. During the dissimilatory reduction of sulphate, APS is reduced to sulphite and AMP. Sulphite (S_2O_3) condenses to form metabisulphite (S_2O_5) which is reduced, via intermediates, to trithionate (S_3O_6). S_3O_6 is cleaved to yield thiosulphate and regenerate sulphite. Finally the thiosulphate is reduced to sulphide and more sulphite.

During the sulphate reduction process ATP is consumed to form APS. The rationale behind this consumption of ATP lies in the fact that for each sulphate mole to be reduced to sulphide the sulphate accepts eight electrons. The redox potential for the SO_4^{2-}/SO_3^{2-} couple is -0.5 V indicating that sulphate is not suitable as an electron acceptor, whereas the redox potential of the APS/AMP + HSO_3^- complex is -0.6 V indicating that APS is more likely to act as an electron acceptor (Hansen, 1988).

The formation of APS is made possible by the hydrolysis of pyrophosphate by the enzyme pyrophosphatase. Pyrophosphatase has the ability to be activated by reducing agents hence becoming inactive in aerobic environments. As a result ATP utilisation and growth cannot occur under aerobic conditions (Postgate, 1984). The APS is converted to AMP and sulphite, catalysed by APS reductase. The sulphite ion is reduced, via a number of intermediates, to the sulphide ion. The exact pathway is unknown but the reduction of bisulphite to sulphide (redox potential $HSO_3^-/HS^- = -0.1$ V) is assumed to proceed

Not all acetate utilisers use the citric acid cycle for metabolism of acetate. For example *Desulfotomaculum acetooxidans* does not possess a citric acid cycle yet utilises acetate. It grows more slowly (doubling time of 30 hrs) than *Desulfobacter* species. *Desulfococcus*, *Desulfovibrio baarsi* and *Desulfobacterium* also oxidise acetate slowly, with a low biomass yield (Colleran *et al*, 1994). They metabolise acetate via a non-cyclic pathway (Figure 2.11) that involves the cleavage of the two-carbon unit into a methyl and carbon monoxide unit. These are then oxidised independently to CO₂. Because the pathway is not cyclic, it involves the consumption of ATP and does not allow substrate level phosphorylation. This could explain the slower growth rates (Hansen 1993). The slow acetate utilisers mentioned do not have 2-ketoglutarate dehydrogenase and do not incorporate carbon sources into amino acids that are indicative of an incomplete citric acid cycle.

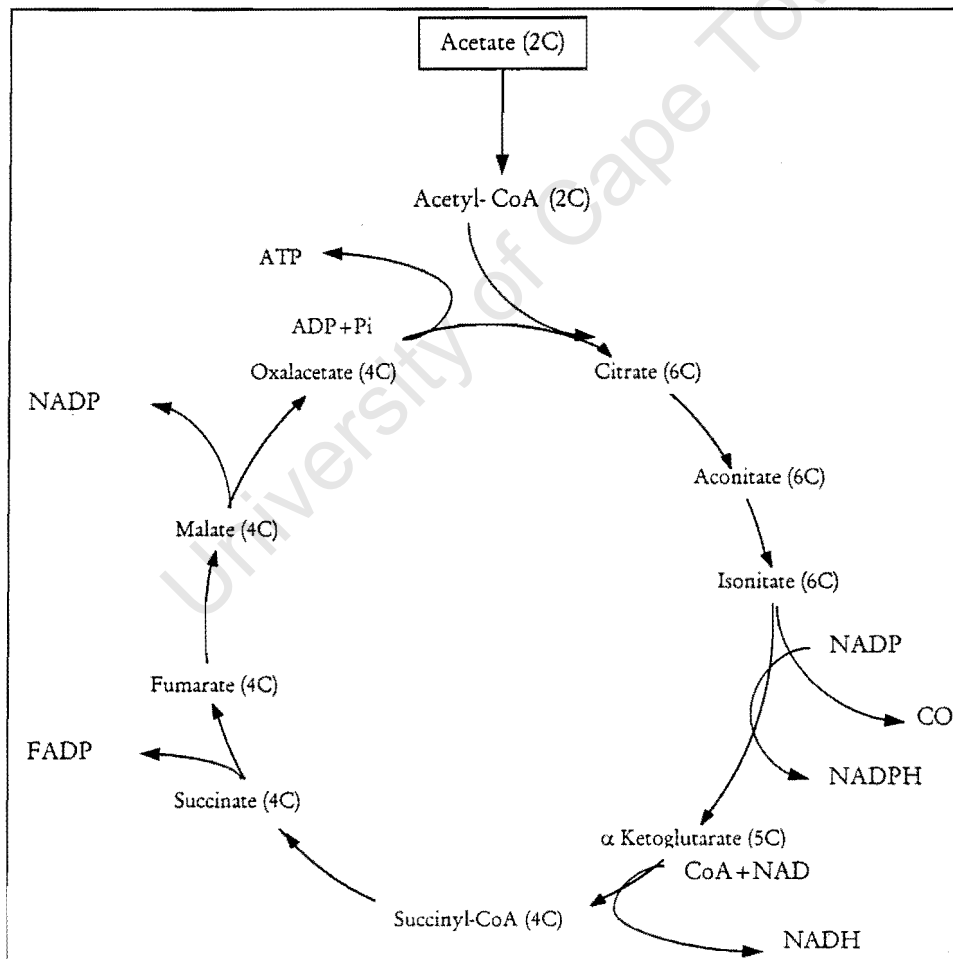


Figure 2.10. Pathway of acetate oxidation via the citric acid cycle in *Desulfobacter postgatei* (Brock and Madigan, 1991; Colleran *et al.*, 1994).

(NADPH: nicotinamide-adenine dinucleotide; ADP: adenosine triphosphate; ATP: adenosine diphosphate; CoA: coenzyme A)

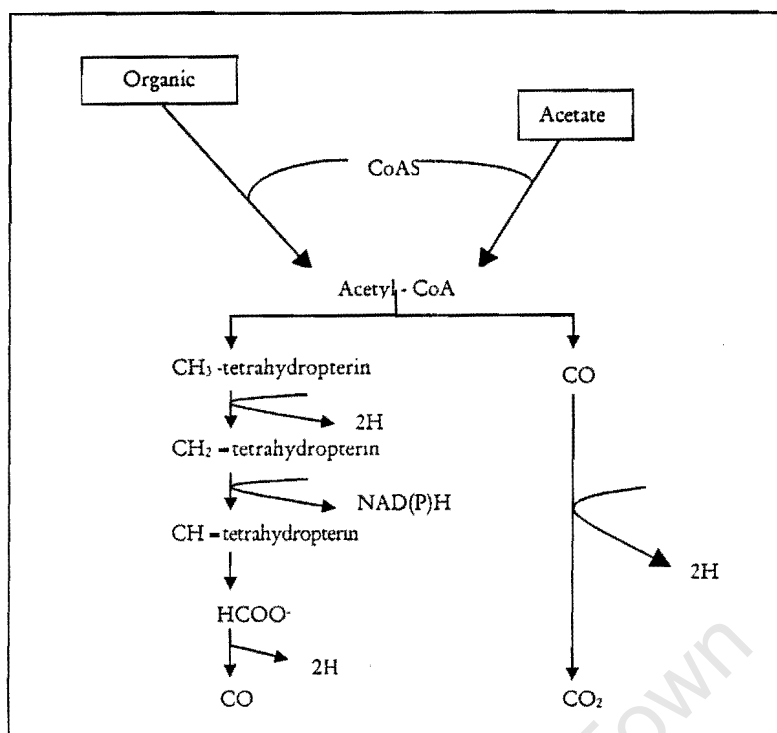


Figure 2.11. Non-cyclic carbon monoxide dehydrogenase pathway for oxidation of acetyl groups by *Desulfotomaculum acetoxidans* and by complete oxidisers growing on higher carbon compounds (Colleran, *et al.*, 1995).

Propionate metabolism

Like acetate, propionate is also a key intermediate in anaerobic digestion of complex organics. The *Desulfobulbus* genus is able to use propionate as the sole carbon and energy source (Gibson, 1990). Propionate undergoes incomplete oxidation by *Desulfobulbus*, to acetate via a pathway known as a randomising pathway (Figure 2.12). The acetate is further converted to carbon dioxide by acetate utilising SRB.

Besides propionate *Desulfobulbus* has been shown to oxidise ethanol, propanol and hydrogen slowly (Widdel, 1988). Several other genera such as *Desulfococcus*, *Desulfonema* and *Desulfobacterium* are capable of complete oxidation of propionate to carbon dioxide (Widdel and Pfennig, 1982).

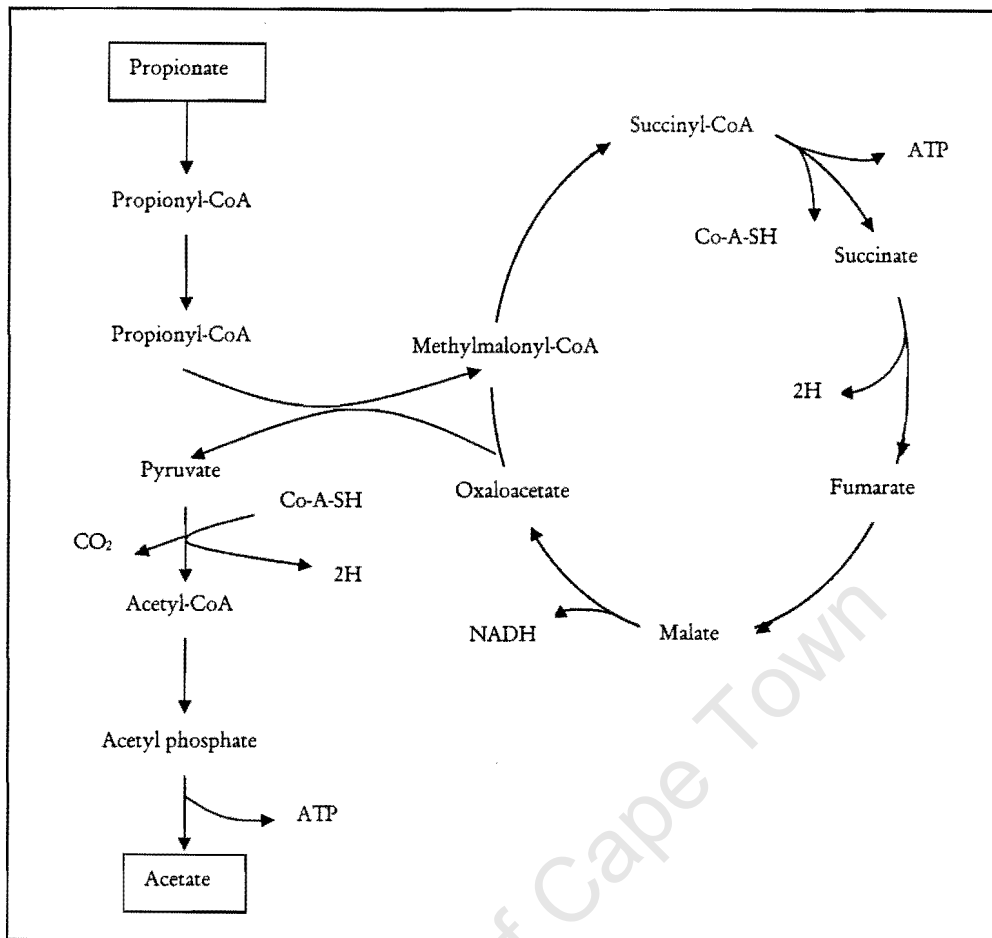


Figure 2.12. Pathway for the incomplete oxidation of propionate to acetate by *Desulfobulbus propionicus* (Colleran *et al.*, 1995).

(ATP: adenosine diphosphate; Co-A: coenzyme A)

Growth of sulphate reducers on butyrate, higher fatty acids and alcohols

Both complete and incomplete oxidisers utilise butyrate and higher fatty acids (Widdel and Pfennig, 1981, Widdel *et al.*, 1983). The incomplete oxidisers convert even numbered fatty acids to acetate and the uneven numbered fatty acids are converted to acetate and propionate. Complete oxidisers utilise fatty acids with C-chain lengths of 10 to 16 to yield carbon dioxide. Both the complete and incomplete oxidisers employ the β -oxidation pathway. Utilisation of alcohols is coupled with the use of acetate or acetate and carbon dioxide as the carbon source (Braun and Stolp, 1985; Colleran *et al.*, 1995).

Hydrogen and formate metabolism

A striking feature of the *Desulfovibrio* species is its ability to use hydrogen in the metabolism of carbon (Postgate, 1951; Badziong and Thauer, 1978a, b). A proton gradient has to be established for the generation of ATP for *Desulfovibrio* as they do not produce ATP through the metabolism of pyrophosphate. In the *Desulfobacter* species the oxidation of acetate yields ATP by substrate level phosphorylation (Postgate, 1984). Thermodynamically (see discussion in Section 2.2.6.1) growth on hydrogen is more favourable than on acetate. It is only the complete oxidisers that can grow autotrophically on hydrogen/carbon dioxide. The incomplete oxidisers require acetate for cell synthesis (Widdel, 1988).

Two models have been proposed for the cycling of hydrogen (Postgate, 1984). Firstly a vectorial electron transfer coupled to the oxidation of hydrogen by hydrogenase and secondly proton translocation coupled to the reduction of specific substrates. The first model (Figure 2.13) is based on observations of hydrogen production during growth, enzyme localization and vectorial electron transfer. Consequently a chemiosmotic hydrogen cycle is proposed. It postulates that hydrogen metabolism is mediated by reversible hydrogenases found in the periplasm (Peck and Legall, 1982; Cypionka, 1995) and the *Desulfovibrio* produce ATP required for growth on lactate and sulphate. On the external surface of the membrane, hydrogen is oxidised by the periplasmic hydrogenase, which requires cytochrome C3. The electrons produced are transferred across the cell membrane into the cytoplasm, leaving the protons on the external surface of the membrane. These electrons are used for the reduction of sulphate to sulphide. The net effect is the transfer of eight protons across the cytoplasmic membrane with the involvement of a vectorial electron transfer in which proton translocation is directly coupled to electron transfer. A proton gradient is thus established.

In the second model illustrated schematically in Figure 2.14, Legall and Fauque (1988) propose that hydrogen production inside the cell controls the redox state of internal electron carriers which are linked to energy conservation and hydrogen utilisation by the periplasmic hydrogenase. In other words the hydrogenase which is situated in the periplasm controls the redox level of ferredoxin and flavodoxin

thereby regulating electron and proton transfer. The second model differs from the first in that energy conservation is attained by the Mitchell loop whereas the first scheme uses vectorial electron transfer for charge separation. Many sulphate reducers capable of growing on hydrogen can also grow on formate as the sole electron and energy source (Colleran *et al.*, 1995). These two models do not account for the complex array of electron transfer proteins which are present in SRB (LeGall and Fauque, 1988), the accumulation of hydrogen by *Desulfovibrio vulgaris Hildenborough* and the failure of molecular hydrogen to inhibit growth on lactate and sulphate i.e. to grow on hydrogen as the sole electron donor. This indicates that a carbon source is vital for the growth of SRB.

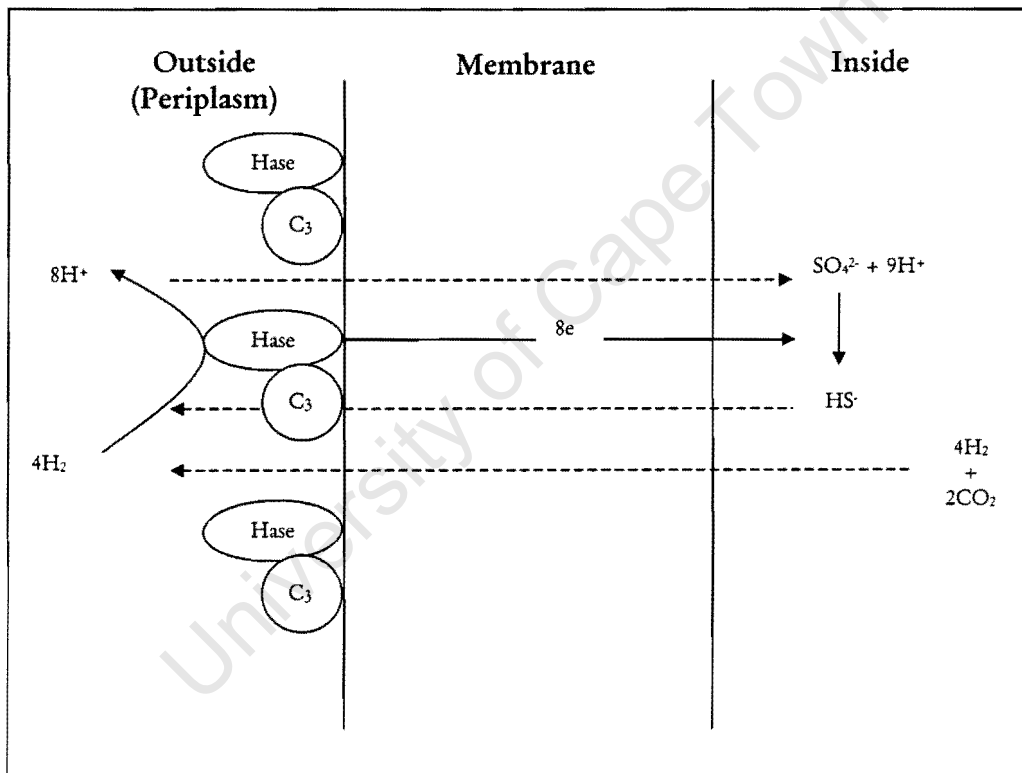


Figure 2.13. Cycling of hydrogen during sulphate reduction- Model I (Peck and Legall, 1982; Cypionka, 1995).

(C₃, tetraheme cytochrome C₃; Hase, hydrogenase)

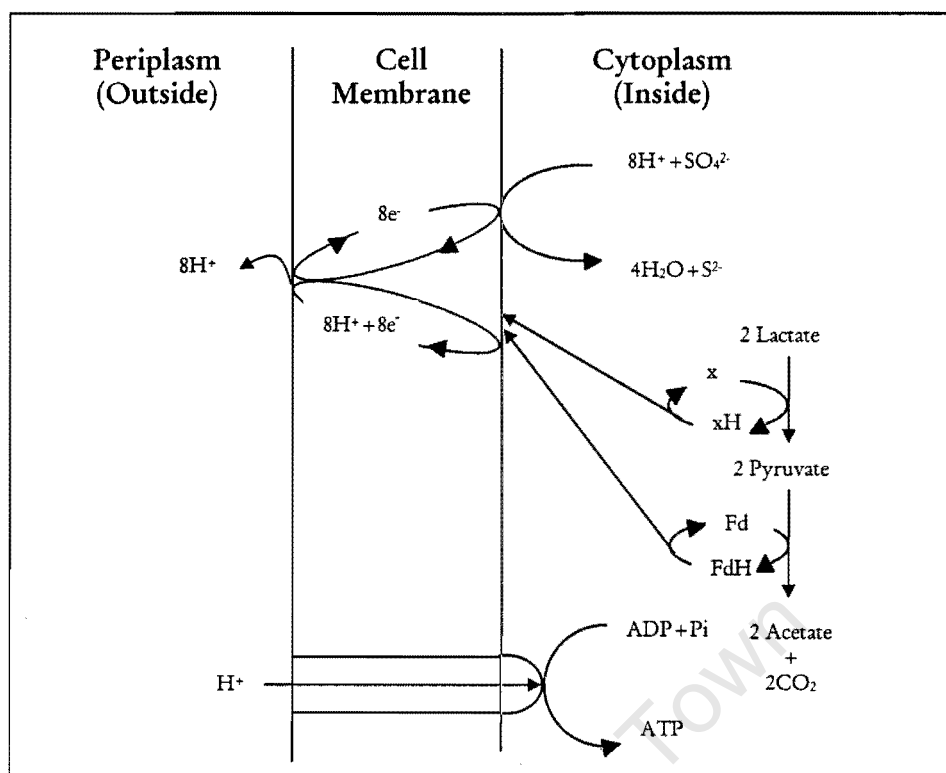


Figure 2.14. Cycling of hydrogen during sulphate reduction – Model II (Legall and Fauque, 1988).
(Fd: ferredoxin).

2.2.4.4 ENVIRONMENTAL CONDITIONS

The SRB are strict anaerobes. The pH and temperature ranges suitable for their growth are similar to those of methanogens (Brown *et al.*, 1973; Cappenberg, 1979; Lowe *et al.*, 1993). The important environmental factors that affect the activity of sulphate reducers include pH, temperature, nutrient requirements and inhibitors such as sulphide and metallic ions (Rintala and Lettinga, 1992; Soto *et al.*, 1993; Hao, 2000). The effect of these factors on the growth and morphology of SRB is discussed in the following sections.

pH

The various genera of sulphate reducers function in different pH ranges (Table 2.8). Generally SRB prefer slightly alkaline conditions in the range 7.0 – 7.8 (Fauque and Legall, 1995) but can tolerate pH values ranging from 5.5 to 9.0 (Visser, 1995). Visser (1995) found that sulphate reducers in sludge from a UASB reactor had an optimal range of 6.9 to 8.5 and were active up to a pH of 10. The species were not

identified. Furthermore the pH optimum for sulphate reduction is reported to be culture specific (Postgate, 1984). The reduction of sulphate in acid mine drainage, which usually has a pH in the range 2.5 to 3.6, has been reported by Tuttle *et al.* (1969). This indicates the ability of SRB to function at low pH values. Acidophilic strains of SRB have been isolated from a stream draining a copper mine (Johnson *et al.*, 1993; Johnson, 2000). Johnson *et al.* (1993) have observed sulphate reduction in sediments at pH 2.8 to 4.4 and in a liquid stream at pH 2.3 draining an abandoned copper mine. A gram-negative sulphate reducer able to grow at pH 4.0 was isolated from wastewater muds and a copper mine with methanol as the electron donor (Hard and Babel (1997) in Johnson, 2000)

Temperature

Mesophilic strains of SRB, with growth temperatures of 28 to 45°C (Okabe and Characklis, 1992 a & b) as well as thermophilic strains with a growth temperature range of 54 to 70°C, have been isolated (Wiegant *et al.*, 1986; Rintala and Lettinga, 1992; van Houten *et al.*, 1997; Weijma *et al.*, 1999). The thermophiles were isolated from geothermal environments. The rate of sulphate conversion using by a mixed SRB population has been shown to increase with temperature in the range 20 to 32°C (Middleton and Lawrence, 1977; Barmes *et al.*, 1992a). Currently, the biological reduction processes employed on a commercial scale are primarily mesophilic.

Nutrient Requirements

Sulphate and organic/electron source

Sulphate and an organic source and electron donor are the primary nutrient requirements of the SRB. The *Desulfovibrio* species have been shown to utilise thiosulphate, tetrathionate and sulphite as an electron acceptor, in the absence of sulphate (Postgate, 1951). In most cases the organic source is also the electron donor. In the case when hydrogen is used as the electron donor, carbon monoxide or carbon dioxide is used as the carbon source. A discussion of the various organic sources and electron donors has been presented in Section 2.2.4.2.

Nitrogen

In most cases ammonia is the principal nitrogen source. Nitrite and hydroxylamine can also be utilized (Senez & Pichinoty, 1958a, b; in Postgate, 1984). It has been shown that in the absence of sulphate certain *Desulfovibrio* species were able to reduce nitrate to ammonia (McCready *et al.*, 1983 in Postgate 1984). In media employed on an industrial and lab scale the nitrogen component of the media is added as the ammonium ion. Typical media used for the growth and maintenance of SRB both on a lab, pilot and industrial scale is shown as Table 2.10.

Table 2.10. Various media used for the growth and maintenance of SRB.

Component	Concentration (kgm ⁻³)			
	Postgate (1986) ¹	Barber (1999) ²	Rose (1996) ³	Barnes <i>et al.</i> (1992a, b) ⁴
K ₂ HPO ₄	0.5	4.0	1.0	2.0
NH ₄ Cl	1	-	0.5	0.8
CaSO ₄	1	-	-	-
Na ₂ SO ₄	-	-	0.5	-
Na ₂ HCO ₃	-	163.0	-	-
MgSO ₄ .7H ₂ O	2	-	-	-
CaCl ₂ .2H ₂ O	-	-	0.1	-
FeSO ₄ .7H ₂ O	0.5	-	0.1	-
Ascorbic acid	0.1	-	-	-
Thioglycollic acid	0.1	-	-	-
Yeast extract	1	-	-	-
Peptone	-	40.0	-	-
Meat Extract	-	13.0	-	-
Sodium lactate	3.5	-	-	-
Sugar	-	134.0	-	-
Trace metals	Tap water	yes	no	no

¹ general purpose media used for culturing and detecting *Desulfotomaculum* and *Desulfovibrio*; ² media used to run lab scale anaerobic baffled reactors reducing sulphate; ³ media used to run a pilot scale upflow anaerobic reactor removing sulphate; ⁴ industrial media used for the reduction of sulphate in a minerals processing effluent

Trace metals

As with most bacteria the SRB require trace amounts of metals as cofactors for the activation of their enzymes (Postgate, 1984). In most instances tap water is used to prepare the media required for growth and consequently no trace metals are added to the media. In cases where distilled or deionised water is used to prepare the media the trace metals showed in Table 2.11 have been added.

Table 2.11. Trace metal solutions cited in literature for the growth of SRB.

Component	Concentration (kgm ⁻³)		
	Postgate (1984)	Visser (1995)	O'Flaherty <i>et al.</i> (1998)
FeCl ₂ .4H ₂ O	1.5	2.0	1.5
ZnCl ₂	0.7	0.5	0.7
MnCl ₂ .4H ₂ O	0.1	-	0.1
MnCl ₂	-	0.5	-
H ₃ BO ₃	0.06	0.05	0.006
CoCl ₂ .6H ₂ O	0.12	0.05	0.19
CuCl ₂ .2H ₂ O	0.015	0.05	0.002
NiCl ₂ .6H ₂ O	0.025	0.05	0.024
Na ₂ MoO ₄ .2H ₂ O	0.025	-	0.036
Cystein-HCl	-	-	0.56
EDTA	-	0.5	-
Na ₂ SeO ₃	-	0.1	-
AlCl ₃	-	0.05	-
(NH ₄) ₆ Mo ₇ O ₂₄ .4H ₂ O	-	0.05	-

Effect of Inhibitory Compounds

Sulphide

While the SRB have the highest tolerance compared to other anaerobic microorganisms, to sulphide their activity is nonetheless inhibited by the presence of sulphide (Reis *et al.*, 1991a, b; 1992; Maillacheruvu *et al.*, 1993; Konishi *et al.*, 1996; Kolmert *et al.*, 1997; O'Flaherty *et al.*, 1997, 1998; O'Flaherty and Colleran, 1998, 2000). Table 2.12 shows the inhibitory levels of sulphide on the activity of SRB reported by independent researchers. The mechanism of sulphide inhibition has not been understood but two hypotheses are proposed. The first is that the undissociated sulphide in the environment precipitates any

metals present, the result being that the SRB are deprived of essential trace metals that are required as cofactors for the activation of their enzyme systems (Bharathi *et al.*, 1990). The second theory is that the sulphide is absorbed into the cells and denatures proteins, by acting as a cross-linking agent between the polypeptide chains (Postgate, 1984). The sulphide could also interfere with the metabolic coenzymes through the formation of sulphide bonds. However this theory has been challenged by Parkin and Owen (1986). Sulphide inhibition has been shown to be reversible (Reis *et al.*, 1992; Okabe *et al.*, 1992). For example, after exposure of *Desulfovibrio* to inhibitory concentrations of sulphide, bacterial growth ceased. Once the sulphide was removed bacterial growth proceeded again and reached the same concentration as a control. In contrast to other microorganisms where only the undissociated form of hydrogen sulphide (H_2S) has been shown to be inhibitory, there is debate as to whether total sulphide or only undissociated hydrogen sulphide (H_2S) is inhibitory to SRB (Stucki *et al.*, 1992, Visser, 1995).

Hilton and Oleskiewicz (1991) investigated the degradation of lactate by anaerobic sludge containing sulphate reducers over a pH range of 6.5 to 8.0. From 30 d batch experiments they found that even under alkaline conditions the SRB were inhibited. They concluded that a direct relationship existed between the extent of inhibition and the total sulphide concentration (H_2S and HS^-). In contrast Reis *et al.* (1992) found that only the undissociated form was inhibitory over a pH range of 6.2 to 6.6. The same conclusion was drawn by Visser (1995), whose results show that loss of activity has a better correlation with undissociated sulphide concentration than with total sulphide. This is in accord with the theory that only undissociated H_2S is able to penetrate the cell membrane (Speece, 1983). O'Flaherty *et al.* (1998) have studied the pH dependency of sulphide inhibition on various strains of SRB as well as mixed SRB. The general trend was that as pH is increased from 6.8 to 8.5, the SRB were able to tolerate higher concentrations of sulphide (Figure 2.15). This is in agreement with Visser (1995) who showed that the tolerable level of sulphide increased by about two fold when the pH was increased from 7.1 to 8.1. This further indicates that the undissociated sulphide, which is present at the lower pH range, and not total sulphide is inhibitory to SRB.

Furthermore care should be taken in interpreting the inhibition kinetics obtained from batch tests in which pH, sulphide and the limiting substrate cannot be controlled. Total sulphide has been shown to be inhibitory at concentrations as low as 0.08 kgm^{-3} in batch systems (McCartney and Oleskiewicz, 1991). In contrast to this Okabe *et al.* (1992) found, using *Desulfovibrio desulfuricans* in a continuous bioreactor at pH 7.0 and a dilution rate of 0.2 h^{-1} , that for total sulphide concentrations up to 0.60 kgm^{-3} both lactate utilization and cellular production were strongly inhibited. Further work by Okabe *et al.* (1995) using *Desulfovibrio desulfuricans* in a continuous bioreactor with lactate as the organic source at pH 7.0 showed that the maximum specific growth rate (μ_m) decreased from 0.33 h^{-1} to 0.21 h^{-1} as the total sulphide concentration was increased from 0.026 to 0.438 kgm^{-3} . They also noted that at the higher sulphide concentrations, cell yield decreased from $0.043 \text{ g cell/ g lactate}$ at a total sulphide concentration of 0.108 kgm^{-3} to $0.01 \text{ g cell/ g lactate}$ when the total sulphide was 0.437 kgm^{-3} . The lactate utilisation, however, remained unchanged. They offered two explanations for this phenomenon. Firstly, the decrease in bacterial concentration was attributed to lysis as a result of sulphide presence. Secondly, the energy was used to overcome the inhibition i.e. for maintenance and not for growth. However, above total sulphide concentration of 0.50 kgm^{-3} both lactate utilization and cellular production were inhibited as observed by their earlier work (Okabe *et al.*, 1992). Supporting the trend that sulphate reducers can acclimatise to sulphide in continuous systems, Omil *et al.* (1998) noticed that in the presence of acetate as an organic source SRB are able to acclimatise to a total sulphide concentration of 0.80 kgm^{-3} . In addition, Okabe *et al.* (1995) showed that maximum specific growth rates calculated for batch experiments were lower than those calculated for the chemostat at corresponding sulphide concentrations. Grady *et al.* (1996) note that in a continuous culture selection pressure results in an adaptation of the chemostat culture to changing environmental conditions.

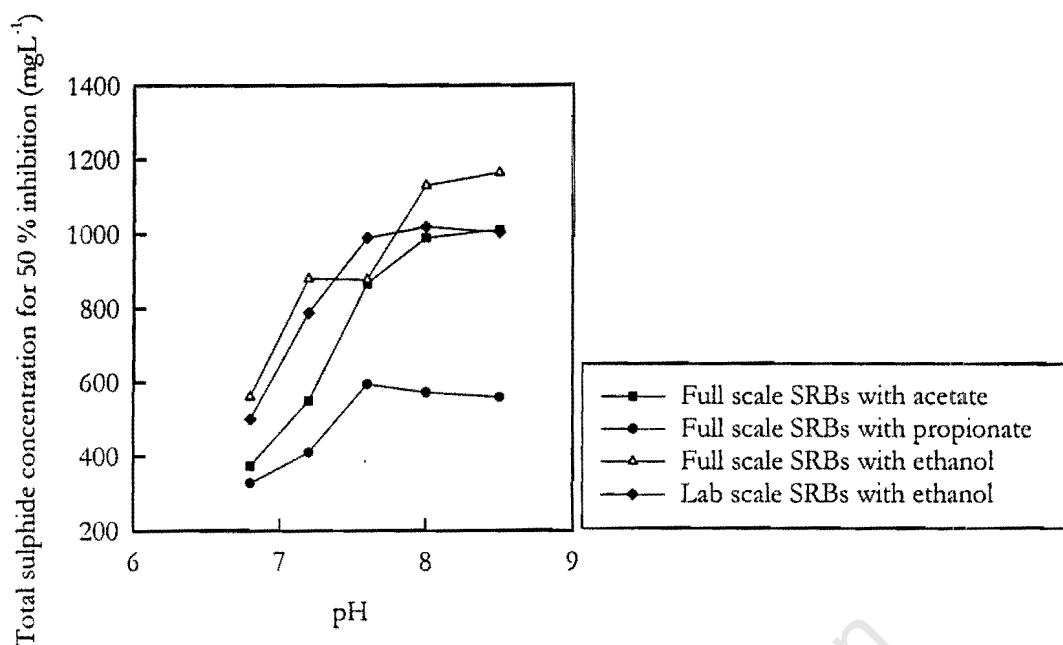


Figure 2.15. Tolerance levels of a mixed SRB culture to total sulphide as a function of pH (O'Flaherty *et al.*, 1998).

Metals

The toxicity of heavy metals on SRB activity has not been extensively studied. Since metal sulphides readily precipitate (Rouse, 1976; Jackson Moss and Duncan, 1990; White and Gadd, 1996). This can be seen from the sulphide solubility products presented in Table 2.13. Furthermore, the toxicity effects of metals are only mediated in the soluble state. Precipitation removes their availability at high concentrations. Consequently the toxicity of metals in a sulphate reducing environment will not be considered further for purposes of this study.

Table 2.13. Solubility products (pK_{sp}) of various metals at 25°C (Barnes *et al.*, 1992a, b).

Metal cation	pK_{sp}
Fe ²⁺	18.1
Cd ²⁺	25.8
Ag ⁺	50.1
Co ²⁺	21.3
Cu ²⁺	36.1
Mg ²⁺	Soluble
Ni ²⁺	19.4
Pb ²⁺	27.5
Zn ²⁺	24.7

Table 2.12. Inhibitory levels of sulphide as reported in different works.

Reference	Microorganisms	Organic	Temperature (°C)	pH	Sulphide (g L ⁻¹)	Reactor and mode	Observation
Karhardkar <i>et. al.</i> (1986)	<i>Desulfotomaculum</i>	lactate	35	7.0	0.064 TS ¹	SF, B	bacterial growth ceased
Hilton & Oleszkiewicz (1988)	mixed SRB (suspended sludge)	lactate	35	7.0	0.400 TS ¹	SF, B	50% decrease in sulphate reduction
McCartney & Oleszkiewicz (1991)	<i>Desulfovibrio desulfuricans</i>	lactate	35	7.2 – 7.6	0.080 TS	SF, B	50% inhibition of sulphate reduction
McCartney & Oleszkiewicz (1993)	mixed SRB (suspended sludge)	lactate	35	7.1 – 7.3	0.300 H ₂ S	STR, B	50% inhibition of sulphate reduction
				8.0	0.185 H ₂ S	STR, B	50% inhibition of sulphate reduction
Reis <i>et. al.</i> (1992)	<i>Desulfovibrio</i>	lactate	37	6.2 – 6.6	0.550 H ₂ S ¹	SF, B	complete inhibition of bacterial growth
Okabe <i>et. al.</i> (1992)	<i>Desulfovibrio desulfuricans</i>	lactate	35	7.0	0.600 TS ¹	STR, C	cellular yield decreased by 95% lactate utilisation decreased by 69%
	<i>Desulfovibrio desulfuricans</i>	lactate	35	7.0	0.440 TS ¹	STR, B	cellular yield decreased by 70% no effect on lactate utilisation
	mixed SRB (suspended sludge)	lactate	35	7.2 – 7.6	0.08 H ₂ S ¹	STR, B	50% inhibition of sulphate reduction

Reactor types: SF, shake flask; STR, stirred tank reactor; Mode of operation: B, batch; C, continuous

Where: TS, total sulphide; ¹ sulphide was added as opposed to produced

Table 2.21 (contd). Inhibitory levels of sulphide as reported in different works.

Reference	Microorganisms	Organic	Temperature (°C)	pH	Sulphide (g L ⁻¹)	Reactor and mode	Observation
Visser (1995)	mixed SRB population (granular)	acetate	35	7.2 – 7.4	0.171 H ₂ S	SF, B	50% decrease in bacterial activity
	mixed SRB population (granular)			8.1 – 8.3	0.057 H ₂ S	SF, B	50% decrease in bacterial activity
O' Flaherty <i>et al.</i> (1998)	mixed SRB adapted to sulphate	acetate	30 - 37	6.8 – 8.5	0.374 – 1.011 TS ¹	UFF, C	50% decrease in bacterial activity
		propionate	30 - 37	6.8 – 8.5	0.328 – 0.559 TS ¹	UFF, C	50% decrease in bacterial activity
		butyrate	30 - 37	6.8 – 8.5	0.593 – 2.059 TS ¹	UFF, C	50% decrease in bacterial activity
		ethanol	30 - 37	6.8 – 8.5	0.561 – 1.164 TS ¹	UFF, C	50% decrease in bacterial activity
		Butyrate	30-37	6.8 – 8.5	0.467 – 0.988 TS ¹	SF, B	50% decrease in bacterial activity
		ethanol	30-37	6.8 – 8.5	0.500 – 1.004 TS ¹	SF, B	50% decrease in bacterial activity
	mixed SRB not adapted to sulphate	butyrate	30-37	6.8 – 8.5	0.489 – 0.960 TS ¹	SF, B	50% decrease in bacterial activity
	ethanol	30-37	6.8 – 8.5	0.0.544 – 1.124 TS ¹	SF, B	50% decrease in bacterial activity	

Reactor types: SF, shake flask; STR, stirred tank reactor; UFF, upflow fixed film; Mode of operation: B, batch; C, continuous

Where: TS, total sulphide; ¹ sulphide was added as opposed to produced

2.2.5 ANAEROBIC TREATMENT OF SULPHATE CONTAINING WASTEWATERS

The biological sulphate reduction process has potential for treatment of wastes containing both metals and sulphates. A generic example of a treatment scheme is shown in Figure 2.16. The first step in the treatment process is the precipitation of metals with the sulphide formed in the second reactor as metal sulphides. In the anaerobic reactor the sulphate is reduced to sulphide and the COD content of the effluent is reduced. Further reduction of the COD occurs in the final stage, an aerobic reactor.

The sulphate reduction process has been implemented on a commercial scale to treat metal containing sulphate effluents despite limited information regarding process kinetics of the reduction process. Studies related to full-scale operation primarily include feasibility studies on treatment of particular wastes, reactor design and choice of electron donor and organic source. These are discussed in the following sections.

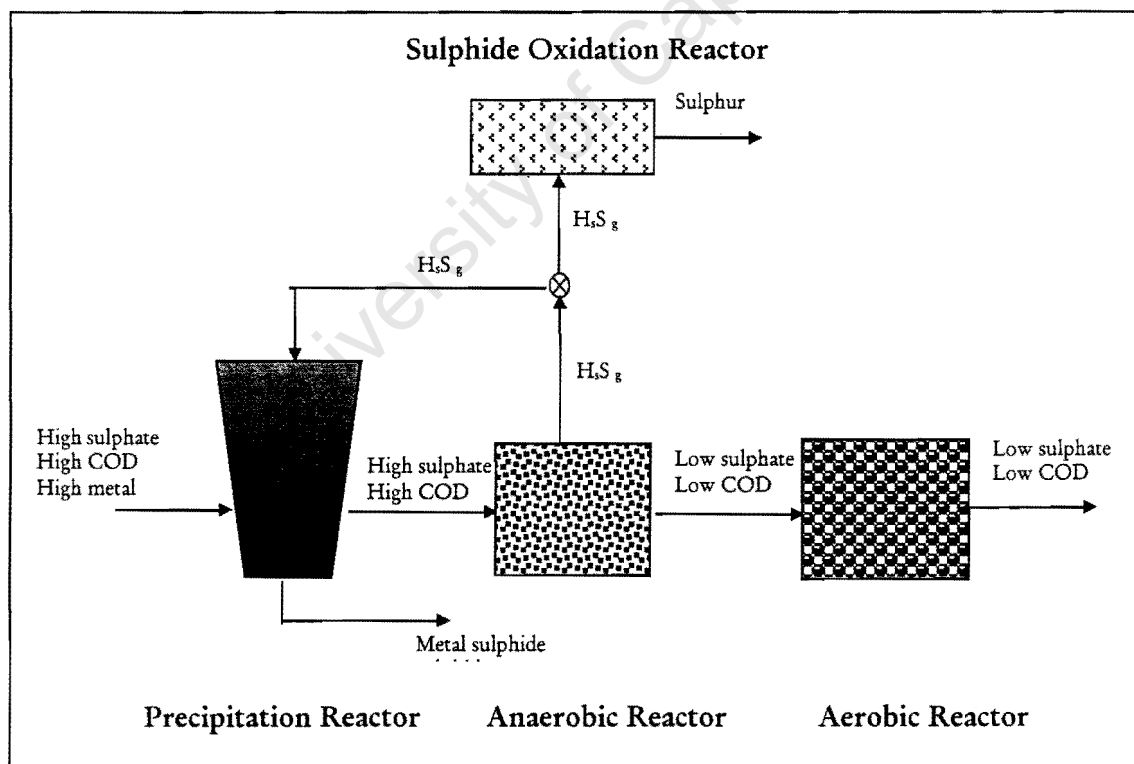


Figure 2.16. Proposed generic treatment scheme for sulphate and metal containing effluents.

2.2.5.1 ELECTRON DONORS AND ORGANIC SOURCES UTILISED FOR THE TREATMENT OF VARIOUS EFFLUENTS

Sewage

The importance of anaerobic sulphate reduction was initially noticed in the treatment of sewage effluents. Initially sulphate reduction was seen as undesirable process, scavenging electron donors that would otherwise be consumed by the methanogenesis. The concomitant reduction of sulphate in treatment of sewage effluent illustrated sewage sludge is a viable organic source and electron donor for sulphate reduction. Maree & Strydom (1985) showed that mine water could be treated in a packed bed reactor using raw sewage effluent as the organic source. At an inlet sulphate concentration of 1.34 kgm^{-3} , 78 % removal was achieved. Zinc refinery effluent has been treated using sewage sludge (Barnes *et al.*, 1992a). Using a synthetic effluent supplemented with sewage sludge, Sanchez *et al.* (1997), achieved 63 % sulphate removal in a 13-L UASB at an inlet sulphate concentration of 0.084 kgm^{-3} . Table 2.14 summarises the work discussed.

Table 2.14. Summary of effluents treated using sewage as the organic source and electron donor .

Reference	Effluent treated
Maree and Strydom (1985)	Mine water
Barnes <i>et al.</i> (1992a)	zinc refinery effluent

Organic Acids

In the late 1970's the utilisation of sulphate reduction as a means for treatment of organic and sulphate containing effluents was studied. The focus was primarily on the choice of low cost carbon sources that favoured sulphate reduction over methane production. Table 2.15 summarises the various organic compounds used. Further observations made by the researches are discussed.

Table 2.15. Summary of organic acids used as the organic source and electron donor for sulphate reduction.

Reference	Organic acid utilised
Middleton and Lawrence (1977)	acetic acid
Isa <i>et al.</i> (1986)	acetic and formic acid
Parkin <i>et al.</i> (1991)	propionic and acetic acid
Gupta <i>et al.</i> (1994a)	acetic and formic acid
Colleran <i>et al.</i> (1994)	citric acid
Omil <i>et al.</i> (1998)	mixed volatile fatty acids

Pioneering work was done by Middleton and Lawrence (1977). These authors examined the kinetics of sulphate reduction in a 9 L CSTR using acetic acid as the organic source. They concluded that a Monod-type kinetic model could describe the growth of SRB in this system. A study on the effect of three organic compounds, acetate, ethanol and formate, on sulphate reduction was carried out by Isa *et al.* (1986). Using a mixed population of methanogens and sulphate reducers, the production of sulphide was insignificant indicating that the methanogens were more active. In the presence of sulphate reducers only, the percentage sulphate removal increased to 71.5 %. The production rate of hydrogen and carbon dioxide was higher in the reactor fed with formate than the reactors fed with ethanol and acetate. Using a UASB fed with propionate and acetate, Parkin *et al.* (1991) showed that the reactor fed on propionate failed (sulphate and propionate removal ceased) sooner than the reactor fed with acetate. This indicates that the propionate utilising SRB are less robust than either the acetate utilising SRB or MPB. Gupta *et al.* (1994a, b), treating a synthetic medium, with acetic acid, methanol and formic acid, showed that acetate resulted in the best sulphate removal (95 %) but with a low biomass growth rate. The reactors fed with methanol showed no signs of sulphate reduction.

Effluent from a citric acid plant was treated by sulphate reduction in a fixed bed reactor (Colleran *et al.*, 1994). The influent sulphate concentration was 3.4 kgm⁻³ and the sulphate removal was 93 %. No metals were present and the bacteria took approximately 3 months to adapt to high levels of sulphide. Recently Omil *et al.* (1998) have investigated whether sulphate reducers dominate during the treatment of volatile fatty acids. The granular upflow sludge bed reactors were run at 30°C and pH 8. In the reactors fed with mixed volatile fatty acids, under sulphate limiting conditions, no lag phase was evident for sulphate reduction and the extent of sulphate reduction was 40 %. When acetate was used a long lag time was

experienced under sulphate limitation and the maximum sulphate removal observed was 70 %. In the presence of excess sulphate no lag phase was observed and the maximum removal was 38 %. A further observation of this work was that sulphate reducers predominated, after prolonged periods of reactor operation, in chemostats fed with acetate.

Ethanol

Ethanol, a cheap and readily available organic source, is used as a carbon source in the Paques Process (Paques Environmental Systems, 1999). The exploratory work carried out by Barnes *et al.* (1992a,b) indicated that sulphate removal up to 91 % could be achieved in a metal refining effluent using ethanol. The reactor was run at a retention time of 28 h and a temperature of 31°C.

Complex Organics

The use of various cheap and readily available complex organic sources has been documented. Table 2.16 details the complex organic compounds used as the organic and electron source for sulphate reduction.

Table 2.16. Summary of complex organic compounds used as the organic source and electron donor for sulphate reduction.

Reference	Complex organic compounds
Maree and Strydom (1985, 1987)	sugar and pulp mill effluent
Maree (1987)	molasses
Hammack <i>et al.</i> (1993)	mushroom compost with lactate

The treatment of mine water using sugar and pulp mill effluents as the organic source was studied by Maree and Strydom (1985) in a packed bed bioreactor. At sulphate concentrations of 2.9 and 1.4 kgm⁻³ in the mine water, 90 % and 67 % sulphate removal were achieved respectively. The mine water contained trace amounts of metals including lead, nickel, aluminium, boron, cobalt, cadmium, iron and manganese. Lead was reduced from 0.09 kgm⁻³ to 0.025 kgm⁻³ and nickel from 0.115 kgm⁻³ to 0.005 kgm⁻³. None of the other metals were removed. In further work reported by Maree (1987) molasses was used as an organic source for sulphate reduction. Using a sludge blanket reactor at a retention time of 15 h and an inlet sulphate concentration of 2.4 kgm⁻³, the sulphate removal was 67 % at a molasses concentration of 2 mL/L. When the molasses concentration was increased to 3 mL/L, the sulphate reduction increased to

92 %. Using a packed bed with dolomite pebbles as bacterial support, the sulphate removal was 42 % at a retention time of 20 h in the presence of 2mL/L molasses as the organic. The packed bed was very sensitive to changes in organic and sulphate loading rates. When the retention time was decreased to 15 h the sulphate removal decreased to 7 %. This clearly shows the dependence of the sulphate reduction process on loading rate. The lower rate observed for the immobilised reactor system is due to the fact that SRB do not attach as readily as methanogens. This is discussed in more detail in Section 2.2.6.

A 98 % sulphate removal was reported from mine water containing 2.0 kgm⁻³ of sulphate when mushroom compost supplemented with lactate was used as the organic source (Hammack *et al.*, 1993).

Hydrogen and carbon monoxide/carbon dioxide

Sulphate reducers are also able to utilise hydrogen and carbon monoxide as the electron donor and carbon source respectively (van Houten *et al.*, 1994). Du Preez and Maree (1994) investigated this on a pilot scale when treating a synthetic effluent in a reactor packed with ash pellets. At flowrates in the range 40 Ld⁻¹ to 120 Ld⁻¹, 95 % sulphate was removed from a feed containing 2.0 kgm⁻³ sulphate. In batch tests complete sulphate removal was achieved in 80 h. Herrera *et al.* (1997) used hydrogen and carbon dioxide as the carbon and energy source in continuous reactors run at a retention time of 12 h and a feed sulphate concentration of 3.36 kgm⁻³. The sulphate reduction under these conditions varied between 12 and 30 %.

2.2.5.2 REACTOR DESIGN

Of the four reactor types available for the treatment of sulphate containing wastewaters the upflow anaerobic sludge bed (UASB) has been the most widely used (Maree and Strydom, 1985; Hilton and Archer, 1988; Barnes *et al.*, 1992; Alphenaar *et al.*, 1993; du Preez and Maree, 1994; Sanchez *et al.*, 1997; Omil *et al.*, 1998). Sulphate reduction rates obtained in the UASB reactor are generally higher than those obtained in CSTRs. The other reactor types, namely the packed bed and the racked sludge bed, were not used as extensively as the UASB and the CSTR.

Table 2.17 compares the performance of these bioreactors across different waste streams containing sulphate. For purposes of direct comparison, performances of the systems are described by the volumetric sulphate reduction rate.

Table 2.17. Performance of various bioreactors used for the treatment of sulphate containing waste streams.

Reference	Effluent treated	Organic utilised	Origin of inoculum	Reactor type and mode	Temp. (°C)	pH	Influent sulphate (kgm ⁻³)	HRT (h)	Volumetric reduction rate (kgm ⁻³ hr)
Burgess & Wood (1961)*	primary sewage sludge	sewage	sewage sludge	-	-	-	-	-	0.188
Sadana & Morey (1962)*	primary sewage sludge	sewage	sewage sludge	-	-	-	-	-	0.100
Rabolina (1971)*	primary sewage sludge	sewage	sewage sludge	-	-	-	-	-	0.116
Middleton & Lawrence (1977)	synthetic	acetic acid	wastewater treatment plant	STR (9 L), C	31	-	1.10	-	0.012
Hilton <i>et. al.</i> (1985)	synthetic	whey	-	UASB (2 L), B	35	-	-	-	0.438
Maree & Strydom (1985)	mine water	sugar	activated sludge	UASB (1 L), C	30	7	2.00	11	0.164
	Mine water	pulp mill effluent (COD = 2.9 kgm ⁻³)	activated sludge	UASB (1 L), C	30	7	1.35	11	0.082
	Mine water	pulp mill effluent (COD = 3.6 kgm ⁻³)	activated sludge	UASB (1 L), C	30	7	1.35	11	0.107
	Mine water	raw sewage sludge (COD = 5 kgm ⁻³)	activated sludge	UASB (1 L), C	30	7	1.35	11	0.095
	Mine water	raw sewage sludge (COD = 5.6 kgm ⁻³)	activated sludge	UASB (1 L), C	30	7	1.35	11	0.118

* Referenced in Middleton and Lawrence (1977). Reactor types: STR, stirred tanks; UASB, upflow anaerobic sludge bed reactor, C; continuous; B, batch

Table 2.17 (contd). Performance of various bioreactors used for the treatment of sulphate containing waste streams.

Reference	Effluent treated	Organic utilised	Origin of inoculum	Reactor	Temp (°C)	pH	Influent sulphate (kgm ⁻³)	HRT (hrs)	Volumetric reduction rate (kgm ⁻³ hr)
Maree (1987)	synthetic	molasses (2 mL/L)	-	PBR (1 L) dolomite pebbles), C	31	7.0	0.90	20	0.019
	synthetic	molasses (2 mL/L)	-	PBR (1 L) (dolomite pebbles), C	31	7.0	0.90	15	0.004
	synthetic	molasses (3 mL/L)	-	PBR (1L) dolomite pebbles), C	31	7.0	0.90	20	0.035
	synthetic	molasses (2 mL/L)	-	SBR(1 L), C	31	7.0	2.50	15	0.113
	synthetic	molasses (3 mL/L)	-	SBR (1 L), C	31	7.0	2.50	15	0.153
Ueki <i>et. al.</i> (1989)	animal waste	acetic acid	-	STR, B	30	-	2.40	-	0.002
	animal waste	propionic acid	-	STR, B	30	-	2.40	-	0.018
Hammack <i>et al.</i> (1992)	synthetic	lactic acid	spent mushroom compost	CR, C	-	4.5	0.20	12	0.001

Reactor types: STR, stirred tanks; UASB, upflow anaerobic sludge bed reactor; PBR, packed bed reactor; SBR, sludge bed reactor; CR, colun reactor, RSB, racked sludge bed, C; continuous; B, batch

Table 2.17 (contd). Performance of various bioreactors used for the treatment of sulphate containing waste streams.

Reference	Effluent treated	Organic utilised	Origin of inoculum	Reactor	Temp (°C)	pH	Influent sulphate (kgm ⁻³)	HRT (hrs)	Volumetric reduction rate (kgm ⁻³ hr)
Barnes <i>et. al.</i> (1992a)	zinc refinery effluent	lactic acid (1.14 kgm ⁻³)	sewage sludge	RSB (0.75 L), C	22	7.0	1.59	33	0.164
	zinc refinery effluent	lactic acid (3.38 kgm ⁻³)	sewage sludge	RSB (0.75 L), C	22	7.0	1.59	31	0.035
	zinc refinery effluent	lactic acid (1.14 kgm ⁻³)	sewage sludge	RSB (0.75 L), C	22	7.0	1.59	5	0.078
	zinc refinery effluent	lactic acid (1.14 kgm ⁻³)	sewage sludge	RSB (0.75 L), C	31	7.0	1.59	29	0.021
	zinc refinery effluent	lactic acid (3.38 kgm ⁻³)	sewage sludge	RSB (0.75 L), C	22	7.0	1.59	28	0.051
	zinc refinery effluent	lactic acid (1.14 kgm ⁻³)	sewage sludge	RSB (0.75 L), C	22	7.0	1.59	5	0.076
Barnes <i>et. al.</i> (1992b)	zinc refinery effluent	ethanol	sewage sludge	UASB (1.5 L), C	35	7.0	1.72	8	0.196
Stucki <i>et al</i> (1993)	sulphuric acid waste	acetate	<i>D. acetoxidans</i> ; <i>D. postgatei</i>	UPB, C	32	7.5 – 8.5	0.17	-	2.700

Reactor types: STR, stirred tanks; UASB, upflow anaerobic sludge bed reactor; UPB, upflow packed bed, PBR, packed bed reactor; SBR, sludge bed reactor; CR, column reactor, RSB, racked sludge bed, C; continuous; B, batch

Table 2.17 (contd). Performance of various bioreactors used for the treatment of sulphate containing waste streams.

Reference	Effluent treated	Organic utilised	Origin of inoculum	Reactor	Temp (°C)	pH	Influent sulphate (kgm ⁻³)	HRT (hrs)	Volumetric reduction rate (kgm ⁻³ hr)
du Preez & Marce (1994)	synthetic	H ₂ /CO	sulphate reducing pilot plant	UASB (pelletized ash)	35	7	2.00	38	0.050
	synthetic	H ₂ /CO ₂	sulphate reducing pilot plant	STR (0.5 L) (raschig rings), B	35	7	1.65	B, 80 d	0.021
Colleran <i>et al</i> (1994)	synthetic	beet molasses	-	UASB (full) C	-	-	3.43	33.6	0.095
	synthetic	beet molasses	-	UASB (lab), C	-	-	4.00	33.6	0.112
Christensen <i>et al.</i> (1996)	mine water	whey	cow manure	CR (2 L), B	15	-	-3.31	B 2d	0.0002
Sanchez <i>et al.</i> , (1997)	synthetic	dairy plant effluent	sewage sludge	UASB (13 L), C	31	7	0.84	19	0.043
Herrera <i>et al.</i> (1997)	synthetic	H ₂ /CO ₂	sewage sludge	STR (1 l), B	30	7	3.36	-	0.033

Reactor types: STR, stirred tanks; UASB, upflow anaerobic sludge bed reactor; PBR, packed bed reactor; SBR, sludge bed reactor; CR, colun reactor, RSB, racked sludge bed, C, continuous; B, batch

Table 2.17 (contd). Performance of various bioreactors used for the treatment of sulphate containing waste streams.

Reference	Effluent treated	Organic utilised	Origin of inoculum	Reactor	Temp (°C)	pH	Influent sulphate (kgm ⁻³)	HRT (hrs)	Volumetric reduction rate (kgm ⁻³ hr)
Omil <i>et. al.</i> (1998)	synthetic	VFA	pilot plant treating acid water	UASB (20 L), C	30	8	1.6	8.8	0.361
	synthetic	VFA	UASB (20 L)	UASB (0.7 L), C	30	8	8.3	6.4	0.783
	synthetic	acetic acid	pilot plant treating acid water	UASB (10 L), C	30	8	2.9	7.3	0.280
	synthetic	acetic acid	UASB (10 L)	UASB (1.7 L), C	30	8	3.5	3.4	0.625

Reactor types: STR, stirred tanks; UASB, upflow anaerobic sludge bed reactor; PBR, packed bed reactor; SBR, sludge bed reactor; CR, column reactor, RSB, racked sludge bed, C; continuous; B, batch; EGSB, expanded granular sludge bed reactor.

2.2.6 INTERACTIONS BETWEEN SULPHATE REDUCERS AND METHANOGENS

To maximise sulphate reduction with minimal use of organic source, the interactions between methanogens and sulphate reducers must be used to the best advantage. Research has focussed on the competition between the two groups for organic substrates such as acetate, propionate and butyrate, and hydrogen (Bryant *et al.*, 1977; Oremland and Polcin, 1982; Schönheit *et al.*, 1982; Capone *et al.*, 1983; Lovley and Klug, 1983; Traore *et al.*, 1983; Hoeks *et al.*, 1984; Lupton and Zeikus, 1984; Yoda *et al.* 1987; Yadav and Archer, 1988; Parkin *et al.*, 1990; Choi and Rim, 1991; Mizuno *et al.*, 1994; Uberoi and Bhattacharya, 1995; Bhattacharya *et al.*, 1996; Li *et al.*, 1996; Omil *et al.*, 1996; 1997; 1998. Raskin *et al.*, 1996; Shin *et al.*, 1996). In addition, the effect of external factors such as sulphide concentration, organic loading rate, ratio of COD to sulphate, pH and reactor configuration has been addressed. These are discussed in the following sections.

2.2.6.1 COMPETITION FOR ELECTRON DONORS

Hydrogen

Thermodynamically the reduction of sulphate using hydrogen is favoured over the formation of methane from hydrogen (Table 2.18). Table 2.19 represents the kinetic coefficients reported for growth of SRB and methanogens on hydrogen, as reported in the different works. It is apparent that the SRB have higher growth rates as well as a stronger affinity for hydrogen. As a result the SRB are able to outcompete the methanogens for hydrogen.

Table 2.18. ΔG° for sulphide and methane production using hydrogen as the electron donor source.

	Products	ΔG° (kJ/mole SO_4^{2-})
SRB	HS, H ₂ O	-152
MPB	CH ₄ , H ₂ O	-135

Table 2.19. Kinetic parameters for the growth of SRB and methanogens on hydrogen.

	K_s mM	μ_{max} d ⁻¹	Y g VSS/mol acetate	pH	Temp °C	Reference
Sulphate Reducers	3.30	1.37	0.85	6.7	37	Robinson and Tiedje (1984)
		4.75	2.50	7.0	37	Visser (1995)
		3.33	2.25	7.0	37	Visser (1995)
	4.00		1.10	7.0	30	Lupton and Zeikus (1984)
		2.56	2.00	7.0	37	Visser (1995)
		1.39	1.75	7.0	34	Visser (1995)
Methanogens	6.6	1.27	0.20	6.7	37	Robinson and Tiedje (1984)
		1.11	1.60	7.0	37	Zeikus <i>et al.</i> , (1975)
		0.34				Visser (1995)
	2.0	2.0	0.80			Visser (1995)
	14		0.60	7.0	30	Lupton and Zeikus (1984)
	38	1.4	0.65	7.0	33	Zehnder <i>et al.</i> , (1977)

Acetate

The consumption of acetate by SRB is coupled to the reduction of sulphate. If sulphate is present, the SRB metabolise acetate. Thermodynamically, in the presence of sufficient sulphate, the SRB should dominate (Table 2.20). Table 2.21 summarises the kinetic coefficients for growth of SRB and methanogens on acetate. In CSTRs the SRB have been shown to dominate as suggested by affinity for sulphate (Middleton and Lawrence, 1977; Visser, 1995). In immobilized cell reactors the situation is not clear. Using reactors with biomass retention Mulder *et al.* (1984) and Rinzema (1988) documented that acetate was completely converted to methane. In CSTRs Rinzema (1988), Choi *et al.* (1991) and Stucki *et al.* (1992) reported the predominance of SRB in the presence of acetate. Various theories have been proposed to explain the apparent advantage of MPB over SRB in biomass retention systems. One explanation is the superior capability of MPB to colonise support material (Isa *et al.*, 1986). Others have proposed that the duration of experiments should be long enough to allow the SRB to dominate under nutrient limited conditions (Omil *et al.* 1996, 1997, 1998)

Table 2.20. ΔG° for sulphide and methane production using acetate as the carbon and electron donor source.

	Products	ΔG° (kJ/mole SO_4^{2-})
SRB	H_2O , CO_2 , HCO_3^- , S^{2-}	-47.6
MPB	CH_4 , HCO_3^-	-31

Table 2.21. Kinetic coefficients for growth of SRB and methanogens on acetate.

	K_s mM	μ_{\max} d^{-1}	Y g VSS/mol Acetate	pH	Temp $^{\circ}\text{C}$	Reference
Sulphate Reducers		1.03	2.56	-	28	Visser (1995)
		0.55	5.52	7.1	36	Widdel and Pfenning (1981)
		1.44	7.55	7.1	36	Widdel and Pfenning (1981)
		0.55	-	7.6	30	Widdel and Pfenning (1981)
	0.10	0.51	-	-	31	Midleton <i>et al.</i> , (1977)
	0.17	0.015	3.70	7.5	30	Yoda <i>et al.</i> , (1987)
Methanogens	0.44	0.11	1.47	7.6	37	Visser (1995)
	1.20	0.69	1.15	7.2	37	Visser (1995)
	0.69	2.40	-	6.3	35	Visser (1995)
	5.60	0.26	3.2	-	30	Lawrence <i>et al.</i> , (1969)
	0.55	0.037	-	7.5	30	Yoda <i>et al.</i> , (1987)

Propionate and butyrate

Propionate is an intermediate produced from amino acid and sugar utilisation (Figure 2.6). Little is reported in the literature on the competition for propionate and butyrate. Smith and Klug (1981) showed that SRB have a higher specific growth rate than methanogens, provided that the sulphate propionate or butyrate are not limiting. Ueki *et al.* (1988, 1989) found that, in a mixed culture growing on lactate and acetate, the presence of SRB is essential for the degradation of propionate.

2.2.6.2 EFFECT OF EXTERNAL PARAMETERS

Sulphide Concentration

It has been reported that SRB are inhibited by the total dissolved sulphide concentration (Section 2.2.4.3), whereas the undissociated hydrogen sulphide was toxic to MPB. Hilton and Oleskiewicz (1988) reported a situation in which the total soluble sulphide (TSS) was high and the unionised hydrogen sulphide low and the formation of methane was dominant over reduction of sulphate.

Maillecheruva and Parkin (1996) proposed that sulphide is increasingly more toxic to the following bacteria: hydrogen utilising MPB < hydrogen utilising SRB < incomplete propionate utilising SRB < acetoclastic MPB < acetate utilising SRB. From this they concluded that the thermodynamic and kinetic advantage of acetate utilising SRB was negated by their sensitivity to sulphide. Uberoi and Bhattacharya (1995) found that the 0.10 kgm⁻³ of unionised hydrogen sulphide was more toxic to SRB than MPB in batch studies. Grown in chemostats at low retention time SRB could tolerate levels of unionised hydrogen sulphide (H₂S) of 0.178 kgm⁻³, suggesting acclimitisation of the SRB.

The debate around the sulphide toxicity remains open, the key points being:

- the nature of the exact species of sulphide that is toxic to SRB remains unknown,
- the sulphide toxicity is pH dependent, and
- the extent of toxicity varies for chemostat and batch cultures.

As a consequence of the above, the outcome of competition between SRB and MPB in a sulphide environment cannot be predicted.

Ratio of COD to sulphate

Theoretically a COD to sulphate ratio of 0.67 indicates a stoichiometrically balanced situation for the complete removal of organic and sulphate components. Above this ratio, in the absence of methanogenic inhibitors (BESA), methanogenesis will dominate and below it sulphate reduction will dominate. In practise, a different scenario exists. Hulshoff Pol *et al.* (1998) have reported a critical COD to sulphate

ratio of 10 below which sulphate reduction predominates. Speece (1996) quotes critical ratios of between 1 and 2 to avoid competition between SRB and MPB. Choi and Rim (1991) reported the predominance of SRB at a COD to sulphate ratio below 0.4, strong competition between SRB and MPB at ratios between 1.7 and 2.7 and the predominance of MPB when the ratio was greater than 2.7.

pH

Sulphate reduction can occur at pH values ranging from 6 to 9 (Section 2.2.4.4) whereas methanogenesis favours pH of 6 – 7 (Section 2.2.3.1). Visser (1995) showed that SRB are less sensitive to pH variations than MPB. Sulphate reduction occurred at pH greater than 8, whereas at pH values between 6.75 and 7.4 both the SRB and MPB were active. In contrast Hilton and Oleszkiewicz (1988) observed the dominance of MPB at high pH values in a UASB at long retention times.

Reactor configuration

Under high acetate concentration MPB have been reported to have superior adhesion properties over SRB (Speece, 1996). Isa *et al.* (1986) reported that planktonic SRB were 30 fold less than those in the reactor biofilm whereas for MPB the ratio was 200 fold. This indicates that MPB have a better ability to be retained in a biofilm than SRB. Hence biofilm reactors provide protection to the MPB over the SRB (Visser *et al.*, 1993b). In other words, reactor design may contribute in determining the dominant population.

2.3 CHAPTER SUMMARY

In this chapter processes for the treatment of sulphate containing effluents were reviewed. It became clear that the anaerobic sulphate reducing process showed potential as an economic method of treating effluents containing sulphates. The general anaerobic degradation process was discussed with particular attention paid to the sulphate reduction process. The sulphate reducing microbes were discussed with regards to the environmental conditions that favour the various species. The treatment of sulphate containing wastes as given in the open in the literature was presented. This took the form of discussing

the research with respect to the organic source utilised. Of importance in the optimisation of the sulphate reduction process was the competition between SRB and MPB for the organic source. The outcome of this competition was shown to depend on numerous factors including sulphide concentration, COD to sulphate ratio, pH and reactor configuration.

This chapter has highlighted the various research studies done on sulphate reduction as a process for treating sulphate containing effluents. It has shown limited kinetic data is available and no rigorous continuous bioreactor kinetic studies on the anaerobic sulphate reduction process have been presented in the open. In the next chapter the objectives of this study (based on the background presented in this chapter) and the experimental programme are presented.

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CHAPTER 3

OBJECTIVES AND EXPERIMENTAL PROGRAMME

In this Chapter the reader is given the rationale for the work presented in the thesis. Against the background of the literature presented in the previous section, the objectives of the research are highlighted. Based on these objectives, the experimental programme is outlined.

3.1 OBJECTIVES OF THE RESEARCH

A review of the literature, presented in the previous chapter, has revealed that despite considerable research in the area of biological reduction of sulphate to sulphide, the kinetics of the reaction have not been studied rigorously and a comprehensive kinetic expression for this biological reaction, which includes dependency of inlet sulphate concentration and temperature, is not available in the literature. Owing to the successful implementation of anaerobic sulphate reduction on a large scale being dependent on the understanding of the reaction kinetics and the factors that affect the kinetics the development of a

model describing the kinetics of reaction over a wide range of physicochemical conditions was set out as the research objective of this work.

Biological reduction of sulphate to sulphide, though attractive compared with chemical alternatives because of its lower environmental impacts, has not yet been extensively used commercially. Numerous laboratory and pilot plant studies have been carried out. These have focussed primarily on the carbon sources to optimise sulphate reduction relative to methanogenesis. Kinetic studies using pure culture have also been performed, but there is a lack of rigorous data regarding the effect of sulphate and temperature on the kinetics of biological sulphate reduction and bacterial growth.

Thus the overall objective of this work was to **investigate and develop a kinetic model to describe the effect of feed sulphate concentration and temperature on the kinetics of a mixed sulphate reducing population in a continuous bioreactor.**

3.2 ACHIEVEMENT OF OBJECTIVES

The objectives of this work was achieved in four phases. The first three phases were experimental and the fourth was modelling. Figure 3.1 provides a diagrammatical of the experimental phase and how this informs the development of the kinetic model.

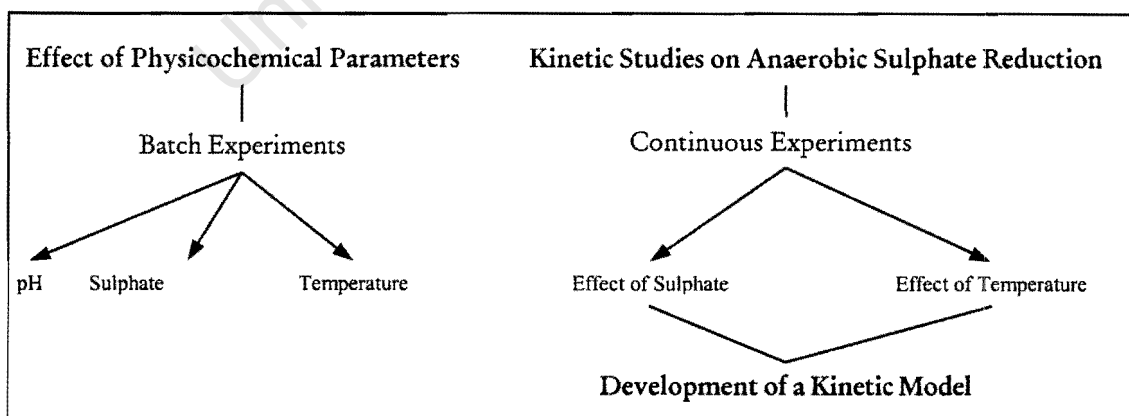


Figure 3.1. Representation of how the objectives of this work were achieved. There were three experimental phases and one modelling phase

3.2.1 EXPERIMENTAL PHASE

From the literature presented in the previous section it was evident that the physical parameters for optimum sulphate reduction was microbial culture specific. Furthermore, the literature review also revealed that the temperature and pH had a significant effect on the sulphate reduction process. Identification of the optimum operating conditions in terms of temperature, pH and feed sulphate concentration for the SRB culture used for this research was therefore the first step in this study. Thereafter the effect of feed sulphate concentration on the kinetics of microbial sulphate reduction was studied in continuous bioreactors. Five feed sulphate concentrations were studied to provide a good spread of data to allow for the development of a meaningful model. Finally, using the temperature range ascertained from the batch studies continuous bioreactor experiments were carried out at four temperatures to ascertain the effect of temperature on the kinetics of anaerobic sulphate reduction.

3.2.2 MODEL DEVELOPMENT

Using the kinetic data obtained from the continuous bioreactors that were operated at five feed sulphate concentrations and four temperatures, a kinetic model was developed. The kinetic model took into account the effect of feed sulphate concentration and temperature on microbial growth and the kinetics of sulphate conversion.

3.3 CHAPTER SUMMARY

In this chapter the objectives of the work and experimental protocol were outlined. The next chapter discusses in detail how the objectives of this study were achieved by presenting the experimental programme, equipment used and analytics techniques.

CHAPTER 4

MATERIALS AND METHODS

This chapter provides the reader with detail on the materials used and experimental methodology. Initially the microorganisms used, their growth media and conditions are discussed. Thereafter the methodology used for the batch experiments is outlined. This is followed by a description of the continuous bioreactor experiments. Finally all analytical techniques used during the experimental programme are detailed.

4.1 MICROORGANISMS

A mixed culture of anaerobic bacteria consisting of acid producers, methane producers and sulphate reducers was obtained from a sulphate-reducing reactor in operation at the CSIR (Council for Scientific and Industrial Research) in Pretoria, South Africa. The researchers at the CSIR initially obtained the culture from a sewage plant and gradually acclimatised it to sulphate, as part of an experimental programme (Maree, 1987). The reactors used at the CSIR to acclimatise the bacteria to sulphate were run on a complex organic medium.

4.1.1 MEDIUM

A soluble complex medium was used (Table 4.1). It is biodegradable and has been used to grow anaerobic microorganisms for long periods of time (Bull, *et al.*, 1984; Grobicki, 1989). All reagents were analytical grade. Sulphate was added in the form of sodium sulphate, the concentration of which varied depending on the experiment being done. The organic source used, glucose or acetate, and its concentration are specified for each experiment.

The medium was sterilised by autoclaving at 121°C and 15 psig for 20 min. To prevent reaction and precipitation, the organic compounds, mineral salts and metal salts were autoclaved separately and combined when cool. Lab-Lemco, an Oxoid product was added to provide nitrogen as protein.

Table 4.1. Composition of medium used for growth of anaerobic mixed culture.

Components	Weight (g)
Peptone	0.400
Lab-Lemco	0.133
K ₂ HPO ₄	0.040
NaHCO ₃	1.250
Deionised Water	500 ml
Trace Metals	
CoCl ₂ .6H ₂ O	0.00595
FeCl ₂ .4H ₂ O	0.03925
MnCl ₂ .4H ₂ O	0.00188
NaMoO ₄ .2H ₂ O	0.00188
NiCl ₂ .6H ₂ O	0.00225
Deionised Water	500 mL

4.1.2 CULTURE CONDITIONS

A stock anaerobic culture was maintained in 1-litre bottles, sealed with O-rings, using 500 mL of liquid medium and 500 mL inoculum. The media used is presented in Table 4.1. Glucose, at a concentration of 1.33 kgm⁻³ was used as the organic source and the sulphate concentration was 2.50 kgm⁻³. The cultures

were incubated under anaerobic conditions at 35°C and at 160 rpm on a rotary shaker. Sub-cultures were prepared, at ten day intervals as inocula and for stock culture maintenance. The stock culture was incubated for a period of ten days and kept at 4°C. Nitrogen was used to strip any air, hydrogen sulphide, methane, and carbon dioxide from the culture directly after inoculation. If necessary, the pH of the inoculated media was adjusted to pH 7.0 using hydrochloric acid or sodium hydroxide.

4.1.3 EXPERIMENTAL TECHNIQUES FOR IDENTIFICATION OF MICROORGANISMS IN THE BIOREACTOR

Batch experiments were done using the mixed anaerobic population i.e. acid producers, methane producers and sulphate reducers. It was assumed that for the duration of the batch run the population did not vary markedly.

The continuous reactors were initially inoculated with the mixed anaerobic culture. After running the reactors continuously on sulphate and acetate for a period of 4 months the acid producers and methanogens were washed out, as there was no substrate for them. A mixed sulphate reducing consortia remained in the continuous bioreactor. Experiments were done to ascertain the presence or absence of acid producers and methane producers.

4.1.3.1 ANALYSING FOR THE PRESENCE OF METHANE PRODUCERS

A test for the presence of methane producers is to analyse the biogas produced for the presence of methane. A 20 µL gas sample was taken from the reactor headspace using a gas tight syringe and analysed for methane by means of gas chromatography.

4.1.3.2 ANALYSING FOR THE PRESENCE OF ACID PRODUCERS

To verify whether there were acid producing bacteria in the continuous reactors, batch tests were done on the bacteria from each reactor. A volume of 180 mL feed solution (Table 4.1) consisting of glucose, at a concentration of 1.33 kgm⁻³, as the organic source was inoculated with 20 mL bacteria (10 % v/v). The inoculated feed solution was placed in a 250 mL Schott bottle sealed with a rubber bung. The rubber bung

provided for a liquid sample port and a gas outlet bottle and the solution was sparged with nitrogen to remove any oxygen. The bottles were placed on a rotary shaker at 130 rpm and 35°C for a period of 15 d. Samples were taken on a daily basis and analysed for glucose. To ensure that the system remained anaerobic, nitrogen was sparged through the system after sampling. An abiotic control was used to monitor the chemical hydrolysis of glucose.

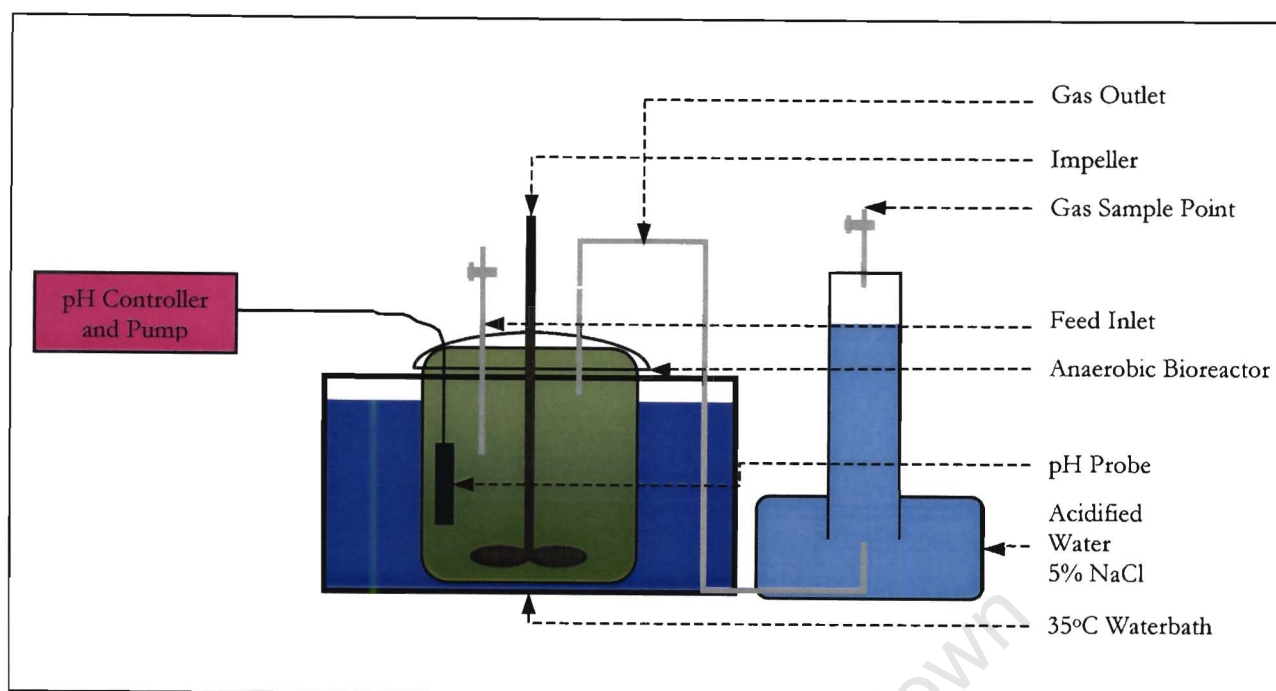
To confirm that the group of glucose utilisers present were the acid producers (since glucose can be utilised by fermentative organisms other than the acid producing consortia) a set of batch tests were done using methanol as the organic source. Only the acid producers can utilise methanol.

4.2 SULPHATE REDUCTION BY A MIXED CULTURE OF ANAEROBIC MICROORGANISMS USING A BATCH STIRRED TANK REACTOR

The effect of temperature, pH and sulphate concentration on the reduction of sulphate was studied in a batch system. A mixed anaerobic culture, described in Section 4.2, was used.

4.2.1 EXPERIMENTAL APPARATUS

The batch experiments were carried out in sealed 1 L stirred tank reactors. Outlets for liquid and gas were provided. Heidolph overhead stirrers driving 2 blade propellers were used to keep the system completely mixed. All fittings and the stirrer shaft were sealed with vacuum grease to prevent any gas leakage. The reactors were placed in a waterbath with temperature control. The pH was controlled continuously by the addition of either sodium hydroxide or hydrochloric acid. Any gas produced was collected by means of the displacement method using acidified water of pH 3 containing 5 % sodium chloride. The reactor set-up is shown schematically as Figure 4.1.



4.2.2 EXPERIMENTAL CONDITIONS

For all the experiments 50 % inoculum (v/v) was added to the reaction medium. The standard condition used for the experiments, unless otherwise specified, where:

- pH: 7.0,
- temperature: 32°C, and
- sulphate concentration: 2.5 kg m⁻³

These are the optimum pH and temperature as reported in literature (this has been discussed Chapter 2).

The effect of initial sulphate concentration on sulphate reduction was studied across the range 0.0 to 5.0 kgm⁻³. To investigate the effect of temperature, experiments were carried out at 25, 30, 35 and 40°C.

By controlling the pH of the system at 6, 6.5, 7, 7.5, 8 and 9, the effect of pH on reduction of sulphate was investigated.

4.3 KINETICS OF CONTINUOUS SULPHATE REDUCTION BY ANAEROBIC SULPHATE REDUCERS

The kinetics of sulphate reduction and the bacterial growth were studied in 1-litre continuous bioreactors using acetate as the organic source. In order to eliminate the effect of the APB and MPB present in the reactor, acetate was used as the organic source as opposed to longer chain organics such as glucose, sucrose and ethanol. Acetate favours the growth of SRB over that of APB and MPB (Visser, 1995).

4.3.1 MEDIUM

The media used to run the continuous reactors is described in Table 4.1. Acetic acid was used as a carbon source at a concentration of 17.5 kgm^{-3} . Unless otherwise specified, sulphate was added at 5.0 kgm^{-3} . The pH of the medium containing 17.5 kgm^{-3} acetic acid was pH 8.0.

4.3.2 MICROORGANISMS

For the continuous studies, the microbial culture was enriched for SRB while MPB and APB were selected against as follows. To inhibit MPB, bromo ethanol sulphonic acid (BESA) was added at a concentration of 3.2 kgm^{-3} (Visser, 1995). In order to acclimitise the organisms to the acetic acid, thereby selecting against APB, the glucose was removed from the feed and replaced with 2.5 kgm^{-3} acetic acid. The reactor was operated in batch mode for a period of 30 d. After 30 d batch operation, the presence of active MPB and APB was tested as described in Section 4.1.3. Subsequently three reactors, run on 1.0 kgm^{-3} , 2.5 kgm^{-3} and 5.0 kgm^{-3} sulphate were inoculated with the microorganisms grown in batch. The reactors were operated continuously and the amount of acetic acid was increased stepwise to 17.5 kgm^{-3} to ensure an excess of acetic acid at the highest sulphate concentration

Microorganisms from the continuous bioreactor with a 5.0 kgm^{-3} sulphate feed were used to inoculate the reactor operated with a 10.0 kgm^{-3} sulphate feed. In turn, the bacteria from the continuous bioreactor with a 10.0 kgm^{-3} sulphate feed were used to inoculate the continuous bioreactor receiving 15.0 kgm^{-3} sulphate.

After two months of continuous operation the presence of MPB and APB was determined in each bioreactor.

Microorganisms were taken from the continuous bioreactor with a feed containing 5.0 kgm^{-3} sulphate in the feed to inoculate the continuous reactors used to investigate the effect of temperature on the bacterial sulphate reduction process. To assess changes in the microbial population when the 10.0 kgm^{-3} bioreactor approached washout the retention time was increased stepwise until a retention time of ten days was achieved and thereafter the retention time was decreased stepwise till washout was achieved. This provided data to determine the reproducibility of experiments.

4.3.3 EXPERIMENTAL APPARATUS

One-litre Quickfit vessels were employed for the continuous experiments. The glass lids of the vessels and the Quickfit adapters were sealed with vacuum grease to ensure that an anaerobic system was maintained. The gas outlet discharged into an inverted cylinder filled with acidified water. The reactor was kept mixed by Heidolph overhead stirrers driving two bladed impellers at a speed of 400 rpm. Fresh medium was fed into the reactor by a variable speed peristaltic pump. To avoid channelling the feed was introduced close to the base of the reactor. The effluent discharged by gravity through a U-shaped overflow tube, designed to maintain the liquid volume in the reactor at 1 L. The effluent was passed through a $0.45 \mu\text{m}$ Millipore filter to separate the microorganisms. A constant temperature was maintained by placing the reactor in a constant temperature waterbath and the pH was controlled at 7.8 (being identified as optimum in batch experiments carried out as part of this study). A pH probe attached to the lid of the reactor vessel monitored the pH. When the pH deviated from 7.8, sodium hydroxide or hydrochloric acid was added manually through the liquid sample port. The experimental apparatus is shown schematically in Figure 4.2.

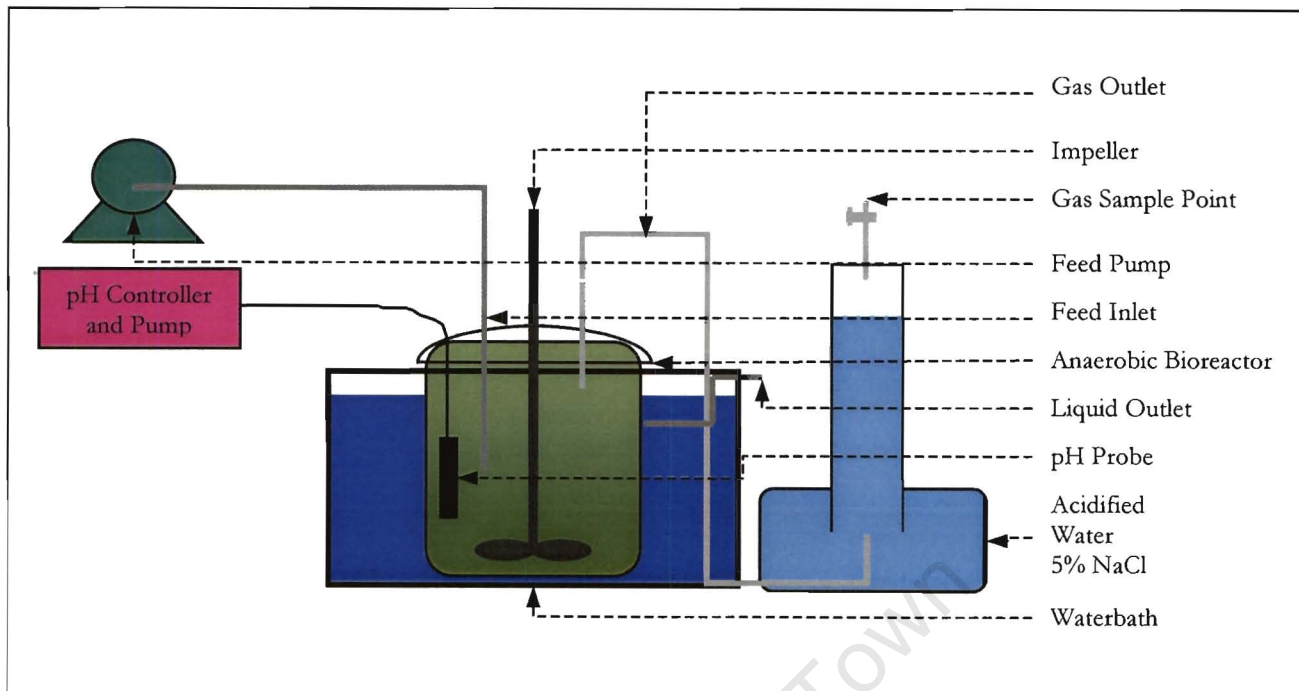


Figure 4.2. Representation of experimental set-up used for continuous experiments.

4.3.4 OPERATION OF THE BIOREACTOR

A volume of 800 mL media was equilibrated to the operating temperature in the reactor prior to adding 200 mL of inoculum. The reactor was operated in batch until the concentration of sulphate decreased to a stable value. Thereafter the reactor was switched to continuous mode of operation. Feed addition was controlled by a peristaltic pump and increased stepwise during the course of the experiment to affect a range of dilution rates. Flow rates in the range 0.0042 to 0.0208 Lh^{-1} were applied representing dilution rates in the range 0.0042 to 0.0208 h^{-1} . 'Stable' state conditions were achieved at each flow rate to provide data to estimate the kinetics of sulphate reduction and bacterial growth. 'Stable' state conditions were assumed when the residual sulphate concentration and bacterial concentration varied by no more than 12 and 15 % respectively for a period of operation equal to one retention time. Liquid samples were taken from the reactor on a daily basis and analysed for sulphate, bacterial and acetate concentrations. Samples were examined under a light microscope to assess the diversity of the bacterial population.

4.3.5 FEED SULPHATE CONCENTRATION

The effects of sulphate concentration and its volumetric loading on the reduction of sulphate were studied at a pH of 7.8 and temperature of 35°C (optimum obtained from batch experiments carried out as part of this study). Five sulphate concentrations (1.0, 2.5, 5.0, 10.0 and 15.0 kgm⁻³) were tested, providing information on volumetric sulphate loading rates in the range 0.0042 to 0.156 kgm⁻³h⁻¹. The acetate was kept in excess (the acetate concentration ranged between 2.5 and 17.5 kgm⁻³).

4.3.6 EFFECT OF TEMPERATURE

Experiments were carried out at 20, 25, 30 and 35°C to establish the effect of temperature on the sulphate reduction process. The initial sulphate and acetate concentrations were 5.0 kg m⁻³ and 17.5 kgm⁻³ respectively. The pH was controlled at 7.8.

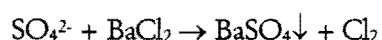
4.4 ANALYTICAL PROCEDURES

The following analytical procedures will be discussed here:

- sulphate concentration,
- acetate concentration,
- glucose concentration,
- biomass concentration, and
- gas composition.

4.4.1 SULPHATE CONCENTRATION

Sulphate was analysed by using the barium precipitation technique (APHA, 1975). Sulphate is precipitated in an acidic medium with barium chloride forming insoluble barium sulphate.



4.4.1.1 PROCEDURE FOLLOWED

After filtering with a 0.45 μ m Millipore filter, samples were diluted as required. After mixing 5 mL of sample with 0.25 mL conditioning reagent (50 mL glycerol, 30 mL concentrated hydrochloric acid, 75 g sodium chloride, 100 mL ethanol and 300 mL deionised water), an excess of finely ground barium chloride was added. The solution was thoroughly mixed on the Vortex Mixer for one minute and the absorbance was measured at 420 nm on a Varian UV/visible spectrophotometer .

Standard sulphate solutions, containing 0.02, 0.04, 0.08 and 0.10 kgm⁻³ sodium sulphate in deionised water were treated in the same manner as the samples. A calibration curve, shown as Figure 4.3, of the concentrations of the standard solutions (0.02, 0.04, 0.08 and 0.10 kgm⁻³) vs. absorbance was prepared and was shown to be linear in the range 0.02 to 0.10 kgm⁻³ sulphate (the correlation coefficient was 0.98). The samples were prepared in triplicate and a 12 % error was observed.

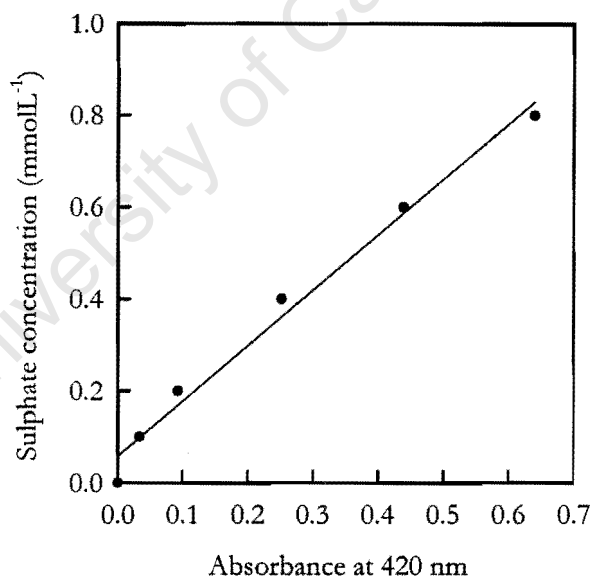


Figure 4.3. Standard curve for the sulphate determination

4.4.2 ACETATE CONCENTRATION

Acetic acid concentrations in the bulk supernatant were analysed using a Beckman System Gold high-pressure liquid chromatograph (HPLC). The separation of components was achieved through a Wakosil II 5C18 RS reverse phase column. The mobile phase was 20 mM H₃PO₄ (adjusted to pH 2.5 with sodium

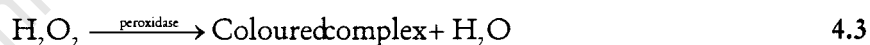
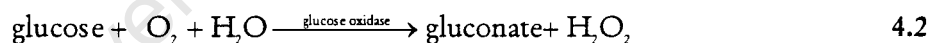
hydrogen phosphate) at a flowrate of 1.0 ml/min. The separated components were detected by changes in the reflective index of the mobile phase, with a Beckman UV detector (model 168) at 210 nm.

Standard solutions were prepared by diluting glacial acetic acid with deionised water. The samples were filtered through a 0.45µm Millipore filter to remove any suspended solids.

The detection limit of the HPLC was 0.01 kgm⁻³. The standard deviation of the standard solution of acetic acid was 4 % on 1.0 kgm⁻³ and 10 % on the same sample over a 5 d period. Thus reproducibility was good, with little drift from the instrument.

4.4.3 GLUCOSE CONCENTRATION

Glucose in the liquid samples was determined using the GOD-Perid test kit from Boehringer Mannheim GmbH Diagnostics (catalogue number 124036). The colorimetric method is based on the enzymatic conversion of glucose to gluconate with the production of hydrogen peroxide (Eqn 4.2). The hydrogen peroxide reacts with di-ammonium 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) to form a green complex (Eqn 4.3) which is measured spectrophotometrically.



4.4.3.1 PROCEDURE FOLLOVED

The sample supernatant is filtered through a 0.45µm Millipore filter and diluted to fall in the 0.0 to 0.5 kgm⁻³ glucose range. The absorbance was read at 610 nm against a reagent blank.

A linear relationship exists between glucose concentration and absorbance (correlation coefficient of 0.99) for the glucose range 0 to 0.50 kgm⁻³. The analysis error, including the dilution error, was 3 %.

4.4.4 BIOMASS CONCENTRATION

Three methods of estimating the bacterial concentration were investigated:

- dry biomass concentration,
- direct counting, and
- absorbance at 660 nm.

The dry biomass method was selected for routine use. The presence of sulphide in the liquid, resulting in a dark colour, prevented use of the absorbance method. Direct counting was hindered by the high motility of the bacteria.

4.4.4.1 DRY WEIGHT

A 1 mL sample was taken from the reactor and centrifuged for 15 min in a pre-dried (80°C for 24 h), pre-weighed microfuge tube using Mikro centrifuge (model 12-24) at 15000 rpm. The supernatant was decanted. The bacterial pellet in the microfuge tube was washed with acidified water to remove any precipitated matter and re-centrifuged. Following removal of the supernatant, the microfuge tube was dried at 80°C for 24 h. After cooling in a desiccator, the microfuge tube and pellet were weighed and the bacterial concentration calculated. The dry biomass analysis was analysed in triplicate, with an average variance of 15 %.

4.4.5 GAS ANALYSIS

Methane, carbon dioxide and hydrogen sulphide concentrations in the gas evolved were measured using a Perkin Elmer Autosystem gas chromatograph with a thermoconductivity detector (TCD). A 2.43 m stainless steel column, packed with HayeSep 60/100 mesh was operated isothermally at 90°C. The carrier gas was helium at 0.0018 m³h⁻¹. The column and detector ports were maintained at 200 and 220°C respectively. A 10 µl gas sample was injected into the injector port using a gas tight syringe. The detection limit for methane, carbon dioxide and hydrogen sulphide was 1.0 ppm and the percentage error was 3 %.

4.5 CHAPTER SUMMARY

In this chapter the experimental programme employed for this study was detailed. The experimental equipment and methodology were discussed and analytical techniques used are outlined. The next chapter is the first of three chapters presenting and discussing the experimental results obtained. Chapter 5 presents the results from batch studies to establish the operating ranges of pH, temperature and feed sulphate concentration for optimum sulphate reduction.

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CHAPTER 5

RESULTS AND DISCUSSION I BATCH EXPERIMENTS

As stated in the literature review the pH and temperature for optimum sulphate conversion by sulphate reducers is culture specific. Results of batch experiments to ascertain operating ranges of pH, temperature and initial sulphate concentration for the optimum conversion of sulphate to sulphide using a mixed anaerobic microbial culture are presented in this Chapter.

5.1 OBJECTIVES OF BATCH EXPERIMENTS

From literature studies presented in Chapter 2 it was apparent that optimum operating conditions, in terms of pH, temperature and sulphate concentration, for mixed anaerobic cultures vary depending on culture history. Hence batch studies were done on the anaerobic culture used for this study. The parameters investigated included:

- pH,
- temperature, and
- feed sulphate concentration.

5.2 SUMMARY OF EXPERIMENTAL CONDITIONS AND VARIABLES

The experiments were carried out in 1 L stirred tank reactors (shown as Figure 4.1 in the previous chapter). A mixed anaerobic population of SRB, MPB and APB reactors was used and run for a period of 30 d. An optical microscope photograph of the inoculum used is presented as Figure 5.1 The carbon source used was glucose, at a concentration of 5.0 kgm⁻³. The COD equivalent of glucose can be calculated as shown below:



$$1 \text{ g C}_6\text{H}_{12}\text{O}_6 \equiv \frac{6 \times 32 \text{ g/mol O}_2}{180 \text{ g/mol glucose}} \times 1 \text{ g COD} \equiv 1.067 \text{ g COD} \quad 5.2$$

This COD equivalent was 5.34 kgm⁻³ (see calculations above). The COD to sulphate ratio was 2.14, greater than the theoretical ratio of 0.67. Sulphate was the limiting substrate.

The standard conditions used were:

- pH: 7.0,
- temperature: 32°C,
- sulphate: 2.5 kgm⁻³, and
- glucose: 5.0 kgm⁻³.

The pH, temperature, and sulphate concentration ranges investigated were:

- pH: 6.0 – 9.0,
- temperature: 20 - 35°C, and
- sulphate concentration: 0.0 – 5.0 kgm⁻³.

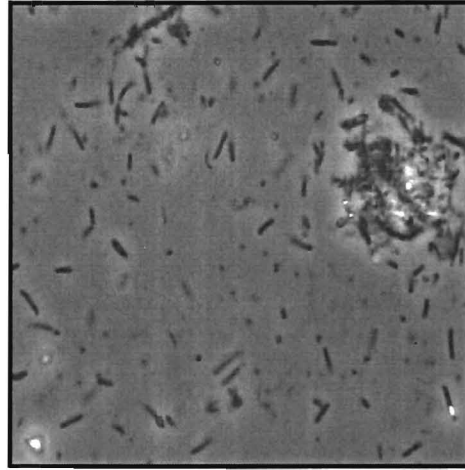


Figure 5.1. Optical microscope photograph of inoculum used for batch experiments showing the different morphology of the various species in the mixed anaerobic culture. (1000X magnification)

5.3 pH

The residual glucose and sulphate concentrations obtained as a function of time across the pH range 6.0 to 9.0 are detailed in Figures 5.2 and 5.3 respectively. Further the duration of the lag phase and the extent of conversion with respect to glucose and sulphate are summarised as a function of pH in Tables 5.1 and 5.2 respectively. With increasing pH, a similar trend of glucose utilisation and sulphate conversion was observed.

Table 5.1. Variation in lag phase and extent of glucose conversion as a function of pH in batch experiments. A mixed population of SRB, MPB and APB at 32°C, feed sulphate and glucose concentrations of 2.5 and 5.0 kg m⁻³ respectively was used.

pH	Lag phase (d)	Glucose conversion (%)
6.0	5	58
6.5	6	71
7.0	5	83
7.5	5	92
8.0	4	92
9.0	9	33

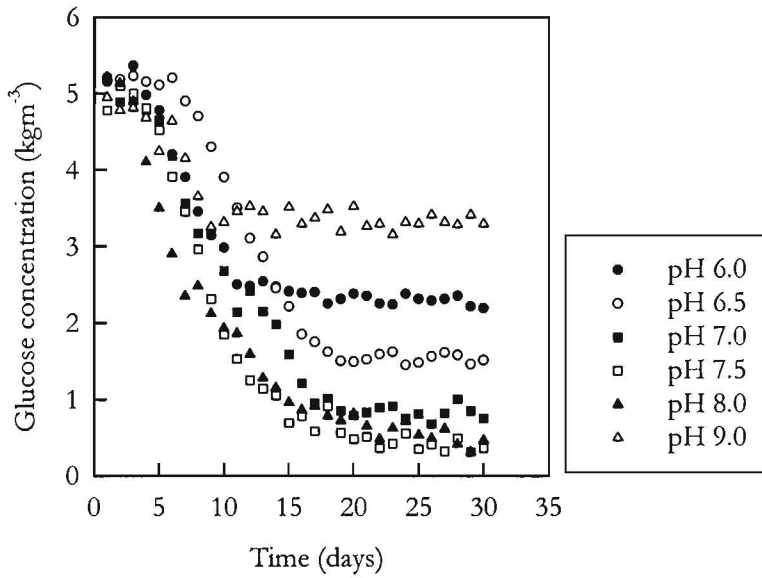


Figure 5.2. Batch glucose concentration profiles as a function of pH. Experiments were carried out at pH 6.0 (●), 6.5 (○), 7.0 (■), 7.5 (□), 8.0 (▲) and 8.5 (△). A mixed population of SRB, MPB and APB at 32°C, feed sulphate and glucose concentrations of 2.5 and 5.0 kg m⁻³ respectively was used

Table 5.2. Variation in lag phase and extent of glucose conversion as a function of pH in batch experiments. A mixed population of SRB, MPB and APB at 32°C, feed sulphate and glucose concentrations of 2.5 and 5.0 kgm⁻³ respectively was used.

pH	Lag phase (d)	Sulphate conversion (%)
6.0	13	13
6.5	10	33
7.0	7	54
7.5	7	72
8.0	6	83
9.0	18	19

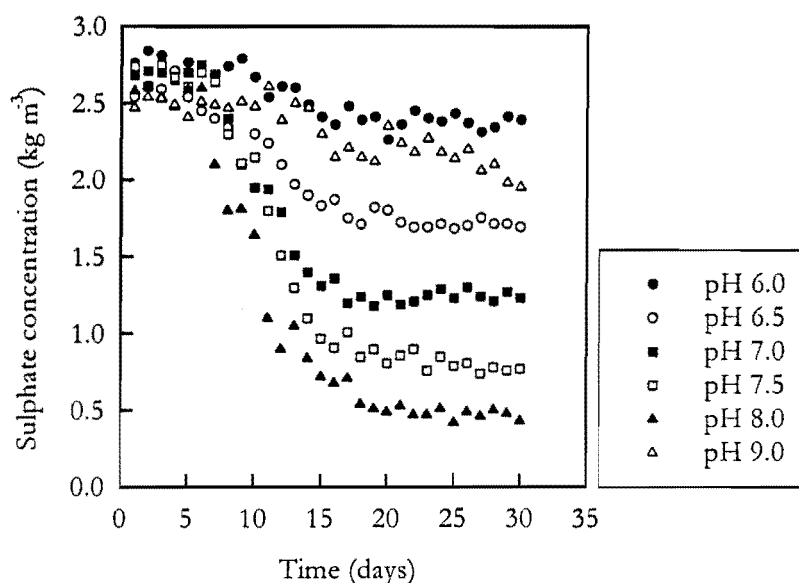


Figure 5.3. Batch sulphate concentration profiles as a function of pH. Experiments were carried out at pH 6.0 (●), 6.5 (○), 7.0 (■), 7.5 (□), 8.0 (▲) and 8.5 (△). A mixed population of SRB, MPB and APB at 32°C, feed sulphate and glucose concentrations of 2.5 and 5.0 kgm⁻³ respectively was used.

The lag phase observed for glucose utilisation was between 4 and 6 d across the pH range 6.0 – 8.0. No distinct dependency on initial pH was evident in this range (Table 5.2). At pH 9.0, the lag phase increased to 9 d. The extent of glucose conversion increased across the pH range 6.0 to 7.5, reaching a plateau of 92 % at pH 7.5 – 8.0. When the pH was further increased to 9.0 the conversion of glucose decreased to 33 % (Table 5.1). Figure 5.2 confirms that the optimum pH for glucose conversion lay in the pH range 7.5 – 8.0.

As the pH was increased from 6.0 to 8.0, the culture displayed a decreasing lag phase for sulphate conversion. At 9.0, the longest lag phase of 18 d was observed (Table 5.2). The percentage sulphate conversion showed an increase with increasing pH in the range 6.0 to 8.0, reaching a maximum conversion of 83 % at pH 8.0. At pH 9.0 the conversion decreased to 19 % (Table 5.2). The optimum pH of 8.0 for sulphate conversion is confirmed in Figure 5.3.

In summary, the optimum pH for sulphate conversion was 8.0. The pH dependence of glucose conversion was less pronounced with an optimum between 7.5 and 8.0. This confirms that sulphate conversion and glucose utilisation are carried out by two different groups of bacteria. Furthermore, the lag phase observed for glucose utilisation was shorter than that for sulphate conversion, suggesting that the sulphate reducers depend on the products of glucose metabolism for carbon source and electron donor (Postgate, 1984; Speece, 1996; Visser, 1995).

From these results, it is proposed that, to optimise sulphate conversion, the pH should lie in the range 7.5 and 8.0. This pH range coincides with the range where sulphide toxicity is reduced because of the reduced concentration of the unionised hydrogen sulphide (O'Flaherty *et al.*, 1998).

5.4 TEMPERATURE

The effect of reaction temperature was investigated at 20, 25, 30, 35 and 40°C. The pH was controlled at 7.0 and the feed sulphate and glucose concentrations were 2.5 and 5.0 kgm⁻³ respectively. Glucose concentration and sulphate concentration profiles are shown as Figures 5.4 and 5.5 respectively.

At a constant pH, increasing the reaction temperature in the range 20 to 35°C resulted in an enhancement of both glucose utilisation and sulphate conversion. The increase is more pronounced for the conversion of sulphate than for glucose utilisation indicating the glucose utilising consortia are able to function over a wider range of temperatures than the sulphate reducers. Furthermore, at 40°C the culture displayed no conversion of sulphate while 22 % of the glucose was utilised. The lag phase for the utilisation of glucose remained constant at 4 d for temperatures of 20, 25 and 30°C and decreased to 3 d when the reaction temperature was 35°C (Table 5.3). The lag phase for the biological conversion of sulphate decreased from 17 to 6 d with increasing temperature in the range 20 to 35°C (Table 5.4). Further increase of temperature to 40°C inhibited sulphate conversion and reduced the rate and extent of glucose conversion.

From the experimental observations it can be concluded that at pH 7.0, the optimum temperature for sulphate conversion is 35°C. Postgate (1984) report optimum growth temperatures of 29 and 36 °C for *Desulfobacter postgatei* and *Desulfotomaculum acetoxidans* respectively.

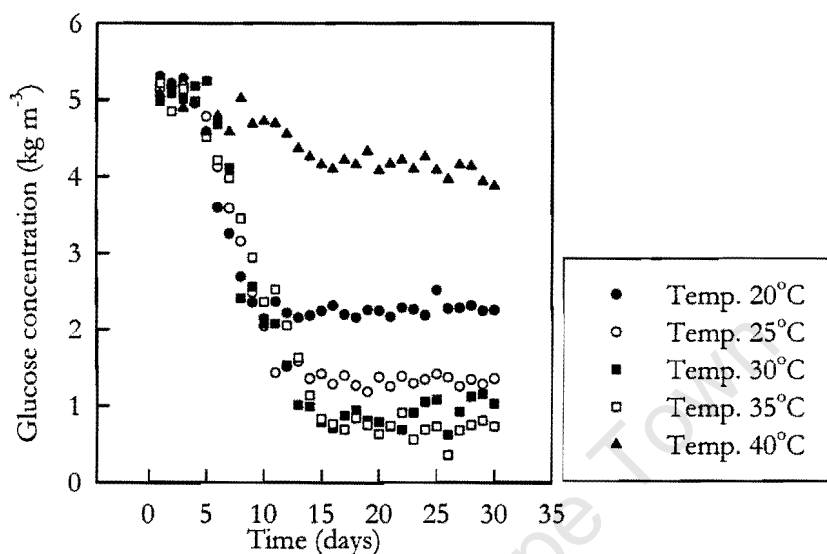


Figure 5.4. Batch glucose concentration profiles as a function of temperature. Experiments were carried out at temperatures of 20 (●), 25 (○), 30 (■), 35 (□) and 40°C (▲). A mixed population of SRB, MPB and APB at pH 7.0, feed sulphate and glucose concentrations of 2.5 and 5.0 kgm⁻³ respectively was used.

Table 5.3. Variation in lag phase and extent of glucose conversion as a function of temperature in batch experiments. A mixed population of SRB, MPB and APB at 32°C, feed sulphate and glucose concentrations of 2.5 and 5.0 kgm⁻³ respectively was used.

Temperature	Lag phase	Glucose conversion
(°C)	(d)	(%)
20	4	57
25	4	75
30	4	80
35	3	85
40	10	22

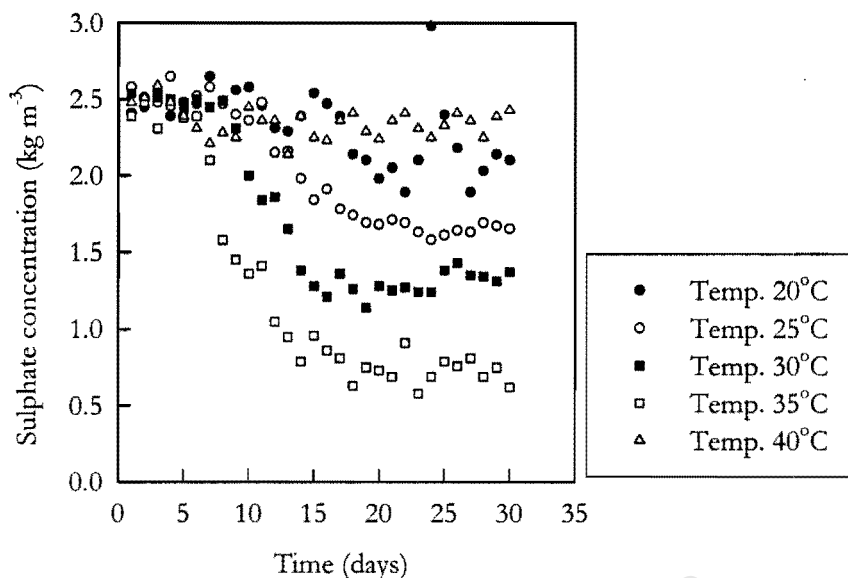


Figure 5.5. Batch sulphate concentration profiles as a function of temperature. Experiments were carried out at temperatures of 20 (●), 25 (○), 30 (■), 35 (□) and 40°C (▲). A mixed population of SRB, MPB and APB at pH 7.0, feed sulphate and glucose concentrations of 2.5 and 5.0 kgm⁻³ respectively was used

Table 5.4. Variation in lag phase and extent of sulphate conversion as a function of temperature in batch experiments. A mixed population of SRB, MPB and APB at pH 7.0, feed sulphate and glucose concentrations of 2.5 and 5.0 kgm⁻³. respectively was used.

Temperature (°C)	Lag phase (d)	Sulphate conversion (%)
20	17	13
25	11	34
30	8	49
35	6	71
40	n/a	0

5.5 SULPHATE CONCENTRATION

The effects of feed sulphate concentration on glucose utilisation and sulphate conversion at pH of 7.0 and temperature of 32°C are shown as Figures 5.6 and 5.7 respectively.

Glucose utilised remained constant for initial sulphate concentrations in the range 0.0 to 2.5 kgm⁻³. The lag phase for glucose utilisation remained constant at 5 d for sulphate concentrations in the range 0.0 to 1.0 kgm⁻³ (Table 5.5). When the feed sulphate concentration was increased to 2.5 kgm⁻³ the lag phase increased to 6 d. Increasing the feed sulphate concentration from 1.0 to 2.5 kg m⁻³ resulted in a decrease in the rate and extent of sulphate conversion. It should be noted that in the culture, which was inoculated with a media containing no sulphate, trace amounts of sulphate were detected due to the residual sulphate present in the inoculum. The lag phase observed for sulphate removal remained constant at 7 d with an increase in feed sulphate concentration from 1.0 to 2.5 kg m⁻³ (Table 5.6). When the feed sulphate was 5.0 kg m⁻³, inhibition of both sulphate and glucose removal was apparent over the 30 d reaction period.

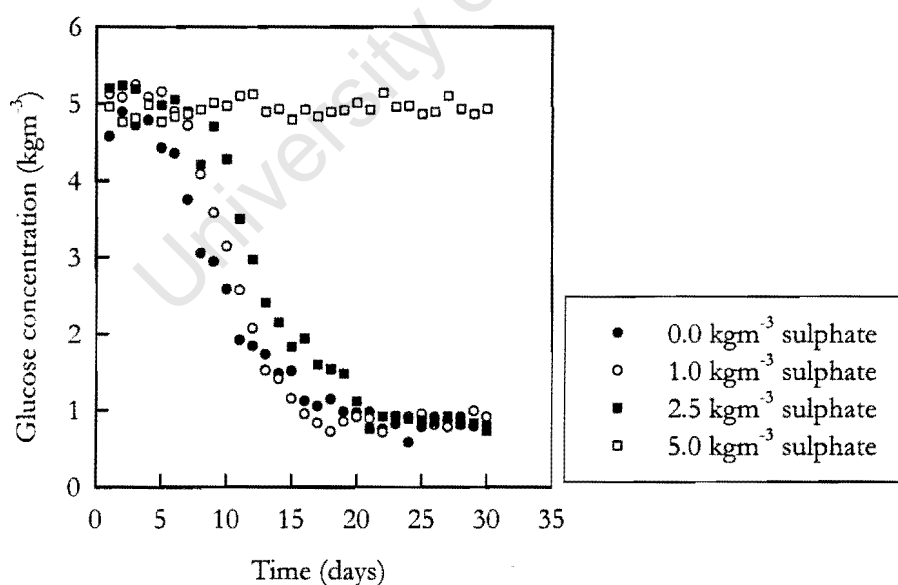


Figure 5.6. Batch glucose concentration profiles as a function of feed sulphate concentration. Feed sulphate concentration used were: 0.0 (●), 1.0 (○), 2.5 (■) and 5.0 kgm⁻³ (□). A mixed population of SRB, MPB and APB at pH 7.0, temperature 32°C and feed glucose concentrations of 5.0 kgm⁻³ was used.

Table 5.5. Variation in lag phase and extent of glucose conversion as a function of feed sulphate concentration in batch experiments. A mixed population of SRB, MPB and APB at pH 7.0, temperature 32°C and glucose concentration 5.0 kgm⁻³ was used.

Sulphate concentration (kgm ⁻³)	Lag phase (d)	Glucose conversion (%)
0.0	5	82
1.0	5	82
2.5	6	85
5.0	> 30	0

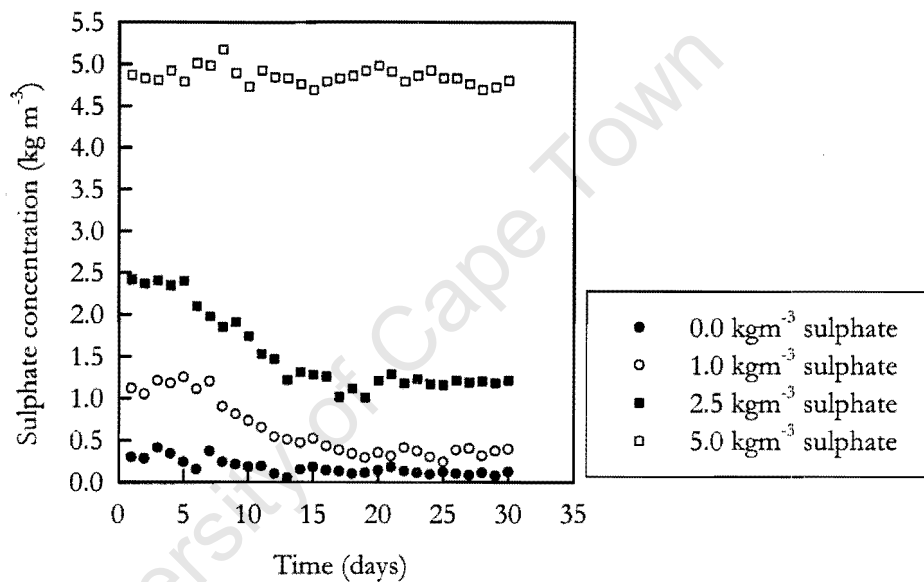


Figure 5.7. Batch sulphate concentration profiles as a function of feed sulphate concentration. Feed sulphate concentration used were: 0.0 (●), 1.0 (○), 2.5 (■) and 5.0 kgm⁻³ (□). A mixed population of SRB, MPB and APB at pH 7.0, temperature 32°C and feed glucose concentrations of 5.0 kgm⁻³ was used.

Table 5.6. Variation in lag phase and extent of sulphate conversion as a function of feed sulphate concentration in batch experiments. A mixed population of SRB, MPB and APB at pH 7.0, temperature 32°C and glucose concentration 5.0 kgm⁻³ was used.

Sulphate concentration (kgm ⁻³)	Lag phase (d)	Sulphate conversion (%)
0.0	7	67
1.0	7	64
2.5	7	49
5.0	> 30	0

5.6 CHAPTER SUMMARY

In this chapter the effects of three physical parameters pH, temperature and initial sulphate concentration were investigated in batch studies using a mixed anaerobic population comprising SRB, MPB and APB. The results confirm the importance of reaction pH, temperature and feed sulphate concentration on the sulphate removal and glucose utilising capacity of the microbial culture.

According to the results of the batch studies, the optimum environmental conditions for optimum sulphate removal are pH 8.0, temperature 35°C and a feed sulphate concentration in the range 1.0 to 2.5 kgm⁻³. The APB, forming the carbon source (for SRB and MPB) from glucose show a pH optimum of 7.5 – 8.0, a temperature optimum of 25 - 35°C and sulphate inhibition at feed concentrations above 5.0 kgm⁻³.

In the next chapter, Chapter 6, the results of continuous bioreactor studies to ascertain the effects of feed sulphate concentration on the kinetics of anaerobic sulphate reduction are presented and discussed.

CHAPTER 6

RESULTS AND DISCUSSION II

FEED SULPHATE CONCENTRATION

The results of the batch experiments presented in the previous chapter showed that optimum sulphate reduction occurred at initial sulphate concentrations of 1.0 to 2.5 kgm⁻³. As sulphate reducers have been shown to acclimatise to operating parameters, including organic and inorganic substrate concentration, (Visser, 1995) the aim of conducting continuous experiments at increasing feed sulphate concentrations was twofold; (i) to ascertain the effect of feed sulphate concentration on the kinetics of anaerobic sulphate reduction and (ii) to determine the maximum sulphate concentration that could be tolerated by the mixed SRB culture used for this study. Against this background the reader is presented with details of results from experiments to determine the effect of feed sulphate concentration and its volumetric sulphate loading on the kinetics of anaerobic reduction of sulphate. The results are presented in five sections. The first section outlines the validation of 'stable' state. The reproducibility of the experiments is presented in the second section. In the third section bioreactor performance following perturbations are detailed. The fourth section provides the 'stable' state kinetic data at each condition employed. A comparison of the 'stable' state kinetic data as a function of residence time is presented in the fifth section.

6.1 VALIDATION OF 'STABLE' STATE

The kinetics of the biological sulphate reduction system was determined from the 'stable' state data. 'Stable' state conditions were achieved when the residual sulphate concentration and the bacterial concentration did not vary by more than 12 and 15 % respectively for at least one retention time. Typical time-course profiles (using a feed sulphate concentration of 5.0 kgm^{-3}) to determine whether 'stable' state had been achieved are shown as Figures 6.1 and 6.2. 'Stable' state is represented by a plateau on the profile of residual sulphate concentration, or bacterial concentration, as a function of the time after perturbation. Table 6.1 details the 'stable' states achieved. Similar time profiles for feed sulphate concentrations of 1.0, 2.5, 10.0 and 15.0 kgm^{-3} are presented in Appendix A. For all retention times employed 'stable' state was established. For the kinetic analysis presented in Section 6.3 the average of the 'stable' state values were used. Washout was represented by a significant change in residual sulphate and biomass concentration as the retention was increased. From the profiles presented (Figures 6.1 and 6.2), it can be seen that washout was not a sudden occurrence. This is evidenced from the gradual increase in residual sulphate concentration and decrease in biomass concentration with increasing retention time and is typical of mixed culture behaviour.

Table 6.1. 'Stable' state data as a function of hydraulic retention time. Data obtained from the bioreactor operating at 30°C and pH 7.8 with a feed sulphate and acetate concentration of 5.0 kgm^{-3} and 17.5 kgm^{-3} respectively.

Hydraulic retention time (d)	Time period of 'stable' state (d)	Residual sulphate concentration (kgm^{-3})	Biomass concentration (kgm^{-3})
10	10	0.34 ± 0.04	2.71 ± 0.13
9	10	0.41 ± 0.05	3.23 ± 0.15
8	8	0.48 ± 0.05	3.10 ± 0.10
7	7	0.49 ± 0.05	3.14 ± 0.16
6	10	0.50 ± 0.55	3.00 ± 0.21
5	10	0.55 ± 0.07	2.92 ± 0.02
4	10	0.57 ± 0.03	2.73 ± 0.01
3.5	7	0.59 ± 0.03	2.70 ± 0.03
3	6	0.61 ± 0.03	2.69 ± 0.04
2.5	8	0.71 ± 0.04	2.54 ± 0.03
2	6	1.50 ± 0.07	1.61 ± 0.04
1.5	6	$2.30 \pm .043$	1.39 ± 0.12

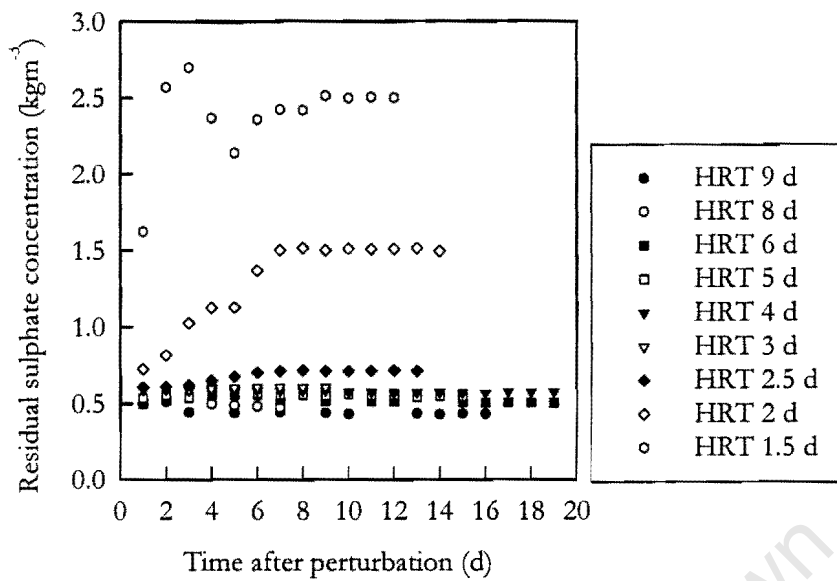


Figure 6.1. Residual sulphate concentration as a function of time after perturbation for the continuous conversion of sulphate at HRTs of 9 (●), 8 (○), 6 (■), 5 (□), 4 (▼), 3 (▽), 2.5 (◆), 2 (◇) and 1.5 (●) d. The continuous bioreactor was operated at 35°C, pH 7.8 with feed sulphate and acetate concentrations of 5.0 and 17.5 kgm⁻³ respectively.

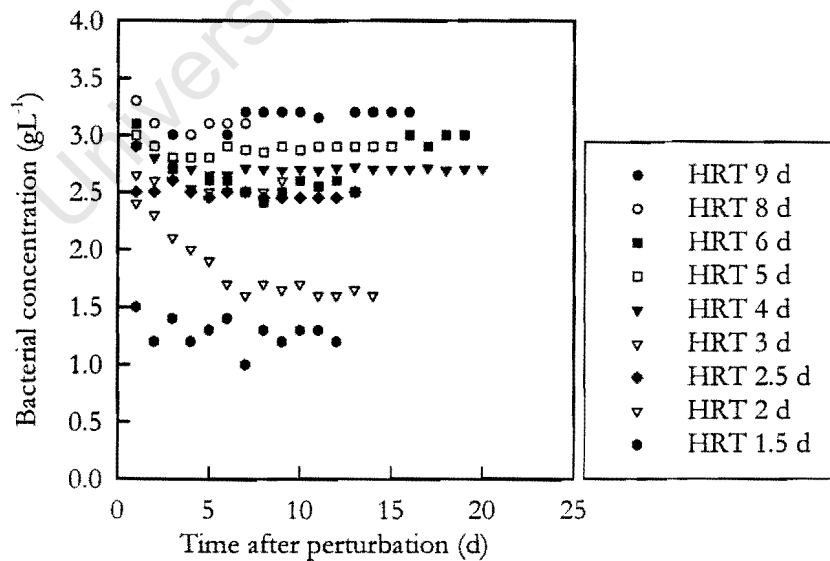


Figure 6.2. Bacterial concentration as a function of time after perturbation for the continuous conversion of sulphate at HRTs of 9 (●), 8 (○), 6 (■), 5 (□), 4 (▼), 3 (▽), 2.5 (◆), 2 (◇) and 1.5 (●) d. The continuous bioreactor was operated at 35°C, pH 7.8 with feed sulphate and acetate concentrations of 5.0 and 17.5 kgm⁻³ respectively.

6.2 REPRODUCIBILITY OF EXPERIMENTS

'Stable' state results of two identical experimental runs at a feed sulphate concentration of 10.0 kgm^{-3} are presented as Figure 6.3. From a paired t-test performed on the transient data it was found that the two sets of data were equal at the 92 % significance level ($n = 18, t = 1.91$). Performing a paired t-test on the two sets of 'stable' state data the data was equal at the 92 % significance level. The experiments were reproducible to within 92 %.

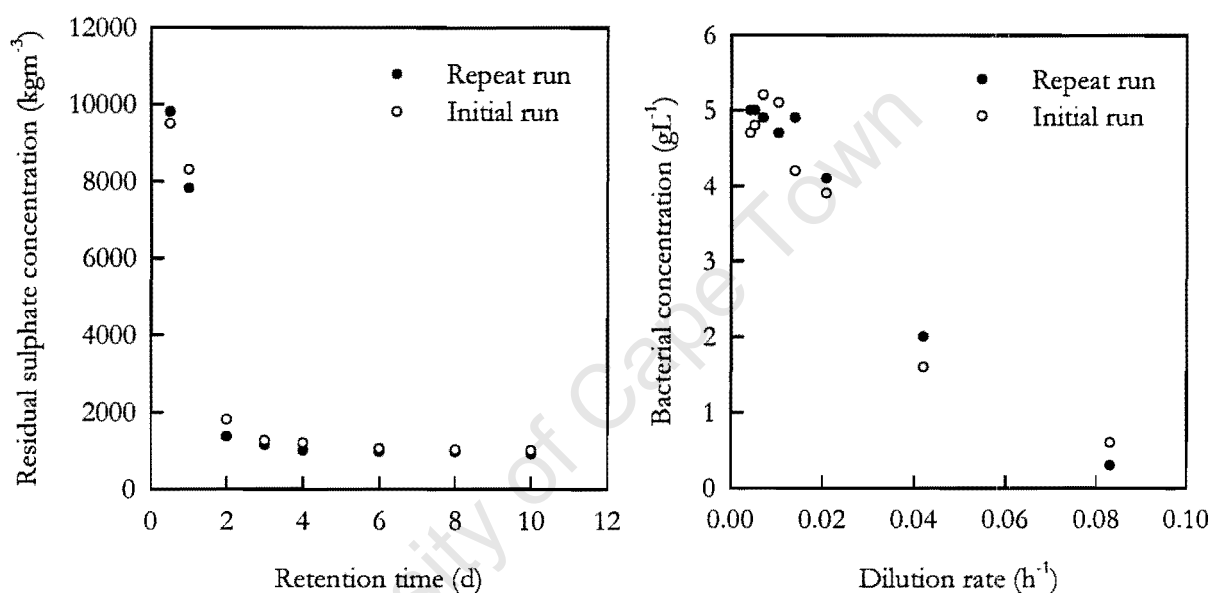


Figure 6.3. Comparison of two sets of 'stable' state data for the continuous bioreactors operating with 10.0 kgm^{-3} sulphate in the feed. The two continuous bioreactors were operated at 35°C and $\text{pH } 7.8$ with a feed acetate concentration of 17.5 kgm^{-3} .

6.3 TRENDS FOLLOWING PERTURBATIONS

Details of the transient performance of continuous bioreactors fed at sulphate concentration of $1.0, 2.5, 5.0, 10.0$ and 15.0 kgm^{-3} are provided in this section. For the bioreactor receiving 1.0 kgm^{-3} of sulphate in the feed, the acetate concentration was 2.5 kgm^{-3} . In order to ensure that acetate was not the limiting substrate for the bioreactors with feed concentrations of $2.5, 5.0, 10.0$ and 15.0 kgm^{-3} sulphate the concentration of acetate in the feed was increased stepwise. The feed acetate concentration was set when no further increase of sulphate conversion with increasing feed acetate was observed. The final

concentration of acetate used was 17.5 kgm^{-3} . The data presented consists of the bacterial concentration, sulphate concentration and the retention time. The operational conditions and general performance of the bioreactors receiving 1.0, 2.5, 5.0, 10.0 and 15.0 kgm^{-3} of sulphate are presented as Figures 6.4 to 6.8.

For the case of all five feed sulphate concentrations the reactor was initially operated batchwise. Once the residual sulphate concentration remained constant and no further reduction was observed the reactor was switched to continuous mode. The retention time was decreased till washout occurred. For the reactor receiving 1.0 kgm^{-3} the maximum retention time was 5 d. The lowest flowrate achievable with the pump employed for this run was 0.14 ml min^{-1} (corresponding to a 5 d retention time). The retention time was decreased in increments of 0.5 d at retention times between 4 and 5 d. For retention times between 3 and 4 d the retention time was decreased in increments of 0.25 d. Below 3 d the retention time was decreased in 0.5 d increments. The bioreactors receiving 2.5 and 5.0 kgm^{-3} sulphate were operated at a maximum retention time of 9 d and the 10.0 and 15.0 kgm^{-3} bioreactor at a maximum 10 d retention time. For bioreactors receiving 2.5, 5.0, 10.0 and 15 kgm^{-3} sulphate the retention time was initially decreased in increments of 1 d in the range 4 to 10 d and then decreased in increments of 0.5 d at retention times below 4 d.

The reactors were in operation for between 120 d (1.0 kgm^{-3} bioreactor) and 204 d (10.0 kgm^{-3} bioreactor). The trends observed for sulphate and bacterial concentrations with changes in retention time were similar for all 5 inlet sulphate concentrations. As expected, decrease in retention time resulted in instability in the system (Bailey & Ollis, 1987). A decrease in retention was associated with fluctuations in residual sulphate and bacterial concentrations. The fluctuation was followed by a stabilisation to the 'stable' state value. Compared to the sulphate concentration, the bacterial concentration was more sensitive to changes in retention time (Figures 6.4 to 6.8). The residual sulphate concentration increased immediately after the perturbation and subsequently decreased to the 'stable' state value. Significant washout of cells was evident at retention times between 0.5 and 2.0 d (Figures 6.1 and 6.2 and Appendix A which contains time-course profiles for the range of feed sulphate concentrations).

The transient profile for the bioreactor receiving 15.0 kgm^{-3} sulphate differed to the other profiles. In this case 'stable' state conditions at each retention time was achieved after a long period. For example, at a 9 d

retention time it took 27 d to reach 'stable' state as opposed to 16 days for the bioreactor receiving 5.0 kgm^{-3} .

The retention times at which washout occurred differed for the 5 bioreactors. For the bioreactors receiving 1.0, 2.5, 5.0, 10.0 and 15.0 kgm^{-3} sulphate washout was approximated to occur at retention times of 2.0, 1.5, 1.0, 0.5 and 4.0 d respectively. The disturbance in trends observed for residual sulphate concentration for inlet sulphate concentrations of 1.0 and 5.0 kgm^{-3} at day 31 and 39 respectively was due to technical problems. For the bioreactor receiving 1.0 kgm^{-3} the reactor became unstable due to failure of the pH control. The residual sulphate concentration increased to 1.0 kgm^{-3} and the bacterial concentration decreased to 0.38 gL^{-1} . Seven days after the pH control was functional the system stabilised. For the bioreactor receiving 5.0 kg m^{-3} sulphate the three fold increase in residual sulphate concentration and 16 % decrease in bacterial concentration was due to a power failure resulting in a decrease reactor temperature and no feed being pumped to the bioreactor. After the power returned (6 h) it took the system 8 d to stabilise.

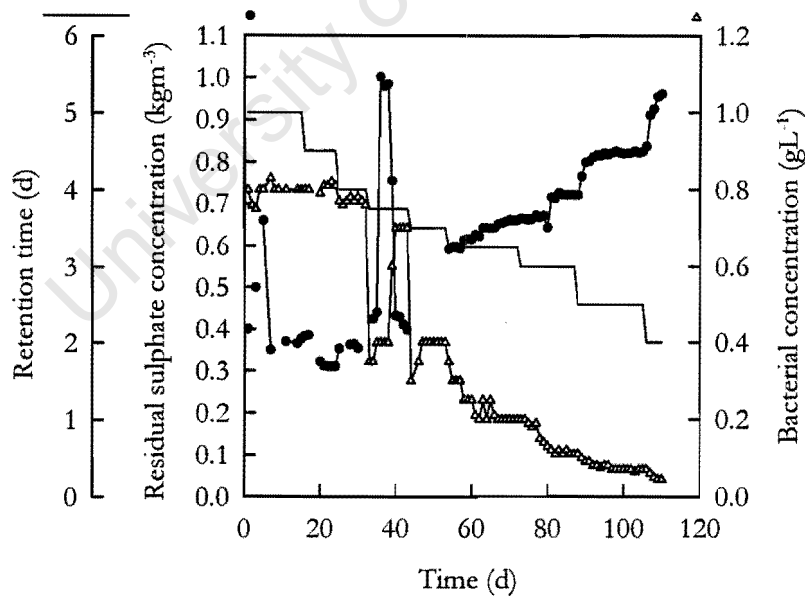


Figure 6.4. Transient profiles of retention time (—), residual sulphate concentration (●) and bacterial concentration (Δ) for the continuous reduction of sulphate with a feed sulphate concentration of 1.0 kgm^{-3} . The continuous bioreactor was operated at 35°C and pH 7.8 with a feed acetate concentration of 17.5 kgm^{-3} .

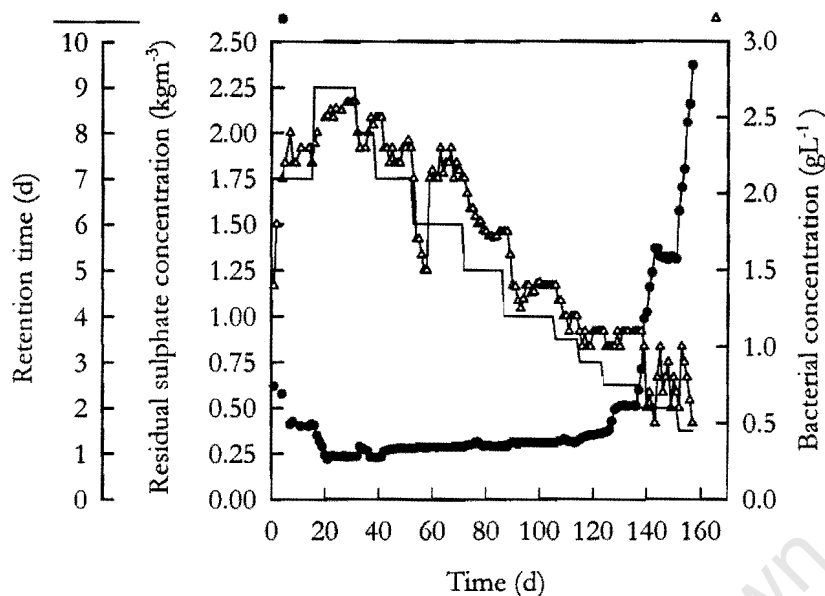


Figure 6.5. Transient profiles of retention time (—), residual sulphate concentration (●) and bacterial concentration (Δ) for the continuous reduction of sulphate with a feed sulphate concentration of 2.5 kgm⁻³. The continuous bioreactor was operated at 35°C and pH 7.8 with a feed acetate concentration of 17.5 kgm⁻³.

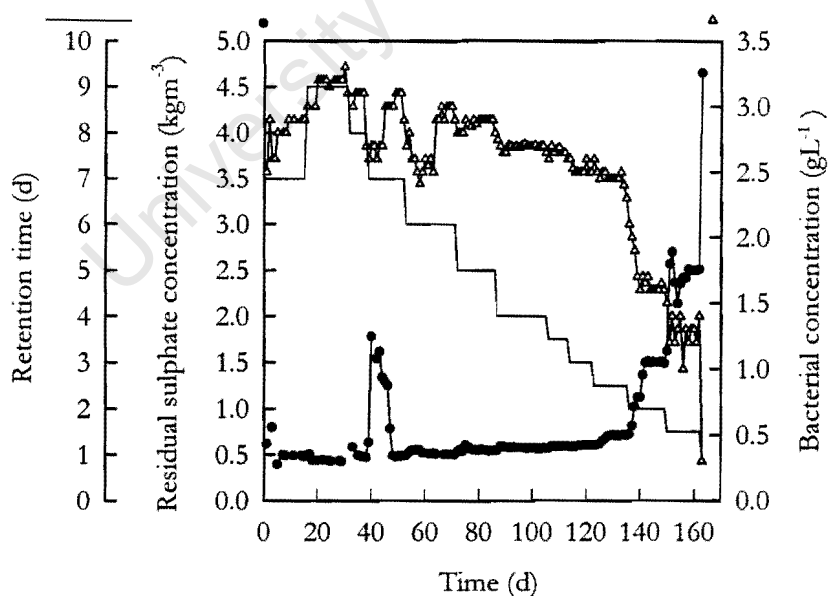


Figure 6.6. Transient profiles of retention time (—), residual sulphate concentration (●) and bacterial concentration (Δ) for the continuous reduction of sulphate with a feed sulphate concentration of 5.0 kgm⁻³. The continuous bioreactor was operated at 35°C and pH 7.8 with a feed acetate concentration of 17.5 kgm⁻³.

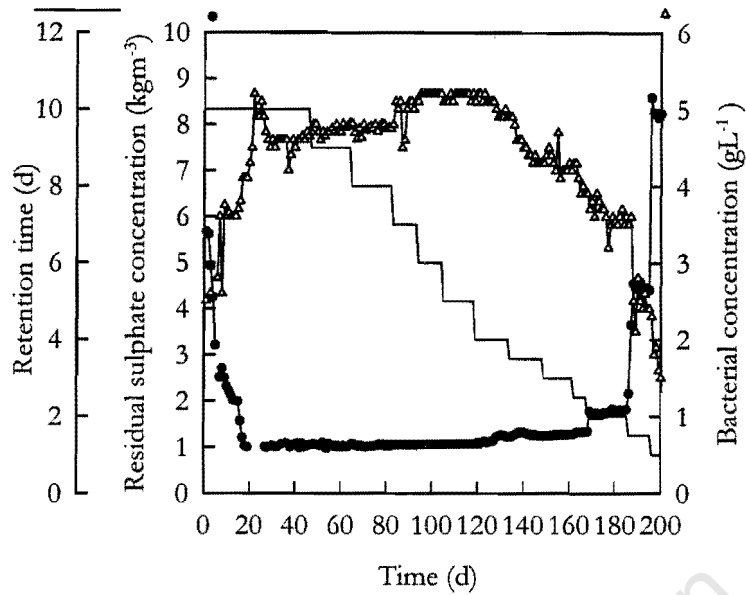


Figure 6.7. Transient profiles of retention time (—), residual sulphate concentration (●) and bacterial concentration (Δ) for the continuous reduction of sulphate with a feed sulphate concentration of 10.0 kgm^{-3} . The continuous bioreactor was operated at 35°C and pH 7.8 with a feed acetate concentration of 17.5 kgm^{-3} .

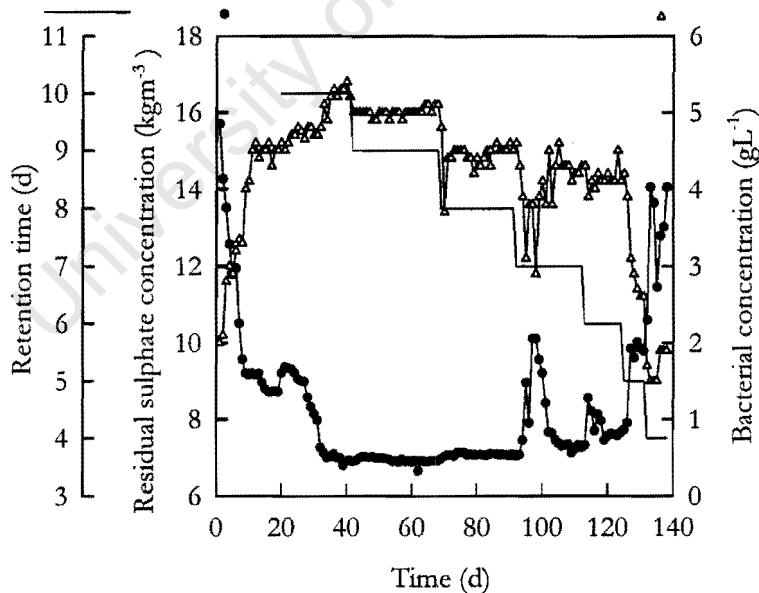


Figure 6.8. Transient profiles of retention time (—), residual sulphate concentration (●) and bacterial concentration (Δ) for the continuous reduction of sulphate with a feed sulphate concentration of 15.0 kgm^{-3} . The continuous bioreactor was operated at 35°C and pH 7.8 with a feed acetate concentration of 17.5 kgm^{-3} .

6.4 'STABLE' STATE RESULTS

In all cases 'stable' state conditions were considered to be achieved when the residual sulphate concentrations and bacterial concentrations did not vary by more than 12 and 15 % respectively over a period of operation equal to a retention time. The volumetric reduction rate of sulphate at 'stable' state conditions calculated, on the basis of initial and final sulphate concentrations, flowrate and working volume was used to quantify the kinetic rate expression (Eqn 6.1).

$$r_s = ([S_o] - [S]) \times \frac{F}{V} \quad 6.1$$

Where: r_s = volumetric sulphate reduction rate ($\text{kgm}^{-3}\text{h}^{-1}$),
 $[S_o]$ = feed sulphate concentration (kgm^{-3}),
 $[S]$ = residual sulphate concentration (kgm^{-3}),
 F = feed flowrate ($\text{m}^3 \text{h}^{-1}$) and
 V = Working volume (m^3).

6.4.1 FEED SULPHATE CONCENTRATION OF 1.0 kgm^{-3}

For the feed medium initially containing 1.0 kgm^{-3} of sulphate (Figure 6.9), the maximum bacterial concentration of 0.98 gL^{-1} was observed at a dilution rate of 0.006 h^{-1} (volumetric sulphate loading rate of $0.006 \text{ kgm}^{-3}\text{h}^{-1}$). Increasing dilution rates in the range 0.006 to 0.011 h^{-1} resulted in a gradual decrease in bacterial concentration. This decreasing trend became pronounced as the dilution rate was increased above 0.011 h^{-1} . However, there was no distinct dilution rate corresponding to washout, owing to the mixed SRB population present. At a dilution rate of 0.014 h^{-1} (volumetric sulphate loading rate of $0.014 \text{ kgm}^{-3}\text{h}^{-1}$) the bacterial concentration was 10 % that of the maximum observed at a dilution rate of 0.006 h^{-1} .

As can be seen in Figure 6.9, 80 to 85 % conversion of sulphate to sulphide was observed at volumetric sulphate loading rates in the range 0.006 to $0.008 \text{ kgm}^{-3}\text{h}^{-1}$ (dilution rates in the range 0.006 to 0.008 h^{-1}).

The corresponding sulphate reduction rate increased across this range from 0.005 to 0.007 $\text{kg m}^{-3}\text{h}^{-1}$. On increasing the volumetric sulphate loading rate in the range 0.008 to 0.011 $\text{kgm}^{-3}\text{h}^{-1}$ (dilution rates in the range 0.008 to 0.011 h^{-1}), a relatively constant sulphate reduction rate of 0.007 $\text{kgm}^{-3}\text{h}^{-1}$, was observed. This represented the maximum reduction rate. The corresponding conversion of sulphate varied between 81 % (volumetric sulphate loading rate of 0.008 $\text{kgm}^{-3}\text{h}^{-1}$) and 61 % (volumetric sulphate loading rate of 0.011 $\text{kgm}^{-3}\text{h}^{-1}$). The constant reduction rate with increasing loading rate was accompanied by a descending trend in conversion of sulphate. Further increase in loading rate led to a sharp decrease in both conversion and reduction rate of sulphate. The trend of acetate utilization was similar to that for sulphate reduction. At low volumetric sulphate loadings, corresponding to a high reduction rate of sulphate, acetate utilization was high. The decrease in reduction rate of sulphate at higher volumetric sulphate loadings led to a decrease in utilization of acetate. The ratio of sulphate reduced to acetate utilized was 0.84 ± 0.015 moles sulphate/moles acetate for the range of applied loading rates.

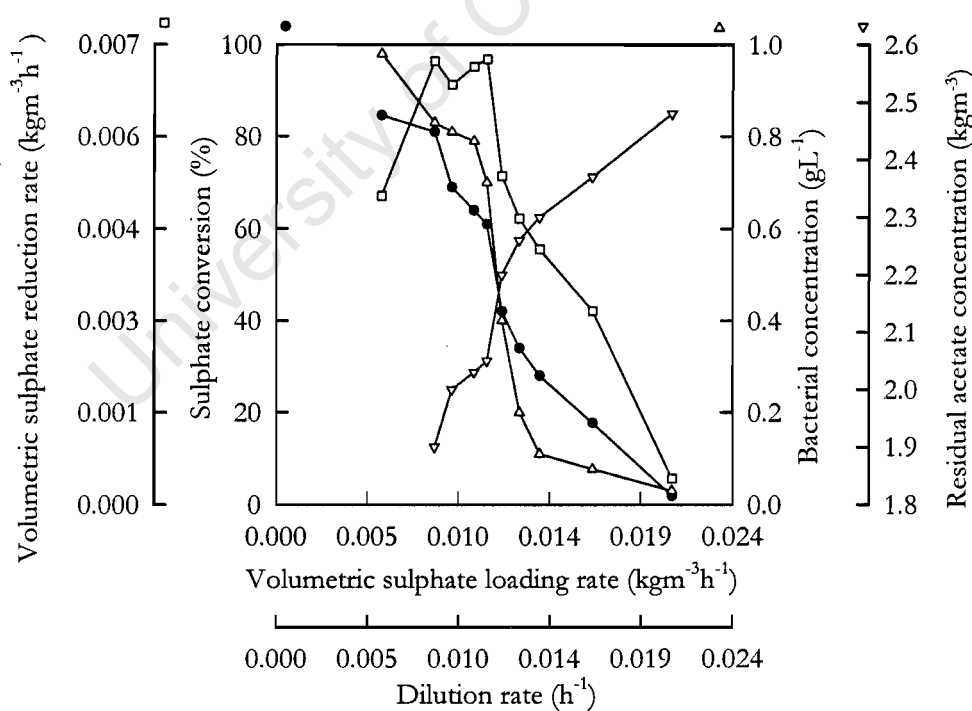


Figure 6.9. 'Stable' state kinetics of continuous reduction of sulphate at 35°C and pH 7.8 in a feed medium containing 1.0 kgm^{-3} sulphate and 2.5 kgm^{-3} acetate. 'Stable' state data presented includes volumetric sulphate reduction rate (\square), sulphate conversion (\bullet), bacterial concentration (Δ) and residual acetate concentration (∇).

6.4.2 FEED SULPHATE CONCENTRATION OF 2.5 kgm^{-3}

With a feed medium containing 2.5 kgm^{-3} of sulphate, shown in Figure 6.10, the bacterial concentration decreased consistently with increasing dilution rate. The maximum bacterial concentration of 2.60 gL^{-1} was observed at a dilution rate of 0.005 h^{-1} (volumetric sulphate loading rate of $0.0130 \text{ kgm}^{-3}\text{h}^{-1}$). The bacterial concentration profile did not indicate any distinct washout value. Sulphate conversion of 80 to 90 % was observed for volumetric sulphate loading rates in the range 0.010 to $0.042 \text{ kg m}^{-3} \text{ h}^{-1}$ (dilution rate of 0.004 to 0.017 h^{-1}). The volumetric sulphate reduction rate increased from 0.009 to $0.032 \text{ kgm}^{-3}\text{h}^{-1}$ with increasing volumetric sulphate loading in this range. The maximum reduction rate of $0.032 \text{ kgm}^{-3}\text{h}^{-1}$ was obtained at a volumetric sulphate loading rate of $0.042 \text{ kgm}^{-3}\text{h}^{-1}$ (dilution rate of 0.017 h^{-1} , retention time of 2.5 d). The conversion of sulphate at this retention time was 80 %. Applying higher volumetric sulphate loading rates led to a substantial decrease in the reduction rate of sulphate. The dependency of acetate utilization and sulphate reduction was similar to the previous run with a constant ratio of 0.77 ± 0.010 mole reduced sulphate/mole utilized acetate.

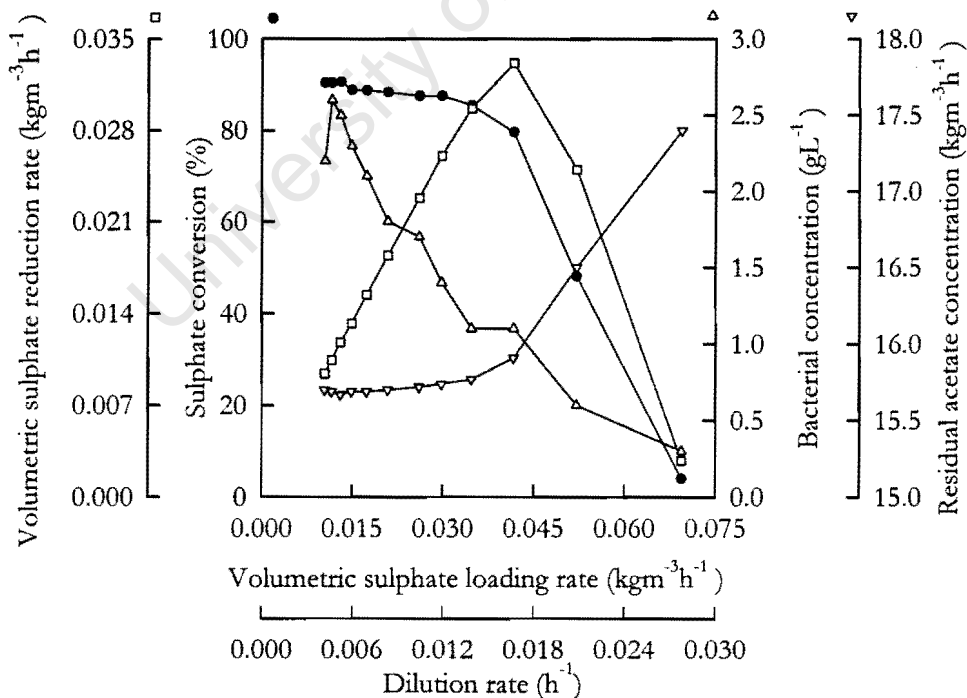


Figure 6.10. 'Stable' state kinetics of continuous reduction of sulphate at 35°C and pH 7.8 in a feed medium containing 2.5 kgm^{-3} sulphate and 17.5 kgm^{-3} acetate. 'Stable' state data presented includes volumetric sulphate reduction rate (\square), sulphate conversion (\bullet), bacterial concentration (Δ) and residual acetate concentration (∇).

6.4.3 FEED SULPHATE CONCENTRATION OF 5.0 kgm^{-3}

For a feed medium containing 5.0 kgm^{-3} sulphate, shown in Figure 6.11, the bacterial concentration decreased gradually from 3.10 gL^{-1} to 2.50 gL^{-1} as the dilution rate was increased from 0.005 to 0.017 h^{-1} (volumetric sulphate loading of 0.026 to $0.083 \text{ kgm}^{-3}\text{h}^{-1}$). This decreasing trend became more pronounced as the dilution rate was increased above 0.017 h^{-1} . No distinct dilution rate corresponding to washout was distinguished. Applying loading rates ranging from 0.021 to $0.07 \text{ kgm}^{-3}\text{h}^{-1}$ (dilution rate of 0.004 to 0.014 h^{-1}) the conversion of sulphate varied from 93 to 86% . Over this range of volumetric sulphate loading rates a linear increase in reduction rate of sulphate from 0.019 to $0.061 \text{ kgm}^{-3}\text{h}^{-1}$ was observed. Further increase of volumetric sulphate loading rate up to $0.14 \text{ kgm}^{-3}\text{h}^{-1}$ (dilution rate of 0.028 h^{-1}) led to a relatively constant region in the reduction rate curve accompanied by a decrease in conversion of sulphate. The maximum reduction rate of sulphate in this set of experiments was $0.075 \text{ kgm}^{-3} \text{ h}^{-1}$, achieved at a volumetric sulphate loading rate of $0.138 \text{ kgm}^{-3}\text{h}^{-1}$ (dilution rate of 0.028 h^{-1}). The corresponding sulphate reduction was 54% . The ratio of sulphate reduced to acetate utilized in this set of experiments was 0.83 ± 0.014 mole reduced sulphate/mole utilized acetate.

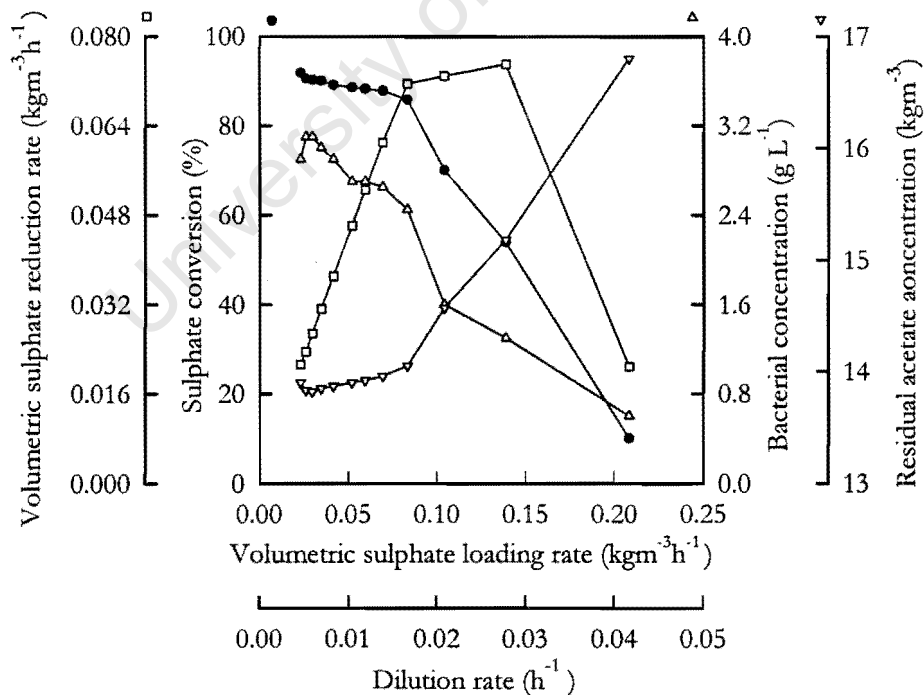


Figure 6.11. 'Stable' state kinetics of continuous reduction of sulphate at 35°C and pH 7.8 in a feed medium containing 5.0 kgm^{-3} sulphate and 17.5 kgm^{-3} acetate. 'Stable' state data presented includes volumetric sulphate reduction rate (\square), sulphate conversion (\bullet), bacterial concentration (Δ) and residual acetate concentration (∇).

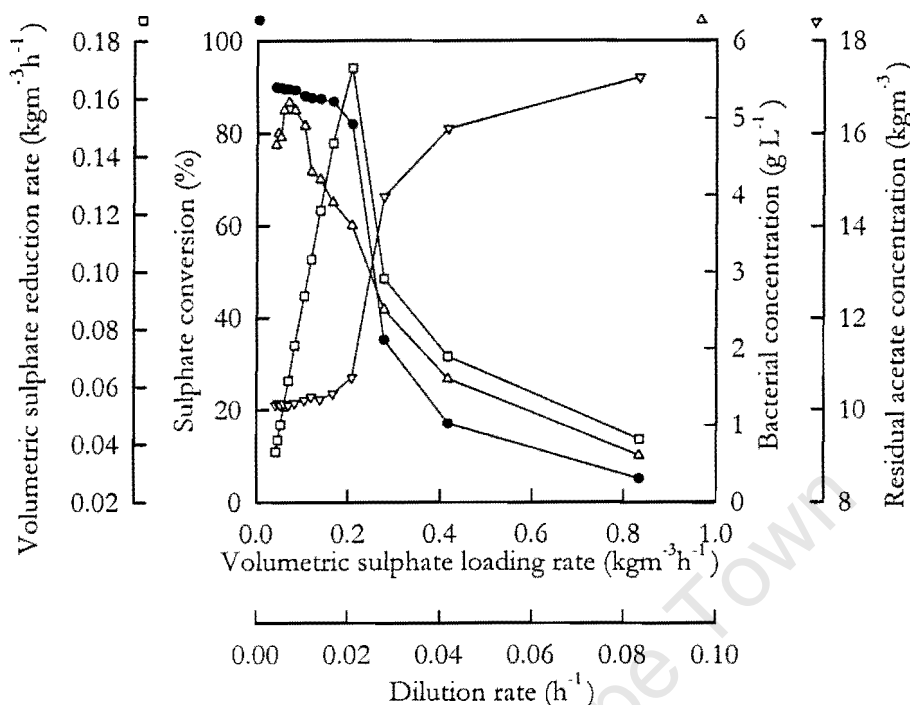
6.4.4 FEED SULPHATE CONCENTRATION OF 10 kgm^{-3} 

Figure 6.12. 'Stable' state kinetics of continuous reduction of sulphate at 35°C and pH 7.8 in a feed medium containing 10.0 kgm^{-3} sulphate and 17.5 kgm^{-3} acetate. 'Stable' state data presented includes volumetric sulphate reduction rate (\square), sulphate conversion (\bullet), bacterial concentration (\triangle) and residual acetate concentration (∇).

With the feed medium containing 10.0 kgm^{-3} of sulphate, illustrated in Figure 6.9, an increase in bacterial concentration from 4.75 to 5.10 gL^{-1} was observed for dilution rates in the range 0.005 to 0.008 h^{-1} (volumetric sulphate loading rates of 0.052 to $0.083 \text{ kgm}^{-3}\text{h}^{-1}$). The maximum biomass concentration was 5.10 gL^{-1} , occurring at dilution rates of 0.006 , 0.007 and 0.008 h^{-1} (volumetric sulphate loading rates of 0.059 , 0.069 and $0.083 \text{ kgm}^{-3}\text{h}^{-1}$). Further increase of dilution rate from 0.008 to 0.083 h^{-1} (volumetric sulphate loading of 0.083 to $0.833 \text{ kgm}^{-3}\text{h}^{-1}$) led to a decrease in bacterial concentration from 5.10 gL^{-1} to 0.60 gL^{-1} . For the volumetric sulphate loading range 0.042 to $0.208 \text{ kgm}^{-3}\text{h}^{-1}$ (dilution rate of 0.004 to 0.021 h^{-1}), a relatively constant and high conversion of sulphate (87 to 90%) was observed. The increase in volumetric sulphate loading in this range, however, led to a notable enhancement of sulphate reduction rate. The maximum reduction rate achieved was $0.171 \text{ kgm}^{-3}\text{h}^{-1}$ at a volumetric sulphate loading of $0.208 \text{ kgm}^{-3}\text{h}^{-1}$ (dilution rate of 0.021 h^{-1}). The corresponding sulphate conversion was 82% . Increasing

the volumetric sulphate loading rate beyond $0.208 \text{ kgm}^{-3}\text{h}^{-1}$ resulted in a decrease in sulphate conversion to 35 % and a reduction in the volumetric sulphate conversion rate to $0.097 \text{ kgm}^{-3}\text{h}^{-1}$. The coupling of acetate utilization and sulphate reduction was observed. Across the range of volumetric sulphate loading rates used, the ratio of sulphate reduced to acetate utilized was 0.76 ± 0.003 mole sulphate /mole acetate.

6.4.5 FEED SULPHATE CONCENTRATION OF 15 kgm^{-3}

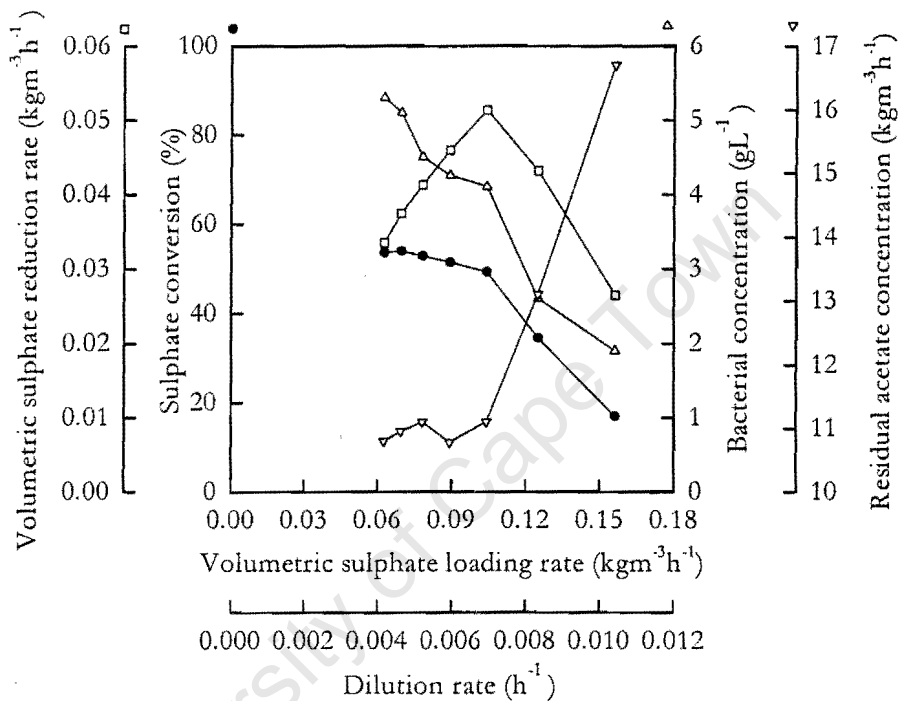


Figure 6.13. 'Stable' state kinetics of continuous reduction of sulphate at 35°C and pH 7.8 in a feed medium containing 15.0 kgm^{-3} sulphate and 17.5 kgm^{-3} acetate. 'Stable' state data presented includes volumetric sulphate reduction rate (\square), sulphate conversion (\bullet), bacterial concentration (Δ) and residual acetate concentration (∇).

With feed a medium using 15.0 kgm^{-3} sulphate (Figure 6.13), the bacterial concentration decreased steadily from 5.30 to 1.90 gL^{-1} with increasing dilution rate in the range 0.004 to 0.014 h^{-1} (volumetric sulphate loading rate of 0.063 to $0.156 \text{ kgm}^{-3}\text{h}^{-1}$). The maximum bacterial concentration of 5.30 gL^{-1} occurred at a dilution rate of 0.004 h^{-1} (volumetric sulphate loading rate of $0.063 \text{ kgm}^{-3}\text{h}^{-1}$). As can be seen in Figure 6.13, the conversion of sulphate was low (54 – 17 %) and showed a descending trend with increasing volumetric sulphate loading rate. The maximum conversion of sulphate was 54 %, achieved at volumetric

sulphate loadings of 0.063 and 0.069 $\text{kgm}^{-3}\text{h}^{-1}$ (dilution rate of 0.004 and 0.005 h^{-1}). For volumetric sulphate loading rates in the range 0.062 to 0.104 $\text{kgm}^{-3}\text{h}^{-1}$ (dilution rates of 0.004 to 0.007 h^{-1}) the sulphate conversion varied between 54 and 49 %. This was accompanied by a linear increase of volumetric sulphate reduction rate from 0.033 to 0.051 $\text{kgm}^{-3}\text{h}^{-1}$. When the volumetric sulphate loading was increased above 0.104 $\text{kgm}^{-3}\text{h}^{-1}$ (dilution rate of 0.007 h^{-1}), both sulphate conversion and volumetric sulphate reduction rate decreased significantly. The maximum reduction rate in this set of experiments was 0.051 $\text{kgm}^{-3}\text{h}^{-1}$ at a volumetric sulphate loading of 0.104 $\text{kgm}^{-3}\text{h}^{-1}$ (dilution rate of 0.007 h^{-1}). The associated sulphate reduction was 49 %. The ratio of sulphate reduced to acetate utilized was 0.73 ± 0.028 moles sulphate/moles acetate for this experimental run.

6.5 COMPARISON OF 'STABLE' STATE RESULTS

In this section, the 'stable' state results, previously detailed for bioreactors employing 1.0, 2.5, 5.0, 10.0 and 15.0 kgm^{-3} of sulphate in the feed, are compared. In particular, attention is paid to sulphate conversion, volumetric sulphate reduction rate, acetate concentration and bacterial concentration.

6.5.1 SULPHATE CONVERSION

In Figure 6.14, sulphate conversion as a function of volumetric sulphate loading is compared across bioreactor cultures with feed concentrations of 1.0, 2.5, 5.0, 10.0 and 15.0 kgm^{-3} sulphate. A similar trend in sulphate conversion with respect to volumetric sulphate loading rate was observed for the five feed sulphate concentrations. Sulphate conversion remained constant for the lower range of volumetric sulphate loadings. As the loading rate was increased beyond the point at which the volumetric sulphate reduction rate passed through a maximum, sulphate conversion decreased. The volumetric sulphate loading at which this decline occurred was unique for each sulphate concentration. The dilution rate corresponding to the decline in sulphate conversion was 0.006 h^{-1} for feed sulphate concentrations of 1.0 and 2.5 kgm^{-3} and 0.004 h^{-1} when the feed sulphate concentration was 5.0, 10.0 and 15.0 kgm^{-3} (Table 6.2). Stucki *et al.* (1992) studied the degradation of sulphuric acid by SRB in a continuous packed bed reactor, using acetate as the limiting organic nutrient. At a feed sulphate concentration of 8.0 kgm^{-3} , increasing loading rates of sulphate in the range 0.20 to 0.33 $\text{kgm}^{-3}\text{h}^{-1}$ resulted in a constant sulphate conversion of

98 %. As the volumetric sulphate rate was increased further to $0.67 \text{ kgm}^{-3}\text{h}^{-1}$ the sulphate conversion decreased to 74 %. A similar trend was reported for a feed sulphate concentration of 16.0 kgm^{-3} .

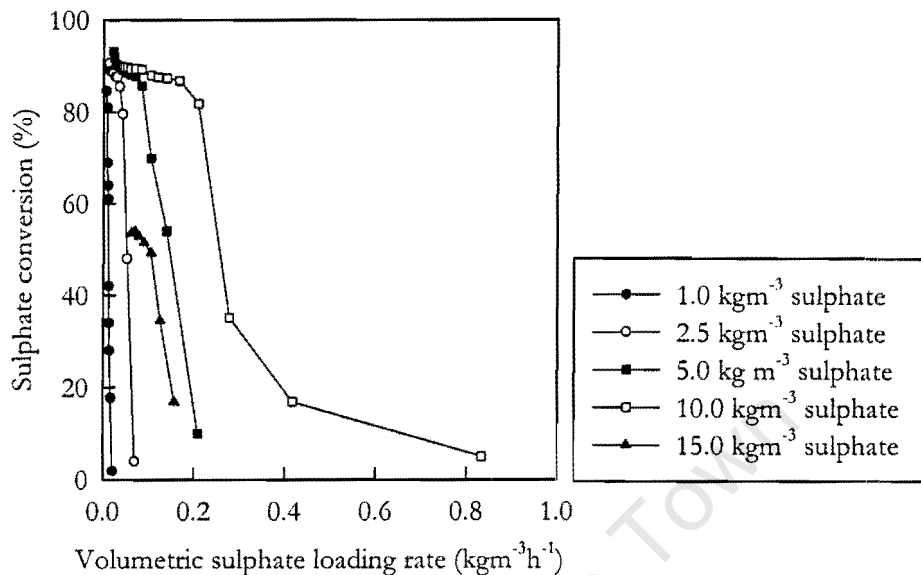


Figure 6.14. Dependency of 'stable' state sulphate conversion profiles on volumetric sulphate loading rate and feed sulphate concentration. Data obtained from continuous bioreactors operating with 1.0 (●), 2.5 (○), 5.0 (■), 10.0 (□) and 15.0 (▲) kgm^{-3} sulphate at 35°C and pH 7.8.

As the inlet sulphate concentration was increased from 1.0 to 10.0 kgm^{-3} the maximum percentage sulphate conversion observed did not vary significantly and was between 85 and 92 % (Table 6.2, Figure 6.14). The volumetric sulphate loading rate at which the maximum sulphate conversion occurred was 0.006, 0.015, 0.023 and 0.042 kgm^{-3} (dilution rates of 0.006, 0.006, 0.004, 0.004 h^{-1}) for feed sulphate concentrations of 1.0, 2.5, 5.0 and 10.0 kgm^{-3} respectively. Work by Grady *et al.* (1972) on *Aerobacter aerogenes* in a continuous system degrading glucose (COD ranging from 0.5 to 1.5 kgm^{-3}) showed that the maximum percentage COD removal remained relatively constant at 99 % regardless of feed COD concentration. Further work by Grady and Williams (1975) using a mixed culture in a continuous system degrading a mixed organic feed with COD ranging from 0.5 to 2.0 kgm^{-3} showed that the maximum percentage conversion of COD was constant at 98 % regardless of the feed COD. Stucki *et al.* (1992) using a continuous packed bed reactor observed a maximum sulphate conversion of 98 %, at a dilution

rate of 0.021 h^{-1} , for initial sulphate concentrations of 0.5 and 8.0 kgm^{-3} . When the inlet sulphate concentration was increased to 16.0 kgm^{-3} they observed a decline in the maximum conversion achievable. Similarly for this work, when the inlet sulphate concentration was increased to 15.0 kgm^{-3} the maximum sulphate conversion decreased to 54% . The corresponding volumetric sulphate loading rate was 0.069 kgm^{-3} (dilution rate of 0.004 h^{-1}). During the continuous reduction of sulphate in a UASB treating a VFA mixture Omil *et al.* (1998) observed a decrease in sulphate conversion from 70.4 to 60.7% as the sulphate concentration in the feed was increased from 2.9 to 3.5 kgm^{-3} . They proposed sulphide, resulting from the conversion of sulphate, to be responsible for the decrease in sulphate conversion at the higher feed sulphate concentrations.

Table 6.2. Variation in maximum sulphate conversion and corresponding volumetric sulphate loading rates as a function of feed sulphate concentration.

Sulphate concentration (kgm^{-3})	Maximum sulphate conversion (%)	Corresponding volumetric sulphate loading rate ($\text{kgm}^{-3}\text{h}^{-1}$)	Corresponding dilution rate (h^{-1})
1.0	85	0.006	0.006
2.5	90	0.015	0.006
5.0	92	0.023	0.004
10.0	90	0.042	0.004
15.0	54	0.069	0.004

The continuous bioreactors were operated at 35°C and $\text{pH } 7.8$.

The decrease in sulphate conversion observed for an inlet sulphate concentration of 15.0 kgm^{-3} could be attributed to either sulphate or sulphide inhibition. For the 5 bioreactors receiving $1.0, 2.5, 5.0, 10$ and 15.0 kgm^{-3} sulphate in the feed, the residual sulphide concentration was not measured directly for all 'stable' state conditions due to unreliability of the measurement technique. Consequently, the total sulphide ($\text{H}_2\text{S} + \text{HS}^- + \text{S}^{2-}$) concentration was calculated from the stoichiometric conversion of sulphate to sulphide. The fraction of each sulphide species ($\text{H}_2\text{S}, \text{HS}^-, \text{S}^{2-}$) present in the liquid phase varies as a function of liquid pH and was quantified using equilibrium relationships ($[\text{H}_2\text{S}]_{\text{aq}} \xrightleftharpoons{K_1} [\text{HS}^-] \xrightleftharpoons{K_2} [\text{S}^{2-}]$). Detailed calculations are given in Appendix B. Figure 6.15 presents

the dependency of the fraction of H_2S , HS^- and S^{2-} on pH. Using the relative fractions of H_2S , HS^- and S^{2-} (at pH 7.8) presented in Figure 6.15 and the total sulphide concentrations calculated at 'stable' state conditions, the concentrations of H_2S , HS^- and S^{2-} for the five bioreactors under were calculated. The dominant species at pH 7.8 are H_2S and HS^- .

'Stable' state residual H_2S and HS^- profiles for bioreactors receiving 1.0, 2.5, 5.0, 10.0 and 15.0 $\text{kgm}^{-3}\text{h}^{-1}$ are presented as Figures 6.16 and 6.17. The residual H_2S and HS^- concentrations present at 15.0 kgm^{-3} varied from 0.03 to 0.26 kgm^{-3} and 0.25 to 2.43 kgm^{-3} (Table 6.3) respectively. This was below the range observed at 10.0 kg m^{-3} (0.25 to 2.43 kgm^{-3} for HS^- and 0.03 to 0.26 kgm^{-3} for H_2S). The similarity in residual HS^- and H_2S concentrations of the bioreactors receiving 10.0 kgm^{-3} and 15.0 kgm^{-3} sulphate indicates that sulphide is not inhibiting the bioreactor receiving 15.0 kgm^{-3} .

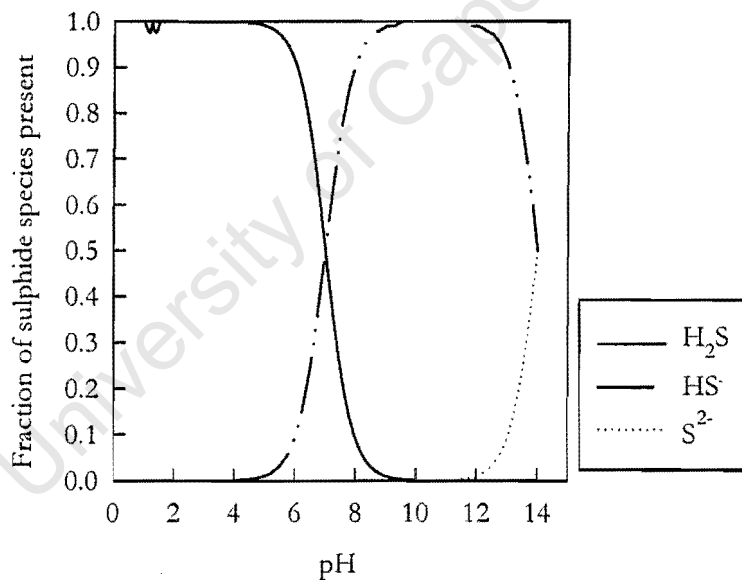


Figure 6.15. The fraction of H_2S , HS^- and S^{2-} present in the liquid phase as a function of pH.

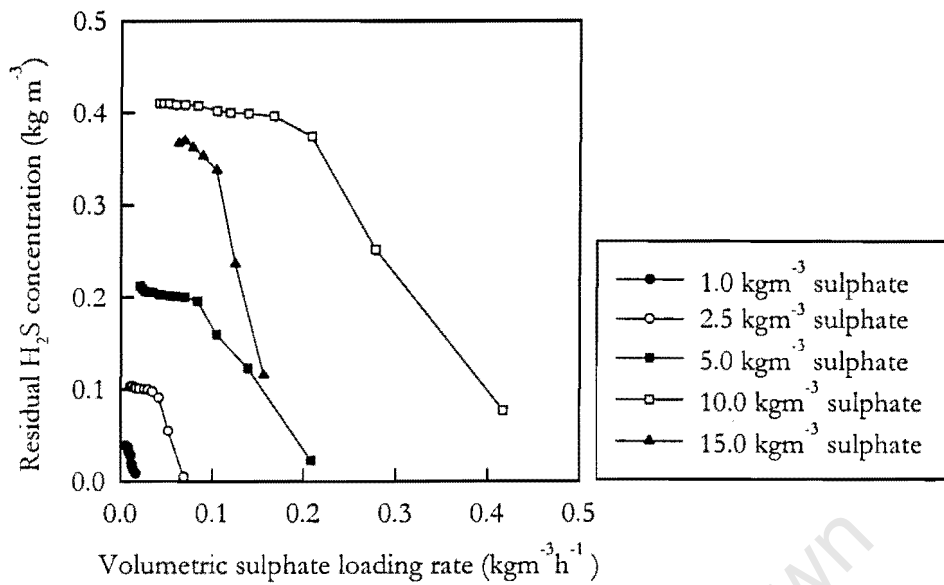


Figure 6.16. Calculated residual H_2S profiles at 'stable' state given as a function of volumetric sulphate loading rate and feed sulphate concentration. Data obtained from continuous bioreactors operating with 1.0 (●), 2.5 (○), 5.0 (■), 10.0 (□) and 15.0 (▲) kgm^{-3} sulphate at 35°C and pH 7.8.

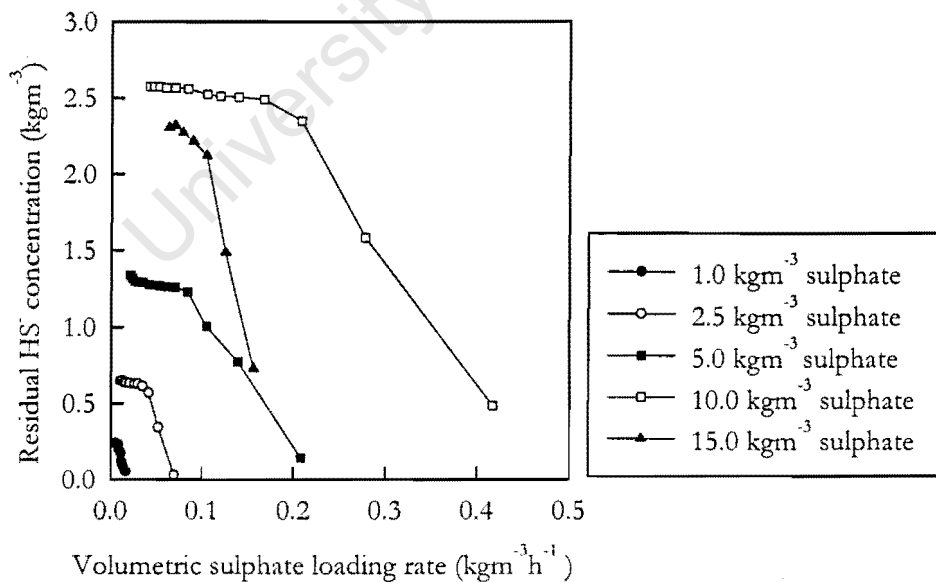


Figure 6.17. Calculated residual HS^- profiles at 'stable' state given as a function of volumetric sulphate loading rate and feed sulphate concentration. Data obtained from continuous bioreactors operating with 1.0 (●), 2.5 (○), 5.0 (■), 10.0 (□) and 15.0 (▲) kgm^{-3} sulphate at 35°C and pH 7.8.

Table 6.3. Comparison of reported H₂S and HS⁻ concentrations at which 50 % inhibition of sulphate reduction occurs with maximum sulphide concentrations observed for this work.

Reference	Microorganism	pH	TS kgm ⁻³	H ₂ S kgm ⁻³	HS ⁻ kgm ⁻³	
Visser (1995)	Mixed SRB	7.3	0.57	0.17	0.40	
		8.2	1.11	0.07	1.04	
O' Flaherty <i>et al.</i> (1998)	<i>Desulfovibrio magus</i>	7.2	0.67	0.22	0.45	
		8.5	0.70	0.01	0.69	
	<i>Desulfovibrio acetoxidans</i>	7.2	0.77	0.25	0.52	
		8.5	1.45	0.03	1.42	
	<i>Desulfovibrio postgatei</i>	7.2	0.93	0.30	0.63	
		8.5	1.29	0.03	1.26	
	Mixed SRB	7.2	0.55	0.18	0.37	
		8.5	1.01	0.02	0.99	
	This work	Mixed SRB (1.0 kgm ⁻³)	7.8	0.28	0.03	0.25
		Mixed SRB (2.5 kgm ⁻³)	7.8	0.75	0.07	0.68
Mixed SRB (5.0 kgm ⁻³)		7.8	1.54	0.15	1.39	
Mixed SRB (10.0 kgm ⁻³)		7.8	2.99	0.29	2.70	
Mixed SRB (15.0 kgm ⁻³)		7.8	2.69	0.26	2.43	

In all cases acetate was used as the organic and electron source.

Where: TS: total sulphide concentration.

This study has shown that the sulphide concentration tolerated by the SRB culture used for this work was relatively high (3.0 kgm⁻³ total sulphide for the bioreactor receiving 10.0 kgm⁻³ sulphate). Studies reported by other researchers on the effect of pH on sulphide tolerance have shown that increasing pH in the range 6.8 to 8.5 enhances the ability of the culture to tolerate the total sulphide present (Table 6.3). The sulphide tolerated is culture specific (Table 6.3). However, from the H₂S concentrations presented in Table 6.3 it can be seen that the H₂S concentrations at which 50 % inhibition occurs are comparable across researchers. Visser (1995) showed the total sulphide concentration for 50 % inhibition of a mixed SRB culture grown batchwise in a UASB using acetate as the organic source to vary from 0.6 to 1.1 kgm⁻³ when the pH varied between 7.2-7.4 and 8.1 to 8.3. Using acetate as the organic source and adding sulphide O' Flaherty *et al.* (1998) reported that the total sulphide concentration for 50 % inhibition of sulphate reduction varied between 0.6 and 1.5 kgm⁻³ when the pH was increased from between 7.2 and 8.5. Pure

sulphate reducing cultures (*Desulfohalobium magnum*, *Desulfohalobium acetoxidans*, *Desulfohalobium postgatei*) were grown in batch. No data has been found in the open literature that provides results from continuous bioreactor studies on mixed SRB utilising acetate as the organic source. The concentration of H₂S observed for this work lies within the range at which 50 % inhibition was observed by other researchers. It has been reported by Omil *et al.* (1998), studying the competition between methanogens and SRB in a continuous upflow granular sludge bed, that the SRB can be acclimatised to increasing sulphide levels and as a result can tolerate a high level of sulphide. A mixture of acetate, propionate and butyrate was used as the organic source.

The counter ion, sodium, has been implicated as having inhibitory effects on the anaerobic reduction process at concentrations above 17.5 kgm⁻³ (Soto *et al.*, 1993; Lens *et al.*, 1998b). Furthermore, Visser (1995) showed that sodium concentrations above 15.0 kgm⁻³ resulted in 50 % inhibition of the activity of SRB from a granular sludge. However, the sodium content of a wastewater is not expected to cause complete process failure (Lens *et al.*, 1998b). The maximum sodium concentration for this study was 7.13 kgm⁻³ corresponding to a feed sulphate concentration of 15.0 kg⁻³ and accumulation of the sodium was not expected in the system since it was continuous. Against this background it is postulated that the sodium content in the bioreactors is not responsible for the lower conversion of sulphate to sulphide observed at feed sulphate concentrations of 15.0 kgm⁻³.

Residual sulphate concentration profiles for the bioreactors operating with 1.0, 2.5, 5.0, 10.0 and 15.0 kgm⁻³ sulphate in the feed medium are shown as Figure 6.18. The highest residual sulphate concentrations are observed in the bioreactor receiving 15.0 kgm⁻³ sulphate in the feed (residual sulphate concentrations ranging between 6.9 and 12.5 kgm⁻³). The high residual sulphate concentrations could be the cause of the low sulphate conversion observed. Inhibition of sulphate reduction was observed for batch studies when the feed sulphate concentration was 5.0 kgm⁻³ (Chapter 5). Sulphate inhibition has been intimated by White and Gadd (1996) when growing a mixed SRB culture on lactate. They reported that "a significant excess of sulphate may lead to inhibition or cessation of sulphate reduction", citing the positive effect that sulphate has on redox potential as the reason for inhibition of sulphate reduction. The

E_h range for optimal sulphate conversion has been given as -100 to 220 mV (Barnes, 1992a; Postgate, 1984). In the case of this work, the redox potential was kept below -100 mV for all experiments because of the presence of sulphide ions. Substrate inhibition has been observed by Nemati and Webb (1996) studying the biological oxidation of ferrous iron in a packed bed reactor. Similar to the results of this work they noted a decrease in the conversion of ferrous iron as the feed ferrous iron concentration was increased from 5.0 kgm^{-3} to 20.0 kgm^{-3} . The percentage conversion when the feed ferrous iron concentration was 5.0 kgm^{-3} ranged between 95 and 38 % for volumetric ferrous iron loading rates in the range 1.0 to 32.0 $\text{kgm}^{-3}\text{h}^{-1}$ (dilution rates of 0.10 to 6.0 h^{-1}). For a feed ferrous iron concentration of 20.0 kgm^{-3} the percentage conversion was between 90 and 2 % as the volumetric ferrous iron ranged between 1.0 and 57.0 $\text{kgm}^{-3}\text{h}^{-1}$ (dilution rates of 0.20 to 2.8 h^{-1}). Recently Dries *et al.* (1998) studying continuous anaerobic sulphate reduction in an expanded granular sludge bed reactor with a sulphate-acetate medium observed a decrease in sulphate conversion from 84 to 64 % as the feed sulphate concentration was increased from 0.80 to 1.0 kgm^{-3} . Substrate inhibition has been mentioned in the general biotechnology context and attributed to the inhibition of the enzymes catalyzing the reaction (Shuler & Kargi, 1992; Nielsen & Villadsen, 1994; Bailey & Ollis, 1986; Pirt, 1975).

A possible explanation for the inhibitory nature of high sulphate concentrations, as observed for this work, has been proposed by Barton and Tomei (1995) who suggests two systems for the uptake of sulphate by SRB, a low sulphate concentration system and a high sulphate concentration system. At high sulphate concentrations the low sulphate concentration mechanism is switched off and in some cases the uptake of sulphate is stopped to prevent flooding of the cell with sulphate that will ultimately result in activity loss. Consequently, the reduction of sulphate is inhibited. From the present experimental observations, it can be deduced that the bioreactor receiving 15.0 kgm^{-3} sulphate is inhibited by high concentrations of residual sulphate. The potential inhibitory effect of sulphide on the microbial reduction of sulphate cannot be discounted from acting concomitantly with sulphate inhibition.

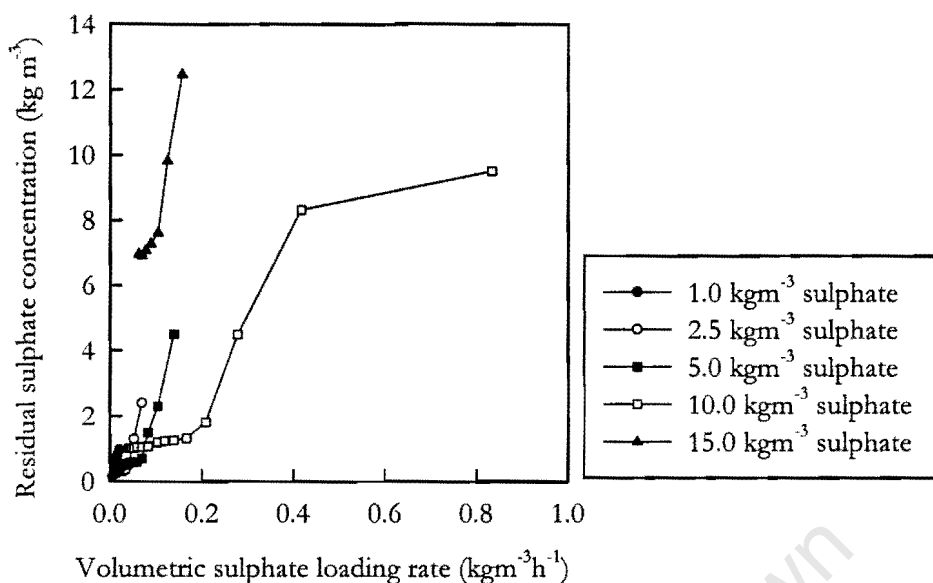


Figure 6.18. Dependency of 'stable' state residual sulphate concentration profiles on volumetric sulphate loading rate and feed sulphate concentration. Data obtained from continuous bioreactors operating with 1.0 (●), 2.5 (○), 5.0 (■), 10.0 (□) and 15.0 (▲) kgm⁻³ sulphate at 35°C and pH 7.8.

6.5.2 VOLUMETRIC SULPHATE REDUCTION RATE

The kinetics of anaerobic sulphate conversion, at 5 different feed sulphate concentrations are compared in Figure 6.19. For sulphate concentrations of 1.0, 2.5, 5.0 and 10.0 kgm⁻³ the volumetric sulphate reduction rate is an increasing linear function of volumetric sulphate loading rate for volumetric sulphate loading rate (and dilution rates) below washout. This indicates decreasing metabolic efficiency with increasing feed sulphate concentration. As the volumetric sulphate loading rate (and dilution rate) was increased for each of these 4 experiments, washout was approached. The onset of washout is gradual because of the mixed SRB population. Where washout was significant, the increasing linear relationship between volumetric sulphate loading and volumetric sulphate reduction rate ceases. The volumetric sulphate reduction rate displays a decrease with further increase in volumetric sulphate loading. For a particular experiment at the same volumetric sulphate loading rates, the volumetric sulphate reduction rates are the same until the dilution rate causes washout for a particular experiment.

Inhibition of the continuous bioreactor receiving a feed stream containing 15.0 kg m^{-3} sulphate causes the volumetric sulphate reduction rate not to be represented by the same function of volumetric sulphate loading rate as observed at feed concentrations of 1.0, 2.5, 5.0 and 10.0 kgm^{-3} sulphate. Additionally, the maximum reduction rate is low (0.051 kgm^{-3}) compared to the bioreactor receiving 10.0 kgm^{-3} sulphate in the feed (0.170 kgm^{-3}) (Table 6.4). For the range of volumetric sulphate loading rates, established at a feed sulphate concentration of 15.0 kgm^{-3} the volumetric sulphate reduction rates ranged between 0.026 and $0.051 \text{ kgm}^{-3}\text{h}^{-1}$. These values are considerable lower than the range observed at a feed sulphate concentration of 10.0 kgm^{-3} ($0.037 - 0.171 \text{ kgm}^{-3}\text{h}^{-1}$). The lowest specific volumetric sulphate reduction rate was observed for the bioreactor receiving 15.0 kgm^{-3} sulphate in the feed, indicating metabolic inhibition.

The maximum volumetric sulphate reduction rate achieved increased as the feed sulphate concentration was increased in the range 1.0 to 10.0 kgm^{-3} (Table 6.4). When the feed medium contained 15.0 kgm^{-3} sulphate the maximum sulphate reduction rate decreased. The volumetric sulphate loading rate at which the maximum reduction rate was observed also increased as the feed sulphate concentration was increased in the range 1.0 to 10.0 kgm^{-3} . The reduction rate trend as a function of initial sulphate concentration was similar to that observed by Stucki *et al.* (1993). Using a mixed population in a fixed bed reactor with glass beads reducing sulphate with acetate as the organic source they found that as the initial sulphate concentration was increased the maximum reduction rate observed increased. Similar to this work, Nemati and Webb (1996) observed an increase of volumetric reduction rate with volumetric loading rate for increasing feed substrate concentrations in a certain range. They found that for the biological oxidation of ferrous iron in a packed bed receiving 5.0, 10.0 and 20.0 kgm^{-3} ferrous iron in the feed the maximum oxidation rates were achieved for the medium containing 5.0 and 10.0 kgm^{-3} ferrous iron. The bioreactor receiving 20.0 kgm^{-3} ferrous iron in the feed exhibited lower oxidation rates indicating an inhibitory effect of ferrous iron on the microbial activity.

Table 6.4. Dependency of maximum volumetric sulphate reduction rate and corresponding sulphate conversion, volumetric sulphate loading rate and dilution rate on feed sulphate concentration. The continuous bioreactor was operated at 35°C and pH 7.8.

Sulphate concentration (kgm^{-3})	Maximum volumetric sulphate reduction rate ($\text{kgm}^{-3}\text{h}^{-1}$)	Corresponding volumetric sulphate loading rate ($\text{kgm}^{-3}\text{h}^{-1}$)	Corresponding retention time (h)
1.0	0.007	0.011	90.9
2.5	0.032	0.042	58.8
5.0	0.075	0.139	35.7
10.0	0.170	0.200	50.0
15.0	0.051	0.104	142.9

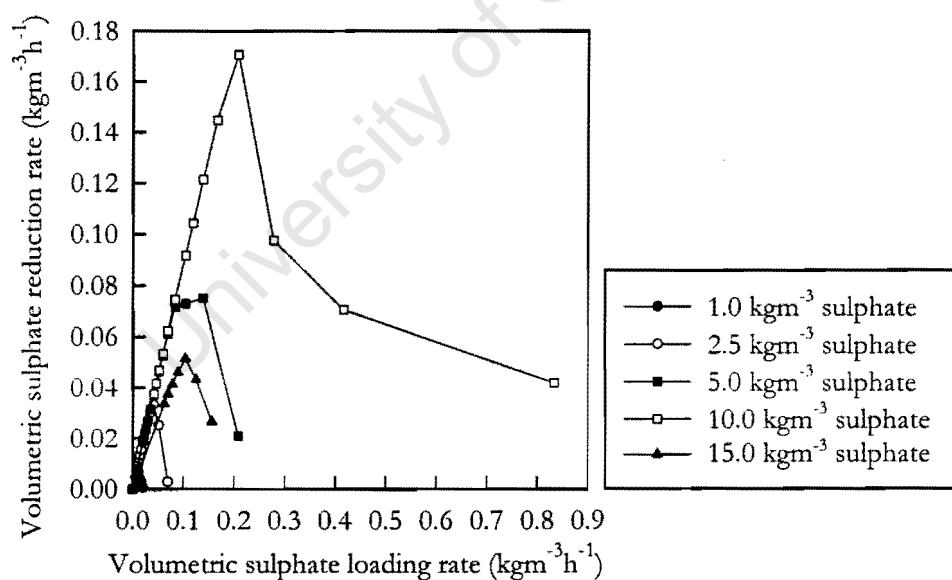


Figure 6.19. Dependency of 'stable' state volumetric sulphate reduction rate on volumetric sulphate loading rate and feed sulphate concentration. Data obtained from continuous bioreactors operating with 1.0 (●), 2.5 (○), 5.0 (■), 10.0 (□) and 15.0 (▲) kgm^{-3} sulphate at 35°C and pH 7.8.

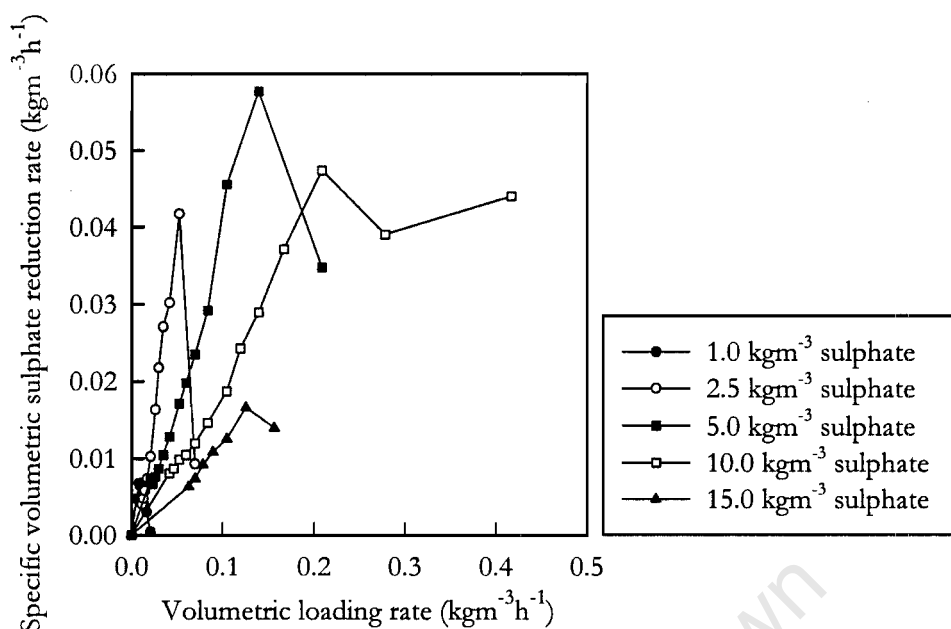


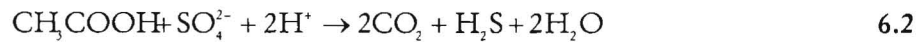
Figure 6.20. Dependency of 'stable' state specific volumetric sulphate reduction rate profiles on volumetric sulphate loading rate and feed sulphate concentration. Data obtained from continuous bioreactors operating with 1.0 (●), 2.5 (○), 5.0 (■), 10.0 (□) and 15.0 (▲) kgm⁻³ sulphate at 35°C and pH 7.8.

6.5.3 RESIDUAL ACETATE CONCENTRATION

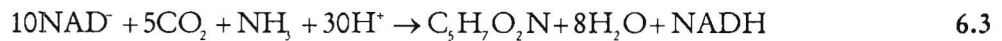
'Stable' state acetate concentrations for the five bioreactors receiving 1.0, 2.5, 5.0, 10.0 and 15.0 kgm⁻³ sulphate concentrations are shown in Figure 6.21. The concentration of acetate in the feed for the bioreactor receiving 1.0 kg m⁻³ sulphate was 2.5 kgm⁻³. For the bioreactors receiving 2.5, 5.0, 10.0 and 15.0 kgm⁻³ sulphate in the feed, an acetate concentration of 17.5 kgm⁻³ was used. For the case of all five bioreactors, the acetate utilization profile followed a similar trend to that of sulphate conversion, indicating a direct relationship between acetate utilised and sulphate reduced.

The acetate utilised provides the electrons required to convert sulphate to sulphide. During this reaction 4 NAD⁺ are formed from NADH. The NAD⁺ is used to build additional biomass with the carbon dioxide via the Calvin cycle. This process is represented graphically as Figure 6.22.

The reaction for the reduction of sulphate using acetate as an organic source can be presented by the following expression:



Eqn 6.3 represents biomass formation.



In all cases, the ratio of reduced sulphate to acetate utilised was calculated to determine whether the feed sulphate concentration had any effect on the stoichiometry of the reaction and to ascertain the metabolic efficiency of acetate (Table 6.3). The ratio of sulphate converted to acetate utilised varied between 0.73 ± 0.028 and 0.84 ± 0.015 indicating that the feed sulphate concentration did not have a significant (statistically significant considering the error of sulphate analysis was 12 %) effect on the stoichiometry of the sulphate reduction reaction nor on the efficiency of acetate utilisation. This observation is reinforced by the published literature. Characklis *et al.* (1989) states that the stoichiometry of the biological reaction should not be affected by the feed substrate concentration. The ratio below 1.00 indicates that for every 1 mole of acetate utilised, between 0.73 and 0.88 moles goes to the provision of electrons for the sulphate of reduction and since no active methanogens were present the balance went elsewhere, e.g. endogenous respiration, maintenance. The average metabolic efficiency of acetate was 79 ± 5 % for the microbial reduction of sulphate by the mixed SRB population employed for this work.

Table 6.5. Dependency of molar ratio of sulphate utilised to acetate utilised on feed sulphate concentration.

Feed sulphate concentration (kgm ⁻³)	Moles sulphate/moles acetate
1.0	0.84 ± 0.015
2.5	0.77 ± 0.010
5.0	0.83 ± 0.014
10.0	0.76 ± 0.003
15.0	0.73 ± 0.028
Theoretical	1.00

Continuous bioreactor operated at 35°C and pH 7.8.

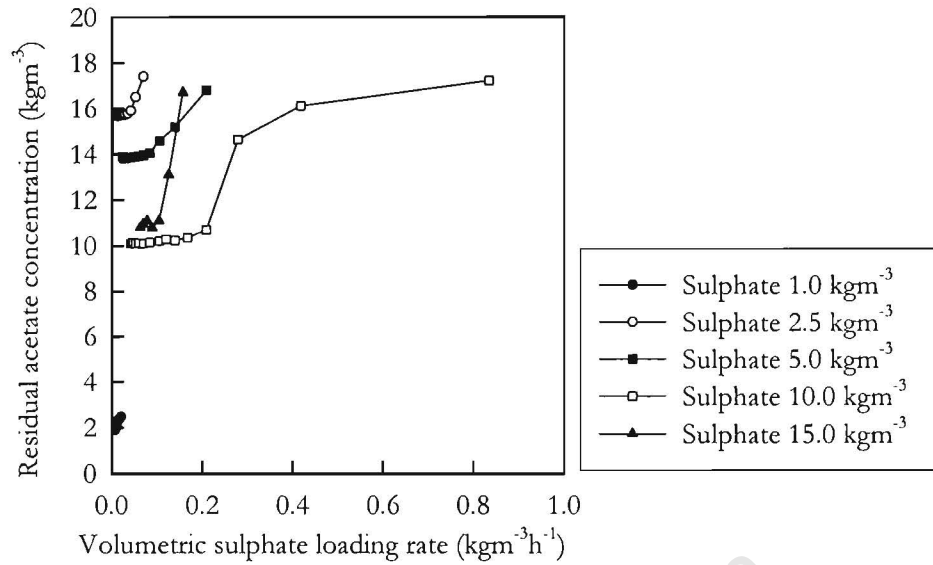


Figure 6.21. Dependency of 'stable' state residual acetate concentration profiles on feed sulphate concentration. Data obtained from continuous bioreactors operating with 1.0 (●), 2.5 (○), 5.0 (■), 10.0 (□) and 15.0 (▲) kgm⁻³ sulphate at 35°C and pH 7.8.

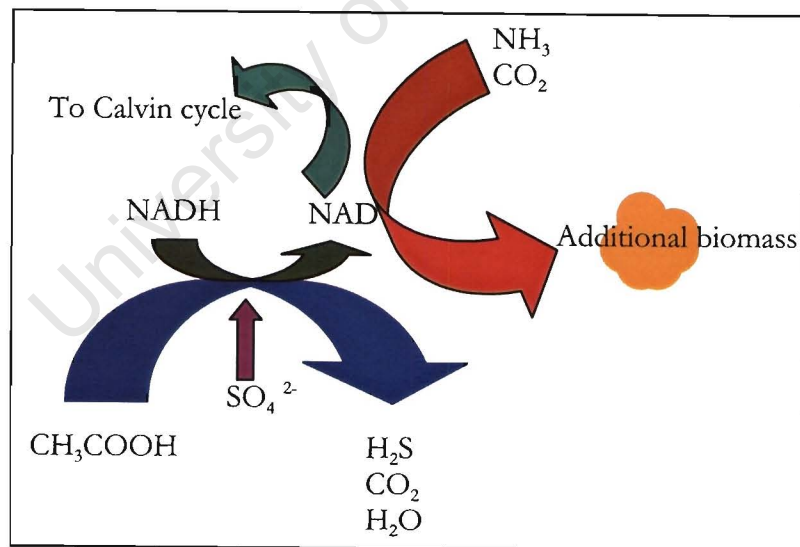


Figure 6.22. Graphical representation of the utilisation of acetate within the cell for the reduction of sulphate and biomass formation.

6.5.4 BACTERIAL CONCENTRATION

Figure 6.23 shows a comparison of 'stable' state bacterial concentrations for bioreactors employing 1.0, 2.5, 5.0, 10.0 and 15.0 kgm⁻³ sulphate. The bacterial concentration decreased with dilution rate for all five feed sulphate concentrations and reached a maximum in the lower range of dilution rates (Table 6.4). The same trend was observed for the growth of a mixed culture on methane (Figure 6.24). Furthermore, for feed sulphate concentrations in the range 1.0 to 10.0 kgm⁻³ there are regions on the curves where the biomass decrease is more pronounced than at other points. This behaviour is observed with mixed cultures where the various species have different maximum specific growth rates and consequently the different species washout at different dilution rates (Chiu *et al.*, 1972a, b). The shape of the curve when the feed sulphate concentration was 15.0 kgm⁻³ is markedly different from those at lower concentrations. There is a sharp and consistent linear decrease with increasing dilution rate. Complete washout was achieved at a lower dilution rate than any other runs, suggesting growth inhibition by high feed sulphate concentrations.

For corresponding dilution rates the bacterial concentration displayed an increase with increasing feed sulphate concentration. When the feed sulphate was further increased to 15.0 kgm⁻³ the bacterial concentration decreased. The dilution rate at which the maximum occurred was similar for all 5 feed sulphate concentrations. Since the microbial population is mixed the onset of washout is characterised by a decline in the biomass concentration after a maximum concentration is achieved. For this set of experiments this occurred at D of 0.005 h⁻¹. It is therefore suggested that μ_m is relatively constant for the mixed SRB population used for the 5 feed sulphate concentration experiments.

Visually (Figures 6.24 to 6.27) as the inlet sulphate concentration was increased in the range 1.0 to 15.0 kgm⁻³ an increase in the size of bacteria could be seen. Clumping was evident for the bioreactor receiving 15.0 kgm⁻³ sulphate, indicating that the system was stressed (Brock and Madigan, 1991).

Table 6.6. Dependency of the maximum bacterial concentration and corresponding dilution rates on the feed sulphate concentration.

Feed sulphate concentration (kgm^{-3})	Maximum bacterial concentration (gL^{-1})	Corresponding dilution rate (h^{-1})
1.0	0.98	0.006
2.5	2.60	0.005
5.0	3.10	0.005
10.0	5.10	0.005
15.0	5.30	0.005

The continuous bioreactor was operated at 35°C and $\text{pH } 7.8$.

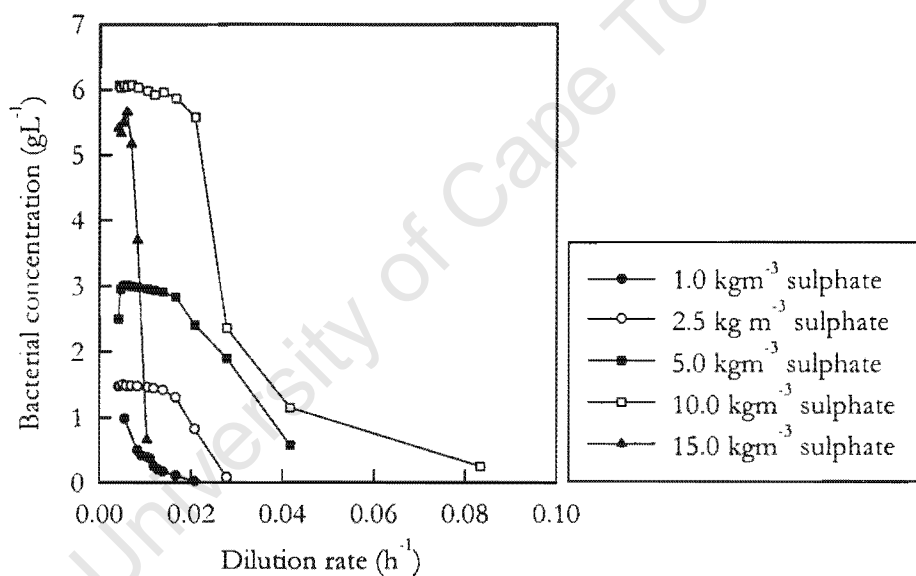


Figure 6.23. Dependency of 'stable' state bacterial concentration profiles on dilution rate and feed sulphate concentration.. Data obtained from continuous bioreactors operating with 1.0 (\bullet), 2.5 (\circ), 5.0 (\blacksquare), 10.0 (\square) and 15.0 (\blacktriangle) kgm^{-3} sulphate at 35°C and $\text{pH } 7.8$.

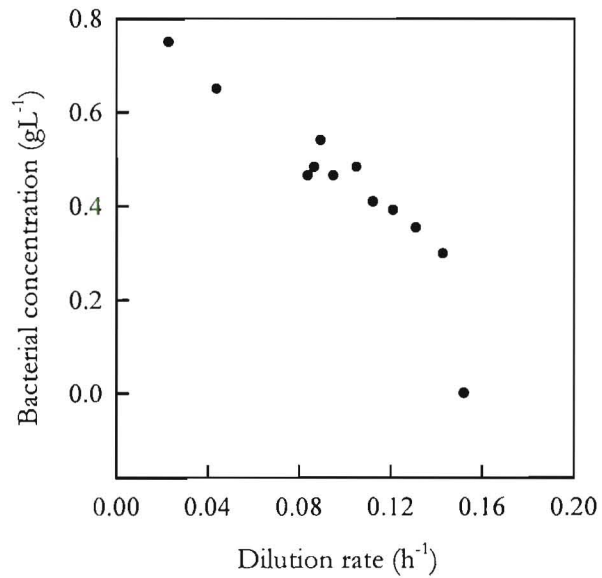


Figure 6.24. Dependency of bacterial concentration on dilution rate for a mixed population utilising methane (Wilkinson *et al.*, 1974).

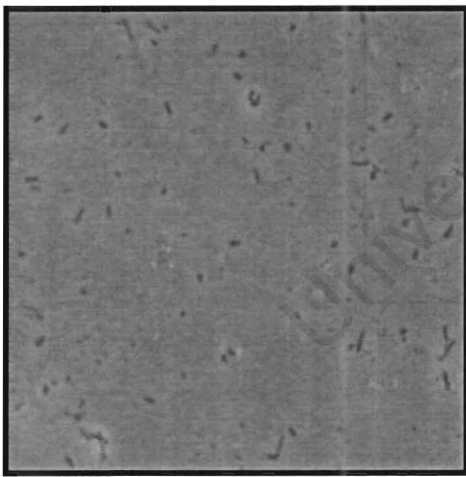


Figure 6.25. Bacteria from bioreactor receiving 1.0 kgm⁻³ sulphate in the feed (1000X magnification).

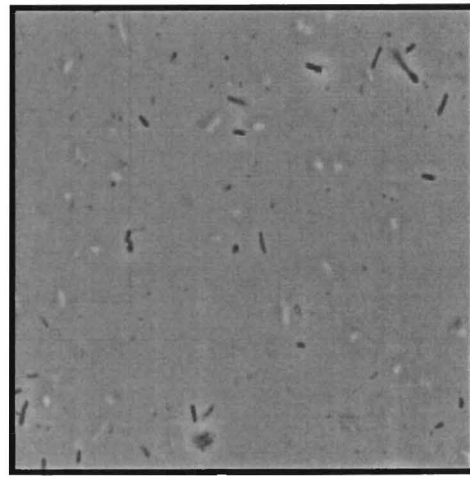


Figure 6.26. Bacteria from bioreactor receiving 2.5 kgm⁻³ sulphate in the feed (1000X magnification).

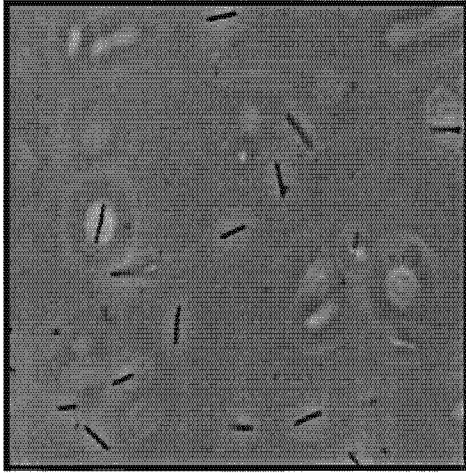


Figure 6.25. Bacteria from bioreactor receiving 5.0 kgm^{-3} sulphate in the feed (1000X magnification).

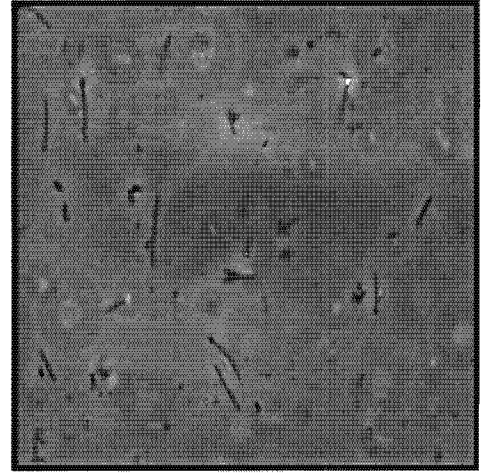


Figure 6.26. Bacteria from bioreactor receiving 10.0 kgm^{-3} sulphate in the feed (1000X magnification).

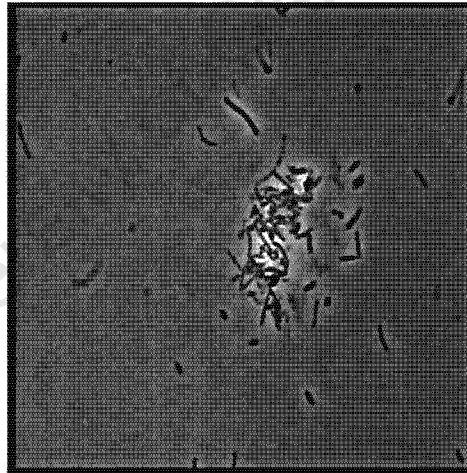


Figure 6.29. Bacteria from bioreactor receiving 15.0 kgm^{-3} sulphate in the feed. (1000X magnification)

The observed increase in bacterial concentration with increasing feed sulphate concentrations in the range 1.0 to 10.0 kgm^{-3} can be explained by considering the theoretical calculation of biomass as proposed by Equations 6.3 and 6.4. In Equation 6.4, $\text{C}_5\text{H}_7\text{O}_2\text{N}$ is the chemical representation for biomass and is stoichiometrically related to the NAD^+ produced when sulphate is reduced to sulphide using acetate as the electron donor. Calculated on a mass basis the theoretical ratio of biomass produced to sulphate utilised is

0.47. Therefore, when the feed sulphate concentration is increased from 1.0 to 15.0 kgm^{-3} and more sulphate is converted to sulphide the biomass production increases. Based on 'stable' state sulphate utilisation and bacterial concentrations, the ratio of biomass formed to sulphate utilized was calculated for each feed sulphate concentration. The ratios are presented in Figure 6.30; the theoretical value of 0.47 is shown as a dotted line. The ratios are scattered above and below the theoretical line indicating the presence of experimental error and fluctuation.

No data is available in the published literature for comparison with the trends presented for bacterial concentration. However, based on the experimental results it can be concluded that an increase in inlet sulphate concentration results in a proportional increase in the utilisation of sulphate and consequently an increase in biomass concentration.

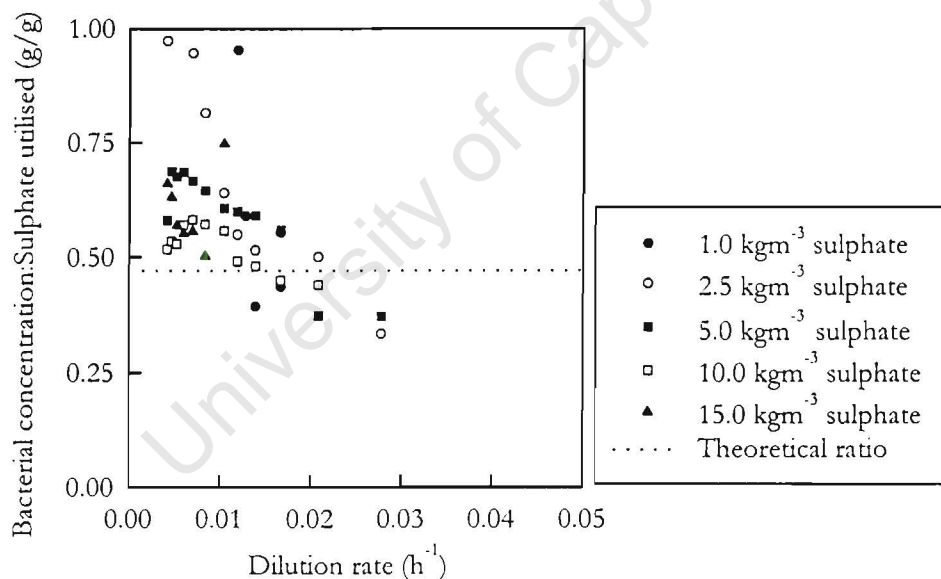


Figure 6.30. Dependency of ratio of bacterial concentration to sulphate utilised on dilution rate and feed sulphate concentration. Data obtained from continuous bioreactors operating with 1.0 (●), 2.5 (○), 5.0 (■), 10.0 (□) and 15.0 (▲) kgm^{-3} sulphate at 35°C and pH 7.8.

6.6 CHAPTER SUMMARY

In this chapter results from a study to investigate the effect of feed sulphate concentration on the kinetics of anaerobic sulphate reduction are presented. Continuous one litre bioreactors were operated at five sulphate concentrations viz. 1.0, 2.5, 5.0, 10.0 and 15.0 kgm⁻³ with a mixed microbial population enriched for SRB with acetate as the organic and electron source. Kinetics of the anaerobic sulphate reduction process was determined at 'stable' state conditions. The results presented in this chapter clearly indicate that the initial sulphate concentration has an effect on the biological sulphate reduction process. As the sulphate concentration was increased in the range 1.0 to 10.0 kgm⁻³ an enhancement of the volumetric sulphate reduction rate was observed. When the initial sulphate concentration was further increased to 15.0 kgm⁻³ the volumetric sulphate reduction rate and the percentage conversion of sulphate decreased. Specific microbial activity displayed a decrease with increasing feed sulphate concentration for feed sulphate concentration in the range 1.0 to 15.0 kgm⁻³. The decrease in metabolic activity was manifest by a decrease in the specific volumetric sulphate conversion rate with increasing volumetric sulphate loading rate. The enhanced volumetric sulphate reduction rates observed with increasing feed sulphate concentration in the range 1.0 to 10.0 kgm⁻³ was a result of the increase in bacterial concentration observed as the feed sulphate concentration was increased from 1.0 to 10.0 kgm⁻³. From the results it is apparent that initial sulphate concentration of above 10.0 kgm⁻³ had an inhibitory effect on the activity of bacteria and the reduction of sulphate.

The following chapter, Chapter 7 presents the results of experiments to determine the effect of reaction temperature on the kinetics of sulphate reduction.

CHAPTER 7

RESULTS AND DISCUSSION III

EFFECT OF TEMPERATURE

The literature stated that temperature affected the activity of SRB. Work by Middleton and Lawrence (1977) and Barnes *et al.*, (1992a) showed that the rate of sulphate reduction by a mixed sulphate reducing microbial group increased with temperature. However, no rigorous data on the effect of temperature on the kinetics of biological sulphate reduction is available in the open literature. Initial batch results presented in Chapter 5 showed that the optimum temperature for sulphate reduction by a mixed anaerobic culture using glucose as the organic source was 35°C, which is consistent with literature. In this chapter the reader is presented with results from experiments to ascertain the effect of temperature and volumetric loading of sulphate on the kinetics of anaerobic reduction of sulphate. Initially the transient data is presented and discussed. This is followed by the 'stable' state results for each reaction temperature. Finally the 'stable' state results are compared and discussed with respect to sulphate, bacterial and acetate concentration and volumetric reduction rate.

In order to study the effect of temperature and volumetric loading of sulphate on the kinetics of sulphate reduction, four independent experimental runs at 20, 25, 30 and 35°C were conducted. This temperature window was chosen from batch studies outlined in Chapter 5. In each experiment, the bioreactor was charged with 800 mL medium and 200 mL inoculum. The inoculum was taken from the stock culture. Acetate, at a concentration of 17.5 kgm⁻³ was used as the organic substrate. The pH was maintained at 7.8 and the inlet sulphate concentration was 5.0 kgm⁻³.

7.1 VALIDATION OF 'STABLE' STATE

Typical plots (for the bioreactor operating at 30°C) of residual sulphate concentration and biomass concentration as a function of time after perturbation are presented as Figures 7.1 and 7.2. The 'stable' state data in terms of time period of 'stable' state, the residual sulphate concentration and biomass concentration as a function of retention time is presented in Table 7.1. Similar plots for the bioreactors operating at 20, 25 and 35°C are presented in Appendix A. These plots indicate the establishment of 'stable' state that is represented by a plateau region. 'Stable' state has previously been defined (Section 6.1) as having been achieved when the residual sulphate concentration and bacterial concentration did not vary by more than 12 and 15 % respectively over a period of at least one retention time.

Table 7.1. 'Stable' state data as a function of hydraulic retention time. Data obtained from a continuous bioreactor operating at 35°C and pH 7.8 with feed sulphate and acetate concentrations of 5.0 and 17.5 kgm⁻³ respectively.

Hydraulic retention time (d)	Time period of 'stable' state (d)	Residual sulphate concentration (kgm ⁻³)	Bacterial concentration (kgm ⁻³)
10	10	1.26 ± 0.12	2.6 ± 0.14
8	8	1.47 ± 0.02	2.9 ± 0.20
6	10	1.63 ± 0.04	2.7 ± 0.06
4	10	1.72 ± 0.03	2.4 ± 0.07
3	6	1.91 ± 0.02	2.2 ± 0.19
2	6	2.30 ± 0.05	1.4 ± 0.12
1.5	6	2.94 ± 0.03	1.0 ± 0.05

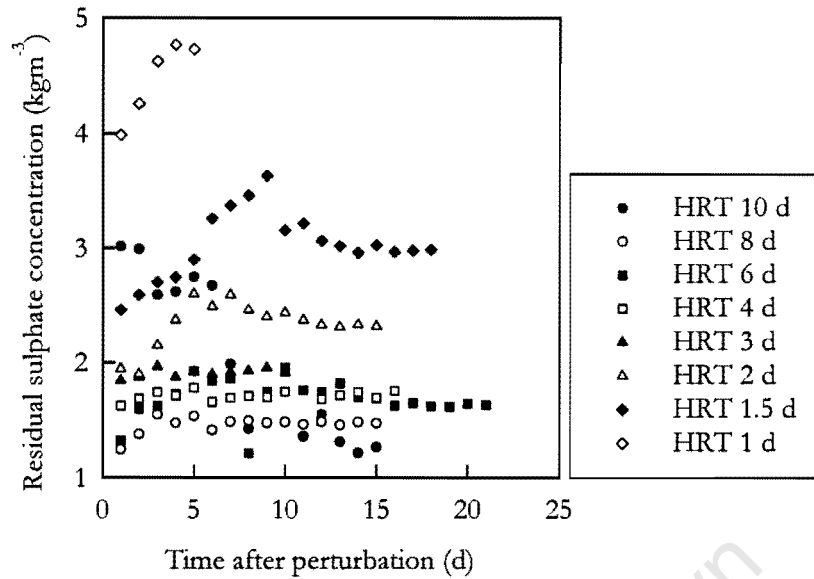


Figure 7.1. Residual sulphate concentration as a function of time after perturbation for the continuous conversion of sulphate at HRTs of 10 (●), 8 (○), 6 (■), 4 (□), 3 (▲), 2 (△), 1.5 (◆) and 1 (◇) d. Data taken from a continuous bioreactor operating at 30°C and pH 7.8 with feed sulphate and acetate concentrations of 5.0 and 17.5 kgm^{-3} respectively.

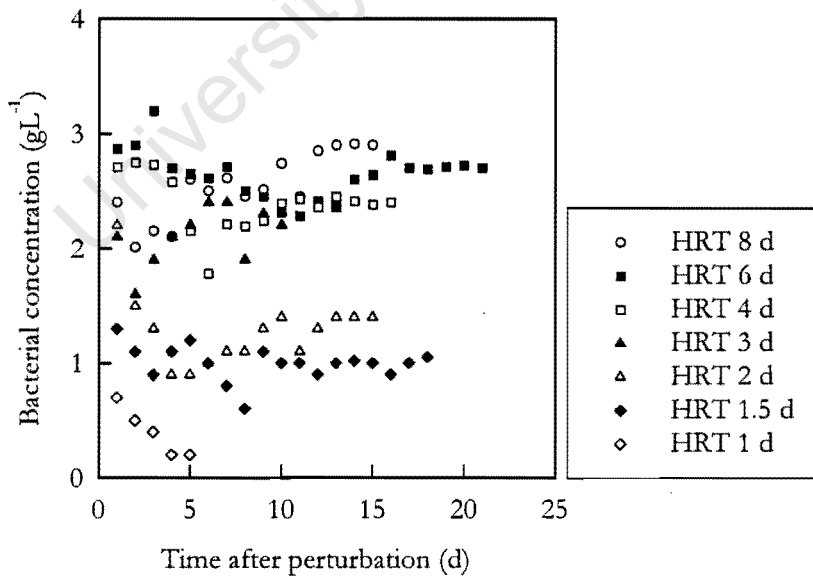


Figure 7.2. Bacterial concentration as a function of time after perturbation for the continuous conversion of sulphate at HRTs of 8 (○), 6 (■), 4 (□), 3 (▲), 2 (△), 1.5 (◆) and 1 (◇) d. Data taken from a continuous bioreactor operating at 30°C and pH 7.8 with feed sulphate and acetate concentrations of 5.0 and 17.5 kgm^{-3} respectively.

7.2 TRENDS FOLLOWING PERTURBATIONS

The transient profiles for reactors operating at temperatures of 20, 25, 30 and 35°C are presented as Figures 7.3, 7.4, 7.5 and 7.6 respectively. Results presented include sulphate conversion, residual acetate concentrations and bacterial concentrations. For the bioreactor operating at 35°C no transient residual acetate concentrations were available. The very low gas evolution could not be readily detected and consequently no gas analysis is presented.

In all cases the reactors were initially operated batchwise. Once sufficient and stable removal of sulphate occurred for a period of at least 7 days the reactors were switched to continuous mode. For the reactors operating at 20, 25 and 30°C the maximum retention time at which the bioreactors were operated was 10 d. The retention time was decreased incrementally once 'stable' state was achieved. The retention time was decreased in increments of 2 d until a retention time of 6 d. Thereafter the retention time was decreased in increments of 1 day until retention time of 2 d was achieved. Finally, the retention time was decreased in 0.5 d increments until total washout was achieved. At each retention time, 'stable' state was achieved. At a reaction temperature of 35°C, the maximum retention time used was 9 d. When 'stable' state was achieved for the 35°C bioreactor, the retention time was decreased in 1 d increments until a 4 d retention time. Thereafter the retention time was decreased in 0.5 d increments. Generally, the bioreactors were in operation between 130 and 160 d.

The trends observed, in terms of residual sulphate concentration, bacterial concentration and acetate concentration was similar in the four temperature runs. Decreasing the retention time led to an initial increase in residual sulphate and acetate concentrations and a decrease in bacterial concentration. This was followed by a decrease in residual sulphate and acetate concentrations and an increase in bacterial concentration and finally 'stable' state was achieved. In all instances the observed trend was more pronounced at the lower retention times. This can be seen from Figures 7.1 and 7.2. Despite the similarity in trends observed for residual sulphate and bacterial concentrations the fluctuations in bacterial

concentration due to perturbations in retention time was more pronounced. The high residual acetate concentration confirms that acetate was always present in excess.

The four bioreactors showed similar response to perturbations in retention time. In the 30°C bioreactor, the decrease in sulphate concentration observed on day 54 of reactor operation is a result of a temperature spike to 34°C. This resulted in a short period of enhanced sulphate reduction with a concomitant decrease in residual acetate concentration. No other change in the overall trend of sulphate reduction with retention time was observed.

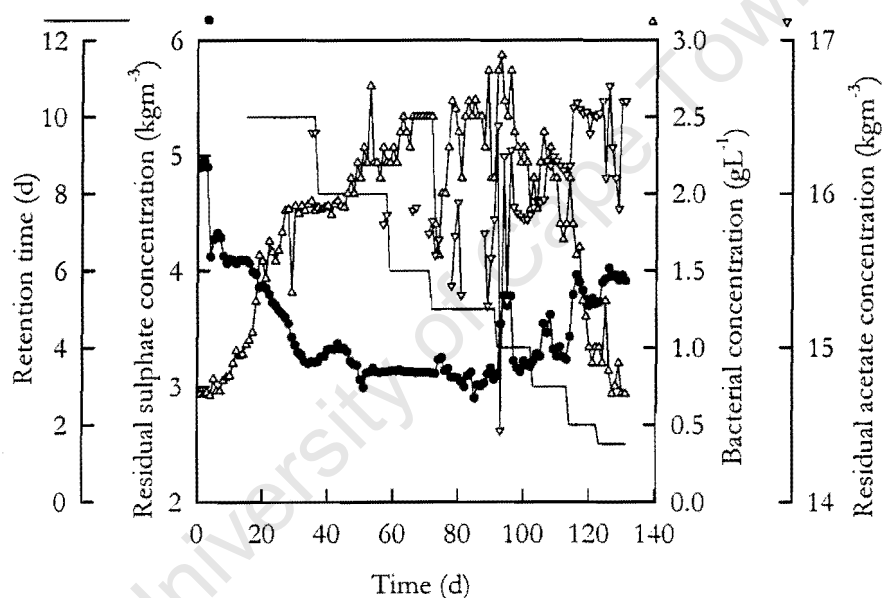


Figure 7.3. Transient profiles of retention time (—), residual sulphate concentration (●), bacterial concentration (Δ) and residual acetate concentration (∇) for the continuous reduction of sulphate at 20°C. Data taken from a continuous bioreactor operating at pH 7.8 with feed sulphate and acetate concentrations of 5.0 and 17.5 kgm^{-3} respectively.

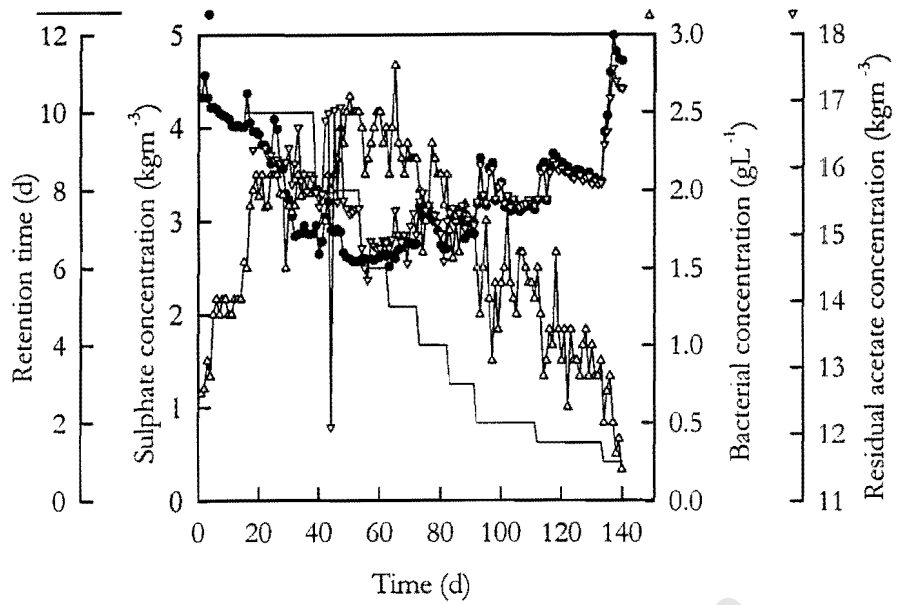


Figure 7.4. Transient profiles of retention time (—), residual sulphate concentration (●), bacterial concentration (Δ) and residual acetate concentration (∇) for the continuous reduction of sulphate at 25°C. Data taken from a continuous bioreactor operating at pH 7.8 with feed sulphate and acetate concentrations of 5.0 and 17.5 kgm^{-3} respectively.

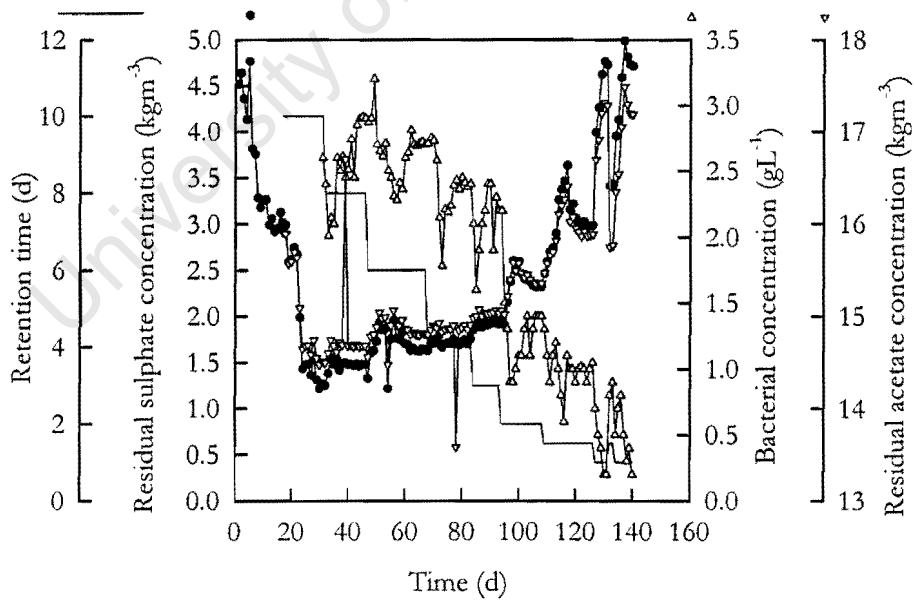


Figure 7.5. Transient profiles of retention time (—), residual sulphate concentration (●), bacterial concentration (Δ) and residual acetate concentration (∇) for the continuous reduction of sulphate at 30°C. Data taken from a continuous bioreactor operating at pH 7.8 with feed sulphate and acetate concentrations of 5.0 and 17.5 kgm^{-3} respectively.

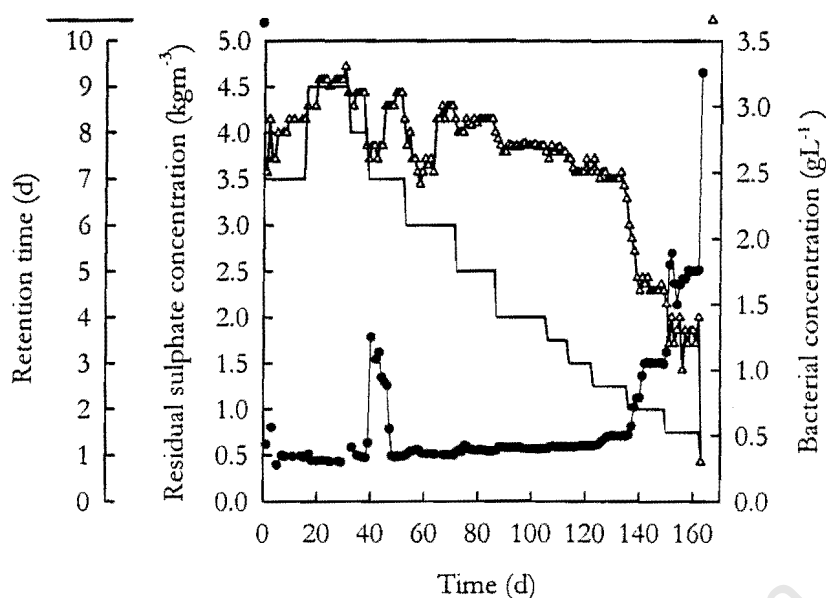


Figure 7.6. Transient profiles of retention time (—), residual sulphate concentration (●) and bacterial concentration (Δ) for the continuous reduction of sulphate at 35°C. Data taken from a continuous bioreactor operating at pH 7.8 with feed sulphate and acetate concentrations of 5.0 and 17.5 kgm⁻³ respectively.

7.3 'STABLE' STATE RESULTS

The 'stable' state kinetics of the continuous biological sulphate reduction in a feed medium containing 5.0 kgm⁻³ sulphate and 17.5 kgm⁻³ acetate at temperatures of 20, 25, 30 and 35°C are presented in this section.

7.3.1 SULPHATE REDUCTION AT 20°C

Kinetics of continuous bacterial reduction of sulphate at 20°C, at an inlet sulphate concentration of 5.0 kgm⁻³ and a pH of 7.8 are shown as Figure 7.7. On operating the bioreactor at 20°C maximum bacterial concentration of 2.5 gL⁻¹ was observed at a dilution rate of 0.007 h⁻¹ (volumetric sulphate loading rate of 0.035 kgm⁻³). Increasing the dilution rate in the range 0.007 to 0.042 h⁻¹ (volumetric sulphate loading rate of 0.035 to 0.210 kgm⁻³) resulted in a decrease in bacterial concentration. As can be seen in Figure 7.7, the conversion of sulphate at 20°C was relatively low, with a maximum value of 39 % at a loading rate 0.042 kgm⁻³h⁻¹ (dilution rate of 0.008 h⁻¹). The increasing of volumetric sulphate loading rate

up to $0.139 \text{ kgm}^{-3}\text{h}^{-1}$ (dilution rate of 0.028 h^{-1}) enhanced the sulphate reduction rate. The maximum volumetric sulphate reduction rate in this set of experiments was $0.03 \text{ kgm}^{-3}\text{h}^{-1}$, achieved at a volumetric sulphate loading rate of $0.139 \text{ kgm}^{-3}\text{h}^{-1}$ (dilution rate of 0.028 h^{-1}). The corresponding conversion of sulphate was 22%. Further increase in volumetric sulphate loading resulted in a dramatic decrease in the volumetric reduction rate of sulphate. The residual acetate concentration profile was similar to that for residual sulphate concentration (residual sulphate concentration profiles not shown). The ratio of sulphate reduced to acetate utilized was relatively constant at 0.80 ± 0.058 moles sulphate/moles acetate for the range of loading rates applied.

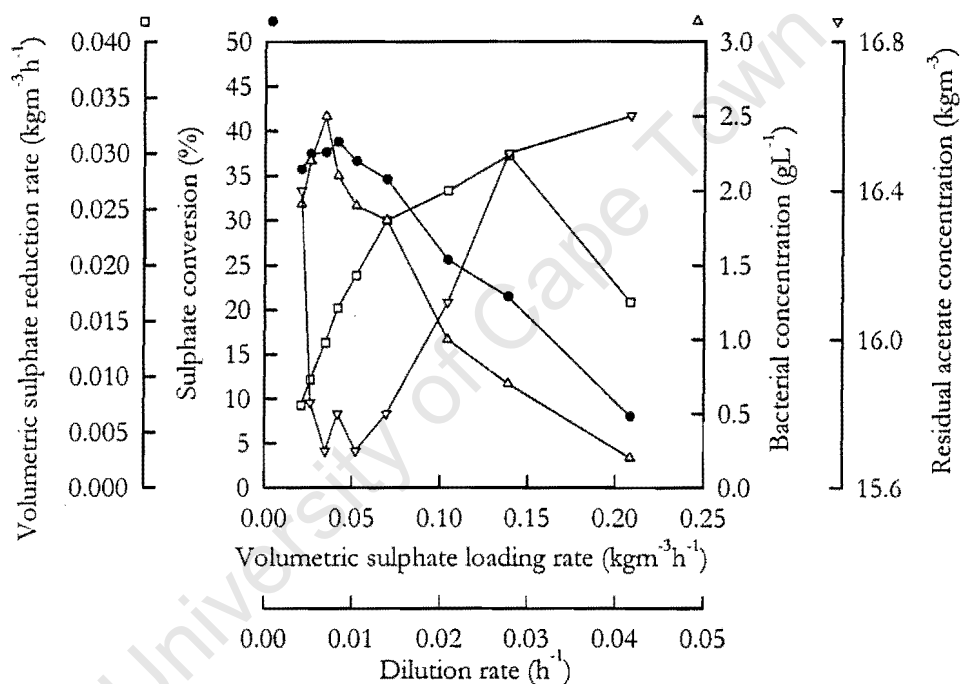


Figure 7.7. 'Stable' state kinetics of continuous reduction of sulphate at 20°C and pH 7.8 in a feed medium containing 5.0 kg m^{-3} sulphate and 17.5 kg m^{-3} acetate. 'Stable' state data presented includes volumetric sulphate reduction rate (\square), sulphate conversion (\bullet), bacterial concentration (Δ) and residual acetate concentration (∇).

7.3.2 SULPHATE REDUCTION AT 25°C

Figure 7.8 presents the kinetic results of the bioreactor operating at 25°C . A maximum bacterial concentration of 2.6 gL^{-1} was achieved at a dilution rate of 0.007 h^{-1} (volumetric sulphate loading rate of

0.035 $\text{kgm}^{-3}\text{h}^{-1}$). On further increase of dilution rate up to 0.042 h^{-1} (volumetric sulphate loading rate of $0.208 \text{ kgm}^{-3}\text{h}^{-1}$) the bacterial concentration decreased. Applying volumetric sulphate loading rates from 0.021 to $0.070 \text{ kgm}^{-3}\text{h}^{-1}$ (dilution rate range of 0.004 to 0.014 h^{-1}) the conversion of sulphate remained constant (44 to 48 %). Over this range of volumetric sulphate loadings, a linear increase in reduction rate of sulphate was observed. Further increase of volumetric sulphate loading up to $0.14 \text{ kgm}^{-3}\text{h}^{-1}$, while accompanied by a decrease in conversion of sulphate, led to a continued increase in sulphate reduction rates. The maximum reduction rate of sulphate in this set of experiments was $0.036 \text{ kgm}^{-3}\text{h}^{-1}$, achieved at a volumetric sulphate loading rate of $0.139 \text{ kgm}^{-3}\text{h}^{-1}$ (dilution rate of 0.028 h^{-1}). The corresponding conversion was 26 %. The ratio of sulphate reduced to acetate utilized in this set of experiments was 0.76 ± 0.047 moles sulphate/moles acetate.

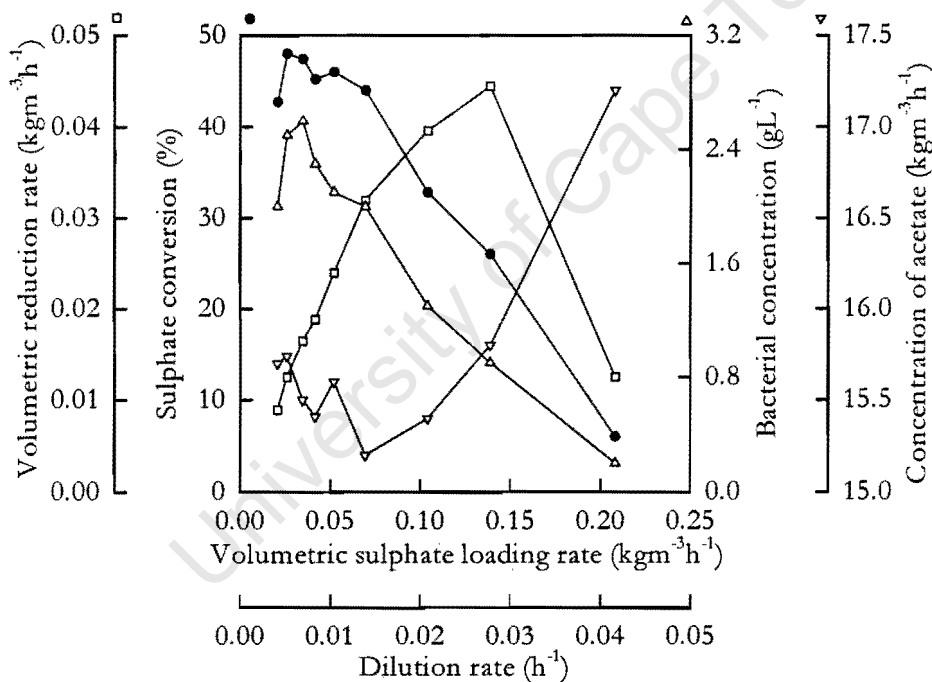


Figure 7.8. 'Stable' state kinetics of continuous reduction of sulphate at 25°C and pH 7.8 in a feed medium containing 5.0 kg m^{-3} sulphate and 17.5 kg m^{-3} acetate. 'Stable' state data presented includes volumetric sulphate reduction rate (□), sulphate conversion (●), bacterial concentration (Δ) and residual acetate concentration (▽).

7.3.3 SULPHATE REDUCTION AT 30°C

Operating the bioreactor at 30°C, the bacterial concentration exhibited a linear decrease over the range of dilution rates employed (Figure 7.9). The maximum bacterial concentration of 2.6 gL⁻¹ was found at a dilution rate of 0.005 h⁻¹ (volumetric sulphate loading rate of 0.026 kgm⁻³h⁻¹). The maximum conversion of sulphate of 75 %, was observed at a volumetric sulphate loading of 0.021 kgm⁻³h⁻¹ (dilution rate of 0.005 h⁻¹). Increasing the volumetric sulphate loading rate in the range 0.021 to 0.10 kgm⁻³h⁻¹ (dilution rate range of 0.004 to 0.021 h⁻¹), while decreasing the conversion of sulphate to 62 %, led to a notable enhancement of reduction rate. The maximum reduction rate achieved was 0.056 kgm⁻³h⁻¹ at a volumetric sulphate loading rate of 0.104 kgm⁻³h⁻¹ (dilution rate of 0.028 h⁻¹). The corresponding sulphate conversion was 55 %. Increasing the volumetric loading rate beyond 0.140 kgm⁻³h⁻¹ (dilution rate of 0.021 h⁻¹) resulted in a dramatic decrease in both sulphate conversion and volumetric reduction rate. For the range of applied volumetric loading rates, the ratio of reduced sulphate to acetate utilized was 0.81 ± 0.104 .

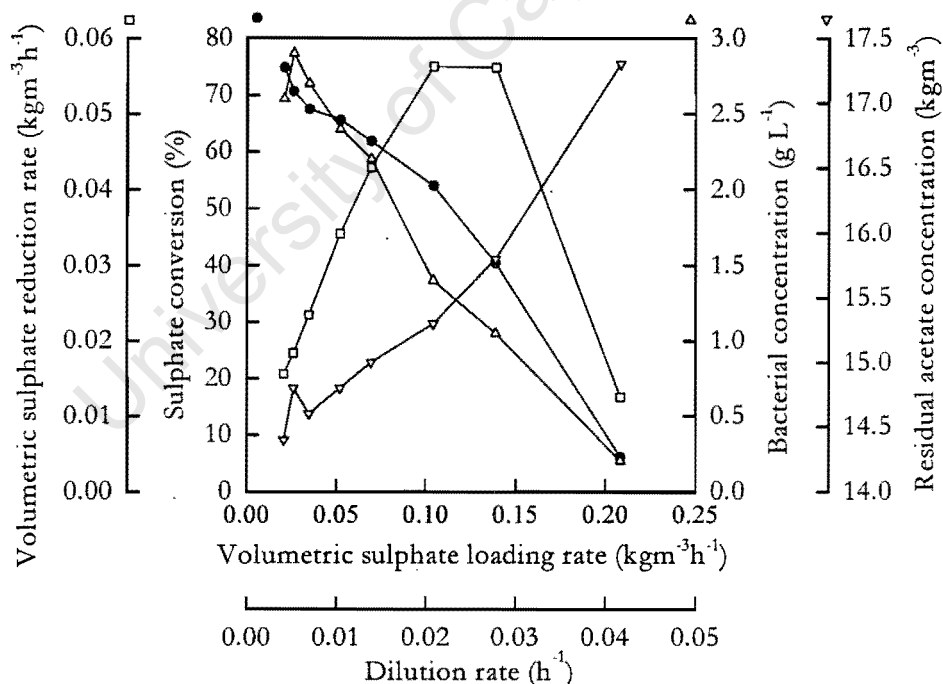


Figure 7.9. 'Stable' state kinetics of continuous reduction of sulphate at 30°C and pH 7.8 in a feed medium containing 5.0 kgm⁻³ sulphate and 17.5 kgm⁻³ acetate. 'Stable' state data presented includes volumetric sulphate reduction rate (□), sulphate conversion (●), bacterial concentration (Δ) and residual acetate concentration (∇).

7.3.4 SULPHATE REDUCTION AT 35°C

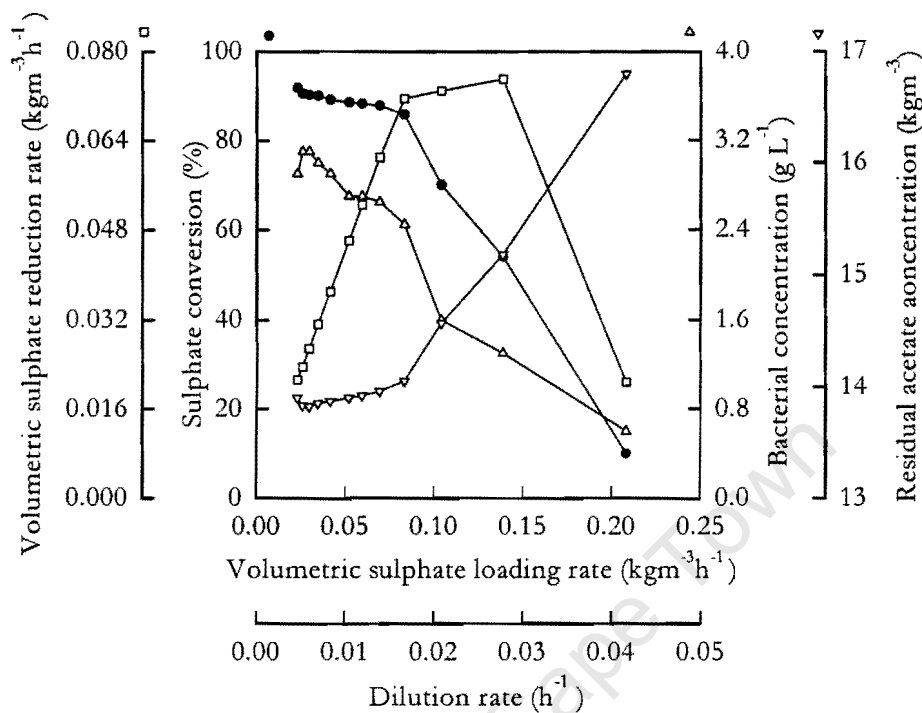


Figure 7.10. 'Stable' state kinetics of continuous reduction of sulphate at 35°C and pH 7.8 in a feed medium containing 5.0 kgm^{-3} sulphate and 17.5 kgm^{-3} acetate. 'Stable' state data presented includes volumetric sulphate reduction rate (\square), sulphate conversion (\bullet), bacterial concentration (Δ) and residual acetate concentration (∇).

During the experiment at 35°C (Figure 7.10), the maximum bacterial concentration of 3.2 $g L^{-1}$ was observed at a dilution rate of 0.004 h^{-1} (volumetric sulphate loading rate of 0.023 $kgm^{-3}h^{-1}$). Bacterial concentration decreased with increasing dilution rate over the range 0.005 to 0.042 h^{-1} (volumetric sulphate loading rate range of 0.026 to 0.0208 $kgm^{-3}h^{-1}$). For volumetric sulphate loading rates in the range 0.021 to 0.083 $kgm^{-3}h^{-1}$ (dilution rate range of 0.004 to 0.017 h^{-1}), a relatively constant and high conversion of sulphate, of 87 to 90 %, was achieved. Over this range of volumetric sulphate loading rates a linear increase in the volumetric sulphate reduction rate from 0.019 to 0.072 $kgm^{-3}h^{-1}$ was found. A further increase of volumetric sulphate loading rate to 0.139 $kgm^{-3}h^{-1}$ (dilution rate of 0.028 h^{-1}), while accompanied by a sharper decrease in conversion, led to a semi-plateau region in the reduction rate curve. The maximum reduction rate of sulphate in this set of experiments was 0.075 $kgm^{-3}h^{-1}$ achieved at a

volumetric sulphate loading rate of $0.139 \text{ kgm}^{-3}\text{h}^{-1}$ (dilution rate of 0.028 h^{-1}). The corresponding sulphate conversion was 54 %. The molar ratio of sulphate reduced to acetate utilized was 0.83 ± 0.014 for the range of applied loading rates.

7.4 COMPARISON OF 'STABLE' STATE RESULTS

The 'stable' state results, for temperatures of 20, 25, 30 and 35°C are compared in this section. The parameters of interest are: sulphate conversion, volumetric sulphate reduction rate, residual acetate concentration and bacterial concentration.

7.4.1 SULPHATE CONVERSION

A comparison of the sulphate conversion profiles for bioreactors operating at 20, 25, 30 and 35°C is presented in Figure 7.11. The trend observed for the dependency of sulphate conversion on volumetric loading of sulphate was similar for all four temperatures applied. The sulphate conversion decreased with increasing volumetric sulphate loading rate. For volumetric sulphate loading rates in the range 0.0208 to $0.069 \text{ kgm}^{-3}\text{h}^{-1}$ the decrease in sulphate conversion was 2, 3, 13 and 7 % for temperatures of 20, 25, 30 and 35°C respectively. As the volumetric sulphate loading was increased further to $0.208 \text{ kgm}^{-3}\text{h}^{-1}$ the sulphate conversion decreased to 8, 6, 6 and 10 % for temperatures of 20, 25, 30 and 35°C respectively. The maximum sulphate conversion obtained for the four temperature runs are given in Table 7.2. As the temperature was increased in the range 20 to 35°C , the maximum sulphate conversion more than doubled. The volumetric loading at which this maximum occurred decreased from 0.042 to $0.021 \text{ kgm}^{-3}\text{h}^{-1}$ as the temperature was increased from 20 to 30°C and remained constant as the temperature was increased further. The volumetric sulphate loading rate at which the maximum sulphate conversion occurred for temperatures of 30 and 35°C was the lowest volumetric sulphate loading rate employed for these runs, indicating a 'stable' decrease in sulphate conversion with increasing volumetric sulphate loading rate. The residual sulphate concentration profiles are shown as Figure 7.12. As expected the residual sulphate concentration decreased with increasing temperature.

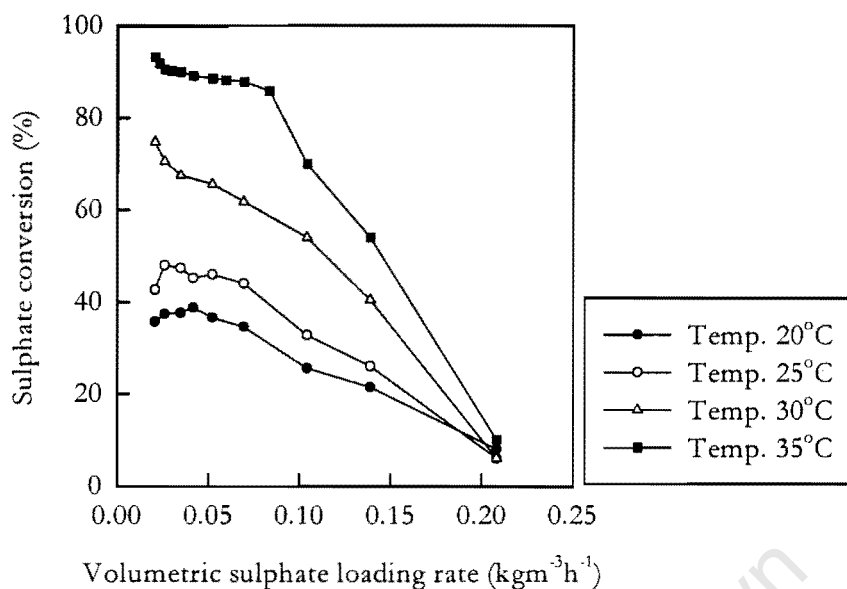


Figure 7.11. Dependency of 'stable' state sulphate conversion profiles on volumetric sulphate loading rate and temperature. Data obtained from continuous bioreactors operating at 20 (●), 25 (○), 30 (△) and 35 °C (■) and pH 7.8 with a feed medium containing 5.0 kg m^{-3} sulphate and 17.5 kgm^{-3} acetate.

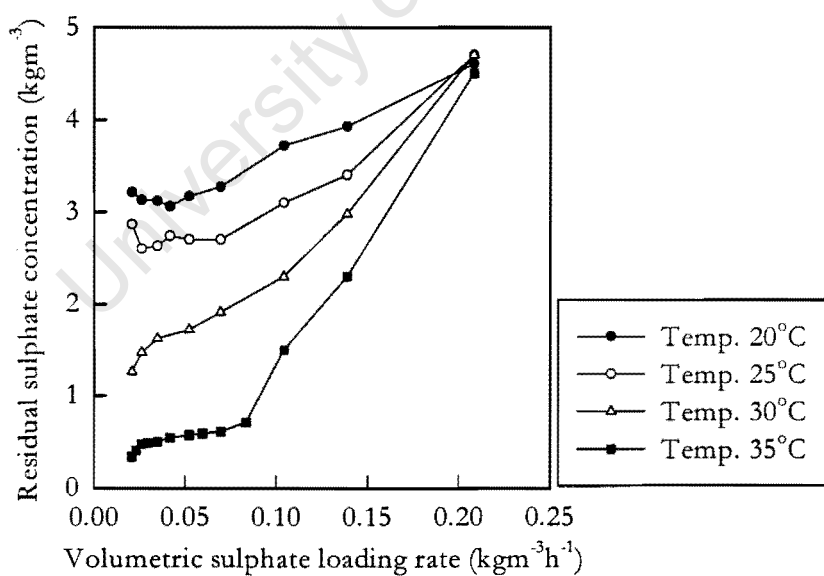


Figure 7.12. Dependency of 'stable' state residual sulphate concentration profiles on volumetric sulphate loading rate and temperature. Data obtained from continuous bioreactors operating at 20 (●), 25 (○), 30 (△) and 35 °C (■) and pH 7.8 with a feed medium containing 5.0 kg m^{-3} sulphate and 17.5 kgm^{-3} acetate

Table 7.2. Variation in maximum sulphate conversion and corresponding volumetric sulphate loading rates and dilution rate as a function of reaction temperature. Data obtained from continuous bioreactors operating at pH 7.8 with feed sulphate and acetate concentrations of 5.0 and 17.5 kgm⁻³ respectively.

Temperature (°C)	Maximum sulphate conversion (%)	Corresponding volumetric loading rate (kgm ⁻³ h ⁻¹)	Corresponding dilution rate (h ⁻¹)
20	39	0.042	0.008
25	48	0.026	0.005
30	75	0.021	0.004
35	93	0.021	0.004

The increase in sulphate conversion with increasing temperature can be attributed to enhanced microbial kinetics at the higher temperatures. An increase in maximum sulphate conversion from 13 to 71 % with increasing temperature in the range 20 to 35°C was observed for batch experiments outlined in Section 5.4. Similarly, continuous sulphate reducing pilot plant studies with ethanol as the organic and carbon source carried out by Barnes *et al.* (1992a) showed that when the reaction temperature was increased from 22 to 31°C the conversion increased from 69 to 90 %. In the review by Speece (1996), the enhanced anaerobic degradation of organic compounds with increasing temperature is presented. Beyond a certain maximum temperature, degradation declines rapidly. The enhanced sulphate conversion may be attributed to the activation of enzymes as the temperature is increased in the range 20 to 35°C (Smith & van Ness, 1987; Bailey & Ollis, 1986). Additionally, the higher sulphate conversion observed as the temperature was increased from 20 to 35°C can be explained by the presence of less H₂S relative to HS⁻ in the liquid phase as the temperature was increased from 20 to 35°C. Both the ionisation constant (K₁) and solubility of hydrogen sulphide are temperature dependent (Table 7.3). As the temperature is increased K₁ increases. Figure 7.13 shows the decreasing ratio of H₂S to HS⁻ with increasing temperature for the four experimental runs. Furthermore the solubility of hydrogen sulphide decreases with increasing temperature. This results in a decrease of H₂S in the liquid phase relative the gaseous phase with increasing temperature in the range 20 to 35°C. The H₂S present in the liquid phase for temperatures of 20, 25, 30 and 35°C is shown in Figure 7.14. It can be seen that there is an increase in H₂S concentration with increasing temperature at volumetric sulphate loading rate in the range 0.0208 to 0.208 kgm⁻³h⁻¹. This is due to the

enhanced conversion of sulphate at the higher temperatures. Consequently the preference for H_2S over HS^- may be a contributory factor to the inhibition of biological conversion of sulphate at lower temperatures, rather than the total sulphide concentrations.

Table 7.3. Ionisation constant and solubility of the H_2S species as a function of temperature.

Temperature (°C)	Ionisation constant (K_1)	Solubility of H_2S (mgL^{-1} per atmosphere of H_2S)
18	9.1×10^{-8}	-
20	-	3850
25	11.2×10^{-8}	3380
30	-	2980
35	14.9×10^{-8}	2650

Ionisation constants from Speece (1996) and solubilities from Lange Handbook of Chemistry (1979).

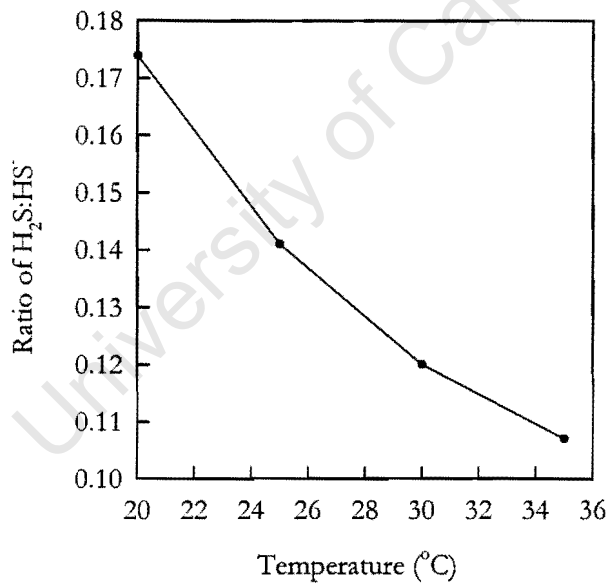


Figure 7.13. Dependency of ratio of H_2S to HS^- on temperature. Data calculated for continuous bioreactors operating at 20 (●), 25 (○), 30 (△) and 35 °C (■) and pH 7.8 with a feed medium containing 5.0 kg m^{-3} sulphate and 17.5 kgm^{-3} acetate

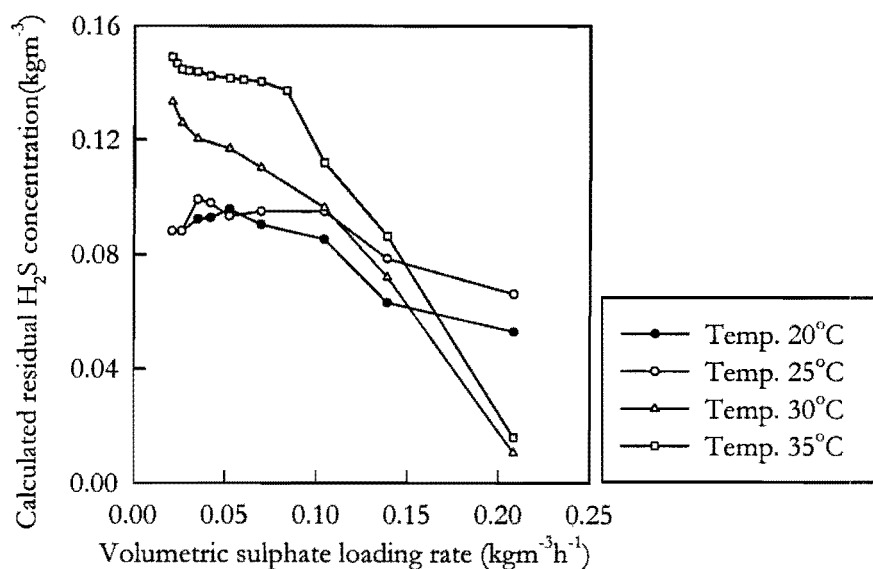


Figure 7.14. Dependency of calculated residual H_2S concentration on volumetric sulphate loading rate and temperature. Data calculated for continuous bioreactors operating at 20 (●), 25 (○), 30 (△) and 35 °C (■) and pH 7.8 with a feed medium containing 5.0 kg m^{-3} sulphate and 17.5 kgm^{-3} acetate

7.4.2 VOLUMETRIC SULPHATE REDUCTION RATE

A comparison of the volumetric sulphate reduction rates at 20, 25, 30 and 35°C is shown as Figure 7.15. For the temperature range, 20 to 35°C, the increasing of volumetric sulphate loading rate from 0.021 to 0.080 $\text{kgm}^{-3}\text{h}^{-1}$ resulted in a linear increase in volumetric sulphate reduction rate. In the range 0.080 to 0.140 $\text{kgm}^{-3}\text{h}^{-1}$ the reduction rate showed a reduced dependence on volumetric sulphate loading rate. Further increase in volumetric sulphate loading rate resulted in a decrease in volumetric sulphate reduction rate linked to washout of biomass. The magnitude of the volumetric sulphate reduction rate increased with increasing temperature. The maximum reduction rates achieved at the different temperatures are shown in Table 7.4. As the temperature was increased in the range 20 to 35°C, the maximum reduction rate increased from 0.030 to 0.075 $\text{kgm}^{-3}\text{h}^{-1}$. The volumetric loading rate at which the maximum volumetric sulphate reduction rate occurred was similar for all temperatures applied. A similar trend of increasing sulphate volumetric loading rate with temperature was observed by Middleton and Lawrence (1977). They investigated the effect on batch microbial growth kinetics of a mixed SRB population at temperatures of 20, 25 and 31°C with acetate was the limiting organic nutrient. An increase in volumetric sulphate reduction rate from 0.0009 $\text{kgm}^{-3}\text{h}^{-1}$ to 0.0019 $\text{kgm}^{-3}\text{h}^{-1}$ was reported as the temperature increased from 20 to 31°C. Investigating the effect of two temperatures (22 and 31°C) on the continuous

conversion of sulphate in a 1.6 l stirred tank using lactic acid as the limiting organic nutrient, Barnes *et al* (1992a) observed a similar response to increased temperature in terms of volumetric sulphate reduction rate with increasing volumetric sulphate loading to that observed for this work. Figure 7.16 shows the effect of temperature on the volumetric sulphate reduction rate as a function of volumetric sulphate loading rate observed by Barnes *et al.* (1992a).

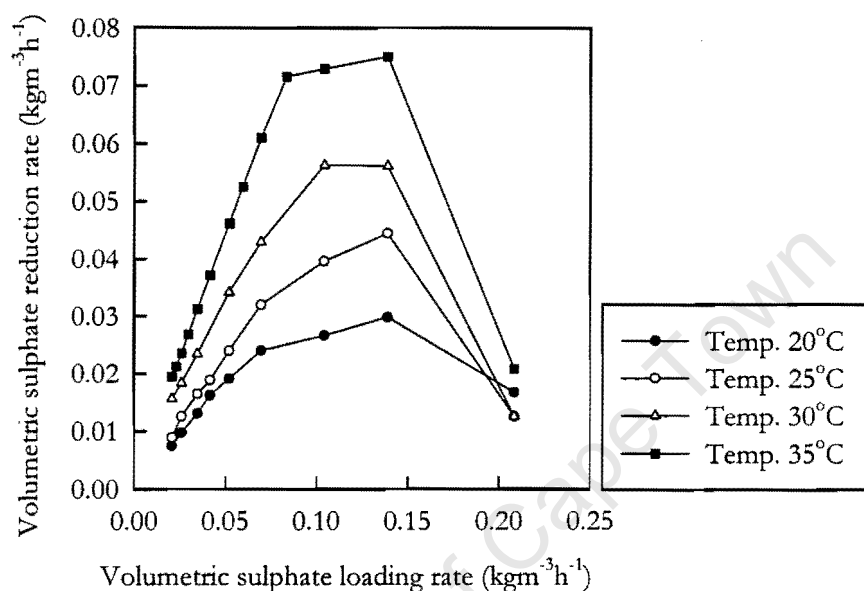


Figure 7.15. Dependency of 'stable' state volumetric sulphate reduction rate profiles on volumetric sulphate loading rate and temperature. Data obtained from continuous bioreactors operating at 20 (●), 25 (○), 30 (△) and 35 °C (■) and pH 7.8 with a feed medium containing 5.0 kg m⁻³ sulphate and 17.5 kgm⁻³ acetate

Table 7.4. Variation in maximum volumetric sulphate reduction rates and corresponding sulphate conversion, volumetric sulphate loading rate and dilution rate as a function of temperature. Data obtained from continuous bioreactors operating at pH 7.8 with feed sulphate and acetate concentrations of 5.0 and 17.5 kgm⁻³ respectively

Temperature (°C)	Maximum volumetric sulphate reduction rate (kgm ⁻³ h ⁻¹)	Corresponding sulphate conversion (%)	Corresponding volumetric loading (kgm ⁻³ h ⁻¹)	Corresponding dilution rate (h ⁻¹)
20	0.030	22	0.139	0.028
25	0.036	32	0.139	0.028
30	0.056	54	0.104	0.021
35	0.075	54	0.139	0.028

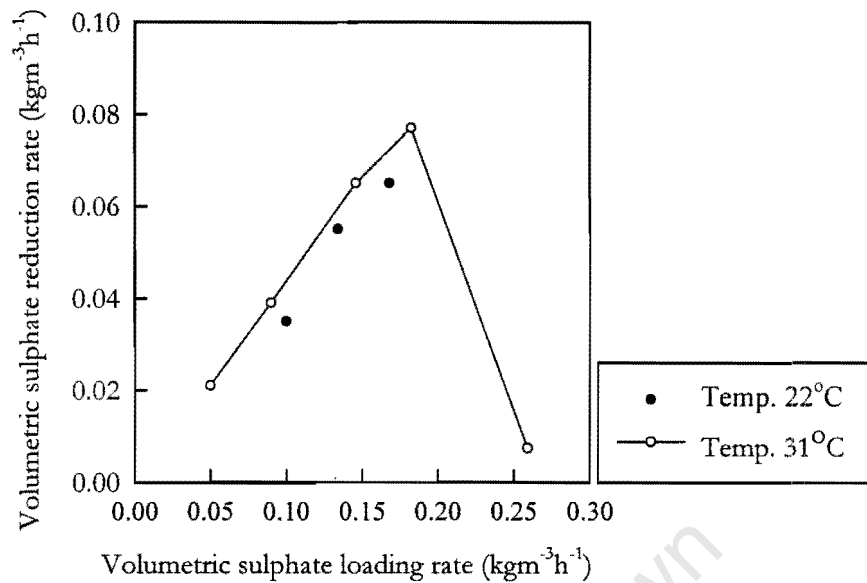


Figure 7.16. Dependency of volumetric sulphate reduction rate on volumetric sulphate loading rate at temperatures of 22 and 31°C for the continuous reduction of sulphate in a stirred tank reactor with lactic acid as the limiting organic nutrient (Barnes *et al.*, 1992a).

An indication of the specific metabolic activity of the microorganisms as a function of temperature is represented by the specific volumetric sulphate reduction rate presented in Figure 7.17. For all cases increasing the volumetric sulphate loading rate resulted in an increase in specific volumetric sulphate reduction rate. For volumetric sulphate rates between 0.021 and 0.035 $\text{kgm}^{-3}\text{h}^{-1}$ (dilution rate of 0.004 to 0.007 h^{-1}) the specific volumetric sulphate reduction rate did not vary significantly for temperatures of 20, 25, 30 and 35°C. As the volumetric sulphate loading rate was increased above 0.035 $\text{kgm}^{-3}\text{h}^{-1}$ the specific volumetric sulphate reduction increased with temperature in the range 20 to 35°C. For example when the volumetric sulphate loading rate was 0.104 $\text{kgm}^{-3}\text{h}^{-1}$ the specific volumetric sulphate reduction rates were 0.026, 0.031, 0.040 and 0.045 $\text{kgm}^{-3}\text{h}^{-1}$ for temperatures of 20, 25, 30 and 35°C respectively. This suggests enhancement of specific metabolic activity with increasing temperature.

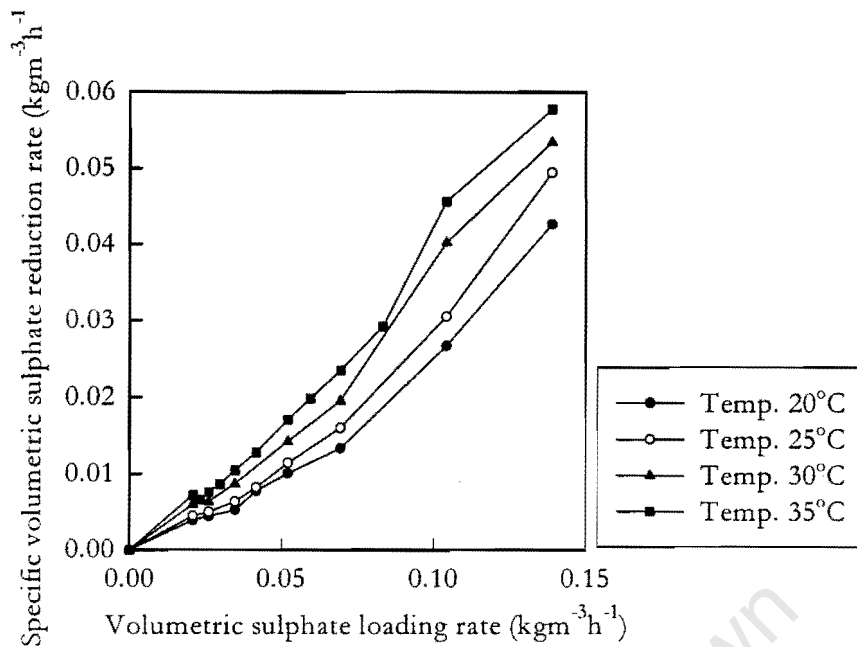


Figure 7.17. Dependency of 'stable' state specific volumetric sulphate reduction rate profiles on volumetric sulphate loading rate and temperature. Data obtained from continuous bioreactors operating at 20 (●), 25 (○), 30 (△) and 35 °C (■) and pH 7.8 with a feed medium containing 5.0 kg m⁻³ sulphate and 17.5 kgm⁻³ acetate.

7.4.3 RESIDUAL ACETATE CONCENTRATION

The residual acetate concentration profiles for reactors operating at temperature of 20, 25, 30 and 35°C are shown as Figure 7.18. For temperatures of 20, 25 and 35°C, increasing the volumetric loading rate of sulphate in the range 0.021 to 0.069 kgm⁻³h⁻¹ caused the residual acetate concentration to remain relatively constant. At temperatures of 20, 25 and 35°C, an increase in volumetric sulphate loading rate from 0.021 to 0.069 kgm⁻³h⁻¹ did not decrease the conversion of sulphate. Consequently the utilization of acetate remained relatively constant. For temperatures of 20, 25 and 35°C as the volumetric loading of sulphate was increased above 0.069 kgm⁻³h⁻¹ the residual acetate concentration increased. When the reaction temperature was 30°C the residual acetate concentration increased with volumetric sulphate loading rate for the full range of applied volumetric sulphate loading rates. This can be attributed to the consistent decrease in sulphate conversion with volumetric sulphate loading rate for the range of applied volumetric sulphate loading rates. Despite the similar trends, observed at temperatures of 20 and 35°C, the residual acetate concentration displayed a marked decrease with increasing temperature for volumetric sulphate loading rates below 0.139 kgm⁻³h⁻¹. For example at a volumetric sulphate loading rate of 0.139 kgm⁻³h⁻¹ the

residual acetate concentrations for temperatures of 20 and 35°C were 16.5 and 15.2 kgm⁻³ respectively. For temperatures of 25 and 30°C the residual acetate concentrations at loading rates above 0.104 kgm⁻³h⁻¹ are similar. Middleton and Lawrence (1977) report a similar observation. In continuous stirred tank studies using acetate as the limiting organic nutrient they found that for operating temperatures of 25 and 31°C the residual acetate concentration was similar at low retention times (high loading rates). At a retention time of 4 d the residual acetate concentration was 0.09 and 0.10 kgm⁻³ for temperatures of 25 and 31°C

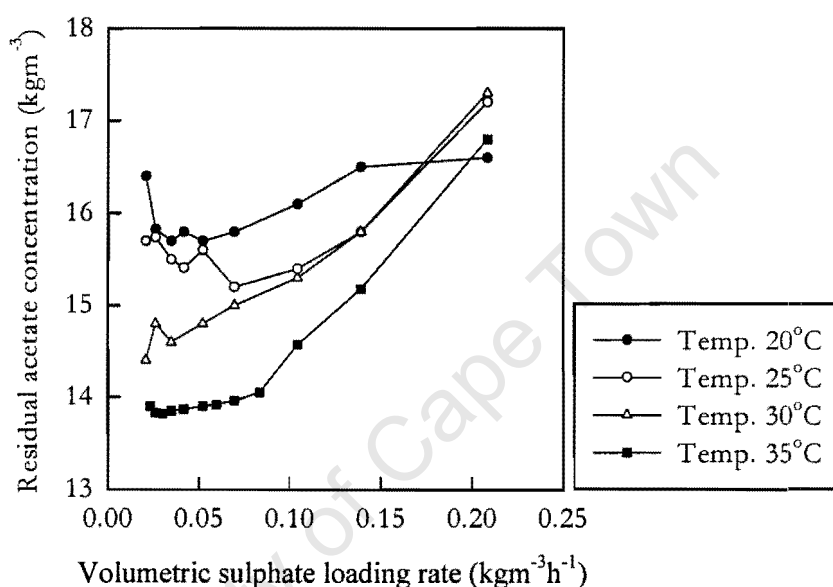


Figure 7.18. Dependency of 'stable' state residual acetate concentration profiles on volumetric sulphate loading rate and temperature. Data obtained from continuous bioreactors operating at 20 (●), 25 (○), 30 (△) and 35 °C (■) and pH 7.8 with a feed medium containing 5.0 kg m⁻³ sulphate and 17.5 kgm⁻³ acetate

The ratio of sulphate reduced to acetate utilised is shown in Table 7.5 for temperatures of 20, 25, 30 and 35°C. The ratio of moles sulphate to moles acetate utilised does not vary significantly for the range of applied temperatures. The theoretical ratio is 1.00 meaning that for every mole of sulphate converted to sulphide one mole of acetate is utilised (Section 6.5.3). A ratio below one indicates that the metabolic utilisation of acetate for the conversion of sulphate is not 100 % efficient. For these experiments the average ratio of sulphate converted to acetate utilised was 0.80 ± 0.03 indicating an average metabolic efficiency of acetate utilisation of $80 \pm 3\%$. As no methane was detected in the gas produced (indicating

the absence of active MPB) it is suggested that the remainder of the acetate was used for maintenance, endogenous respiration etc.

Table 7.5. Dependency of molar ratio of sulphate of sulphate utilised to acetate utilised on temperature.

Data obtained from continuous bioreactors operating at pH 7.8 with feed sulphate and acetate concentrations of 5.0 and 17.5 kgm⁻³.

Temperature (°C)	Moles sulphate/moles acetate
20	0.80 ± 0.056
25	0.76 ± 0.047
30	0.81 ± 0.010
35	0.80 ± 0.014
Theoretical	1.00

7.4.4 BACTERIAL CONCENTRATION

The bacterial concentration profiles for bioreactors operating at temperatures of 20, 25, 30 and 35°C are shown in Figure 7.19. In all cases the bacterial concentration displayed an initial increase as the dilution rate was increased in the range 0.004 to 0.007 h⁻¹, (volumetric sulphate loading rate of 0.021 to 0.035 kgm⁻³h⁻¹). For dilution rates above 0.007 h⁻¹ the bacterial concentration decreased with dilution rate. At a dilution rate of 0.042 h⁻¹, washout was complete (volumetric sulphate loading rate of 0.208 kgm⁻³h⁻¹). Washout did not occur at a distinct dilution rate. This is typical of a mixed culture comprised of species with different maximum specific growth rates. (Bailey & Ollis, 1986, Shuler & Kargi, 1992, Chiu *et al.*, 1972a, b). A similar dependency of biomass concentration on dilution rate was observed by Chiu *et al.* (1972a, b). Studying the continuous breakdown of glucose by a mixed microbial population of sewage origin they found that as the dilution rate was increased from 0.022 to 0.095 h⁻¹ the biomass concentration increased from 0.27 to 0.49 gL⁻¹. Further increase of the dilution rate to 0.40 h⁻¹ result in a relatively constant bacterial concentration of 0.53 gL⁻¹. When the dilution rate was increased from 0.40 to 0.65 h⁻¹ the biomass concentration decreases to 0.07 gL⁻¹ indicating that washout was complete.

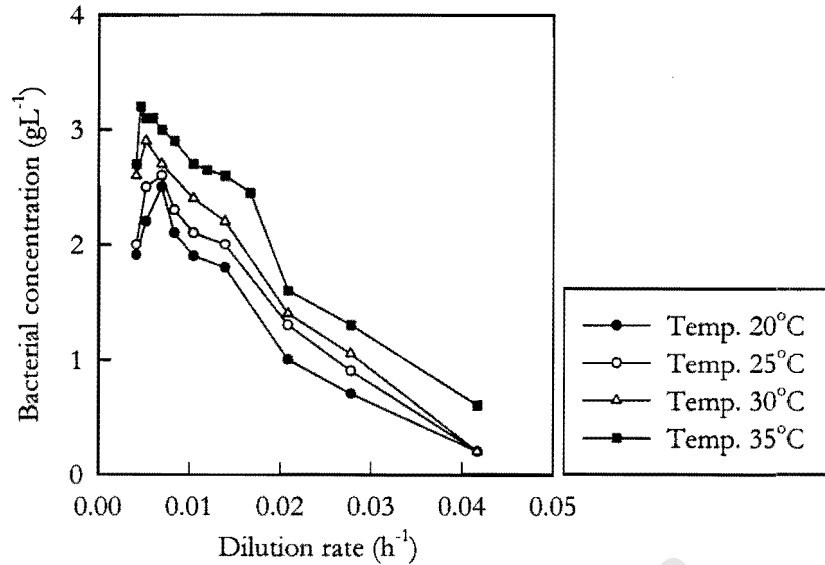


Figure 7.19. Dependency of 'stable' state biomass concentration profiles on dilution rate and temperature. Data obtained from continuous bioreactors operating at 20 (●), 25 (○), 30 (△) and 35 °C (■) and pH 7.8 with a feed medium containing 5.0 kg m⁻³ sulphate and 17.5 kgm⁻³ acetate

The maximum bacterial concentrations achieved for temperatures of 20, 25, 30 and 35°C are given in Table 7.6. The maximum bacterial concentration displayed a 17 % increase as the temperature was increased from 20 to 35°C. This is not very significant, as the experimental error for biomass determination was 15 %. The dilution rate at which the maximum bacterial concentration was observed remained constant for temperatures between 25 and 35°C. This suggests that the onset of washout is similar across the temperature range of 25, 30 and 35°C.

Microscopic observations of the bacteria (Figures 7.20 to 7.23) indicated that the population was morphologically similar across the temperature range. At 20°C the presence of clumps indicates that the system may be stressed (Brock and Madigan, 1991).

Table 7.6 Dependency of the maximum bacterial concentration and corresponding dilution rates on the reaction temperature. Data obtained from continuous bioreactors operating at pH 7.8 with feed sulphate and acetate concentrations of 5.0 and 17.5 kgm⁻³.

Temperature (°C)	Maximum bacterial concentration (gL ⁻¹)	Corresponding dilution rate (h ⁻¹)
20	2.500	0.007
25	2.600	0.005
30	2.900	0.005
35	3.100	0.005

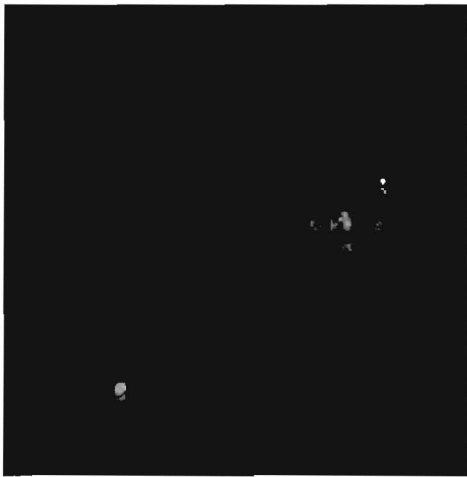


Figure 7.20. Bacteria from bioreactor operating at 20°C. (1000 X magnification)



Figure 7.21 Bacteria from bioreactor operating at 25°C. (1000 X magnification)



Figure 7.22. Bacteria from bioreactor operating at 30°C. (1000 X magnification)

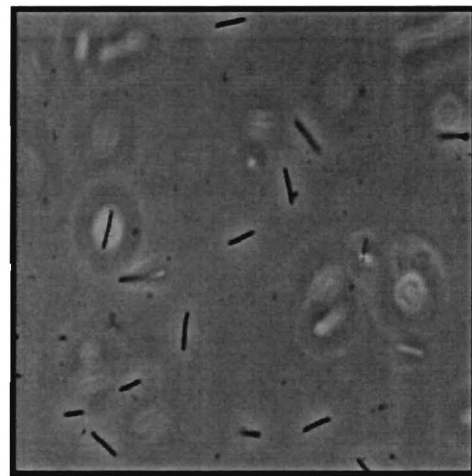


Figure 7.23. Bacteria from bioreactor operating at 35°C. (1000 X magnification)

Biomass formation can be related to the amount of sulphate converted via the NAD^+ formed during the reduction of sulphate with acetate as the electron donor. The NAD^+ is used as the energy source for biomass formation (Section 6.5.4). Using this approach the theoretical yield is $0.47 \text{ (g biomass)/(g sulphate converted)}$. Consequently the enhanced sulphate conversion observed with increasing temperature from 20 to 35°C resulted in an increase in the biomass concentration. The dependency of the observed yield with sulphate on dilution rate is shown in Figure 7.24. As the temperature was increased from 20 to 35°C the ratio of biomass to sulphate converted decreased from 0.89 to 0.50 . The high ratio observed at lower temperatures indicates a deviation from the biomass formation mechanism proposed.

From the biomass results presented in this section, it was seen that the biomass displayed an increase with increasing temperature, with similar trends observed for biomass concentration as a function of dilution rate.

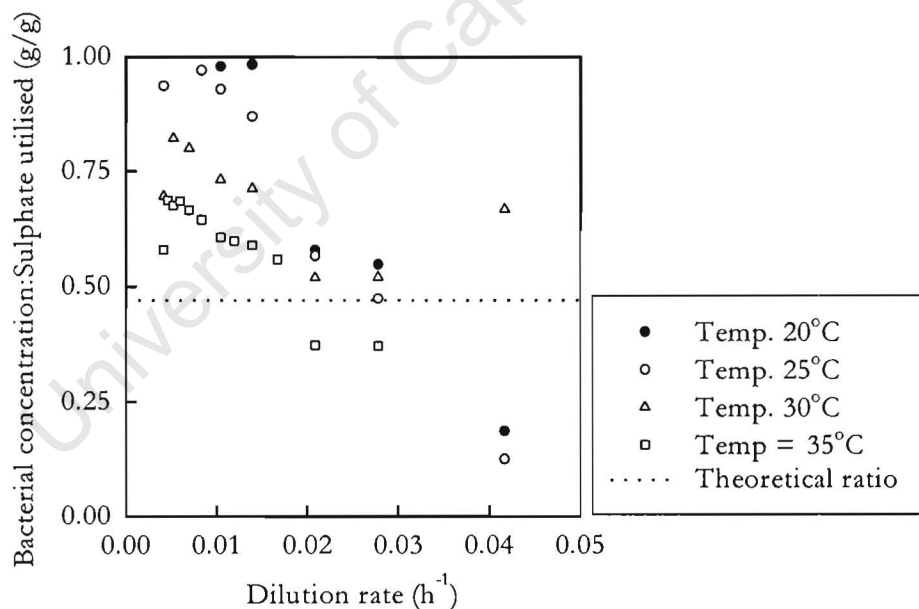


Figure 7.24. Dependency of ratio of bacterial concentration to sulphate utilised on dilution rate and temperature. Data obtained from continuous bioreactors operating at 20 (●), 25 (○), 30 (△) and 35°C (□) and pH 7.8 with a feed medium containing 5.0 kg m^{-3} sulphate and 17.5 kg m^{-3} acetate

7.5 CHAPTER SUMMARY

In this chapter results of a study to determine the effect of temperature on the kinetics of anaerobic sulphate reduction were presented. Continuous bioreactors with a mixed SRB population were operated at four temperatures, 20, 25, 30 and 35°C and the 'stable' state kinetics were used to ascertain what effect temperature had on the activity of sulphate reducers. The results show that increasing the temperature in the range 20 to 35°C resulted in an increase in the conversion and volumetric reduction rate of sulphate. The trends observed in terms of residual sulphate concentration, sulphate conversion, residual acetate concentrations, volumetric reduction rate and bacterial concentration are similar for all four temperatures employed. The enhancement of sulphate conversion in terms of percentage conversion and volumetric sulphate reduction rate with temperature can be attributed to the enzymatic nature of the reduction reaction which is enhanced with increasing temperature in the range 20 to 35°C and to the decrease in H_2S relative to HS^- with increasing temperature.

The results presented in Chapters 6 and 7 are used in the subsequent chapter. In Chapter 8 a model to describe the effect of feed sulphate concentration and temperature on the kinetics of microbial sulphate reduction is developed.

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CHAPTER 8

MODELLING OF EXPERIMENTAL DATA

The results presented in Chapters 6 and 7 have shown that both the feed sulphate concentration and temperature have an affect on the kinetics of the anaerobic sulphate reduction process. The aim of this chapter is to provide the reader with a proposed kinetic model for the anaerobic sulphate reduction process based on the experimental data presented in Chapter 6 and 7. Initially a review of the literature regarding microbial growth kinetics is presented followed by the modelling approach and overview of existing models used to describe anaerobic sulphate reduction. This is followed by the derivation of the kinetic model. Initially the development of the model to account for the effect of feed sulphate concentration on reduction rate is presented and subsequently the model is modified to account of the temperature effects. The model is first calibrated and then validated with an independent set of data.

8.1 MODELLING APPROACH AND REVIEW OF LITERATURE

Modelling of biological processes is valuable in developing an understanding of the system and in enabling prediction. There are two types of models, namely mechanistic and empirical. If the fundamental

processes of a system are known, enabling the growth and product formation kinetics to be described in terms of discrete units in the system, then a structured and segregated approach to modelling can be implemented. These models are described as mechanistic. If the system is complex involving a large number of sub-processes of which the mechanisms are not well understood, an empirical model is used to describe the system. The predictions of the model then represent the basis for experimental work. This approach is unstructured and nonsegregated. The unstructured model assumes a fixed cell composition. This is valid primarily in a single stage steady state continuous culture and the exponential phase of a batch culture but fails during transient conditions. Since the detailed mechanism of sulphate reduction is not fully understood, only the unstructured, nonsegregated models will be considered in this section.

8.1.1 UNSTRUCTURED NONSEGREGATED MODELS FOR MICROBIAL GROWTH

A feature of the unstructured kinetic models is that they attempt to describe the kinetics of cell growth based on cell and nutrient concentration profiles. The bioprocess is modelled by one reaction only. The rate of this reaction is $r_x = \mu X$ and that of the product formation is $r_p = \mu X Y_{p/x}$ where the product is growth associated. Thus modelling of the process involves specification of μ as a function of substrate concentration (S), product (P) and biomass (X).

8.1.1.1 SUBSTRATE LIMITED GROWTH

The simplest approach to modelling biomass growth is to assume that the rate of increase in cell mass is a function of cell mass only (Bailey & Ollis, 1987).

$$\frac{dX}{dt} = f(X) \quad 8.1$$

Malthus' law, Eqn 8.2, is a simple model belonging to the general form given in.

$$\frac{dX}{dt} = \mu[X] \quad 8.2$$

When μ is constant, integration of Eqn 8.2 assuming that at $t = 0$, $[X] = [X_0]$ gives:

$$[X] = [X_0] e^{\mu t} \quad 8.3$$

Bacterial growth which follows Eqn 8.3 is called constant exponential or logarithmic growth. Microbial growth rate is quantified by the specific growth rate (μ).

The first hyperbolic relationship between cell growth rate and a single substrate, S, (Eqn 8.4) was proposed by Jacques Monod in 1949.

$$\mu = \frac{\mu_m [S]}{K_s + [S]} \quad 8.4$$

where: μ = Growth rate (h^{-1})
 μ_m = Maximum specific growth rate (h^{-1})
 S = Limiting substrate concentration (kgm^{-3})
 K_s = Half saturation constant (kgm^{-3})

This function is empirical and based on observations of the growth of *E. coli* at various glucose concentrations. It is not disparate to the Michaelis-Menton function for enzyme kinetics. Since biochemical reactions are controlled by enzymes, this is not unexpected. In Eqn 8.4 μ_m is the maximum growth rate when [S] greatly exceeds K_s . K_s is the value of the limiting substrate concentration at which the specific growth rate is half its maximum value. When [S] exceeds $2K_s$, the growth rate μ is independent of [S]. A diagrammatic representation of the Monod model is shown as Figure 8.1.

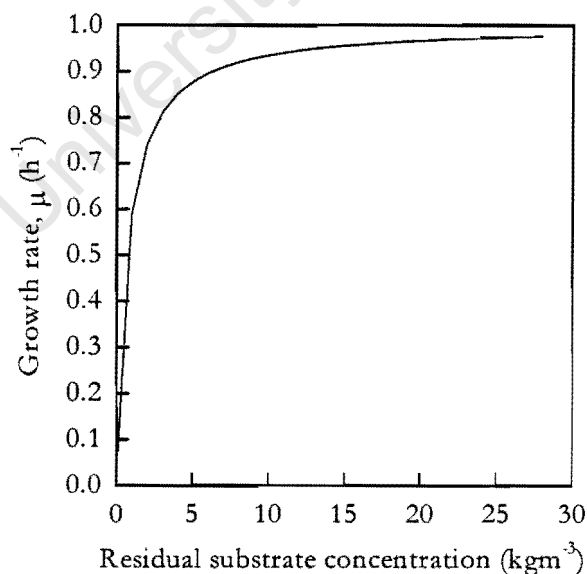


Figure 8.1. Dependency of microbial growth rate on residual substrate concentration as described by the Monod model.

Parameters: $K_s = 0.7 \text{ kgm}^{-3}$, $\mu_m = 1 \text{ h}^{-1}$, $[S_0] = 28 \text{ kgm}^{-3}$, $Y_{X/S} = 0.5$.

The Monod model has been used extensively to model cell growth in anaerobic systems (Andrews, 1973, 1975; Andrews and Graef, 1971; Barthakur *et al.*, 1991; McCarty and Mosey, 1991). Middleton and Lawrence (1977) used it to explain the growth of a mixed SRB population with acetate as the growth limiting substrate. In addition, the Monod model was used when modelling the kinetics of the interactions between MPB and SRB using various organic substrates as the growth limiting substrate (Robinson & Tiedje, 1984; Yoda *et al.*, 1987; Costello *et al.*, 1991; Harada *et al.*, 1994; Gupta *et al.*, 1996; Bhattacharya *et al.*, 1996, Steyer *et al.*, 2000). The growth of SRB in pure culture was also modelled using the Monod relation. The growth of *Desulfovibrio* and *Desulfobacter postgatei* with lactate and acetate as the limiting substrate respectively was shown to obey the Monod function (Ingvorsen *et al.*, 1984). Chen *et al.* (1994) also used the Monod model to determine the kinetic parameters of *Desulfovibrio desulfuricans* grown on lactate as the limiting nutrient.

The Monod equation describes substrate-limited growth only when growth is slow and population density is low. At high population levels, the build up of toxic metabolic by-products becomes more important and the following rate expression has been proposed for rapidly growing dense cultures (Pirt, 1975):

$$\mu = \frac{\mu_m [S]}{K_{s_0} [S_0] + [S]} \quad 8.5$$

The unstructured models discussed above assume the presence of one limiting substrate. In many instances, multiple substrates limit the microbial growth rate. In these cases complex interactions occur and the above kinetic models, for a single limiting substrate become void. This is true for anaerobic sulphate reduction when both the organic source and sulphate can be limiting. Shuler and Kargi (1992) cite three techniques for modelling bacterial growth dependent on more than one limiting substrate.

1. Interactive or multiplicative forms:

$$\mu = \mu_m \left[\frac{[S_1]}{K_{s_1} + [S_1]} \frac{[S_2]}{K_{s_2} + [S_2]} \dots \frac{[S_n]}{K_{s_n} + [S_n]} \right] \quad 8.6$$

2. Additive forms

$$\mu = \mu_m \left[W_1 \frac{[S_1]}{K_{s_1} + [S_1]} + W_2 \frac{[S_2]}{K_{s_2} + [S_2]} + \dots W_n \frac{[S_n]}{K_{s_n} + [S_n]} \right] \quad 8.7$$

where W_1, W_2, \dots, W_n are weighing functions

3. Noninteractive forms

$$\mu = \frac{\mu_m [S_1]}{K_{S1} + [S_1]} \text{ or } \frac{\mu_{m2} [S_2]}{K_{S2} + [S_2]} \text{ or } \dots \frac{\mu_{mn} [S_n]}{K_{Sn} + [S_n]} \quad 8.8$$

In cases 1 and 2 above the bacterial population remains unchanged hence one μ_m is used. The interactive or multiplicative dual substrate model has been successfully used to determine the batch growth kinetic parameters (K_{S1}, K_{S2} and μ_m) of *Desulfovibrio desulfuricans* with both lactate and sulphate as the limiting nutrients (Konishi *et al.*, 1996).

If the limiting substrate is unknown then the logistic equation can be used to describe growth.

$$\mu = \mu_m \left(1 - \frac{[X]}{[X_{max}]} \right) \quad 8.9$$

In the logistic equation, a maximum biomass population is assumed, and this population is not explicitly controlled by growth limitation.

8.1.1.2 MODELING GROWTH INHIBITION

Substrate inhibition

High substrate concentrations may inhibit growth or support such high biomass concentrations that suspension characteristics may affect μ .

The model proposed to describe microbial growth in the presence of substrate inhibition is based on the kinetics of substrate inhibition of enzyme activity (Pirt, 1975). Substrate inhibition is a form of uncompetitive inhibition. Mechanistically this implies that the substrate (S) and the enzyme (E) have reacted to form the enzyme-substrate complex (ES) additional substrate is taken up resulting in SE^2 preventing

the formation of product (P). The product is formed from ES only. By observation microbial inhibition follows similar trends to enzymatic inhibition. Since microbial growth is the sum of enzymatic reactions, the same form of equation as the enzymatic reaction can be used to describe competitive inhibition. The proposed model is:

$$\mu = \frac{\mu_m [S] K_I}{[S] K_I + K_s K_I + [S]^2} \quad 8.10$$

where: K_I = substrate inhibition constant (kgm^{-3})

The relationship between growth rate and residual substrate concentration given by Eqn 8.10 is shown in Figure 8.2. Growth rate increases with substrate concentration up to the critical value $[S]_{\text{crit}}$, thereafter the inhibitory concentration becomes dominant and the growth declines with increasing residual substrate concentration till washout.

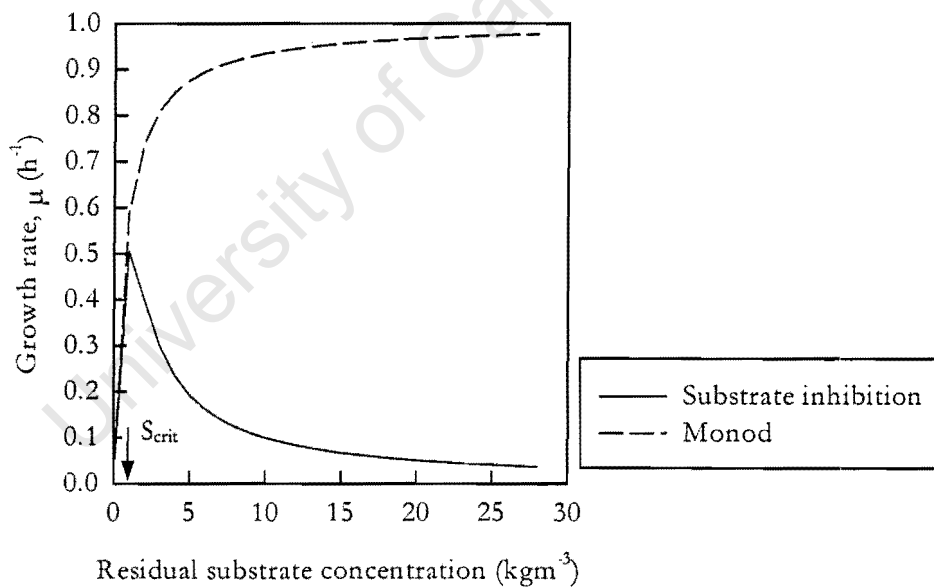


Figure 8.2. Prediction of microbial growth rate as a function of residual substrate concentration according to the Monod equation and the substrate inhibition model (Eqn 8.10).

Parameters: $K_s = 0.7 \text{ kgm}^{-3}$, $K_I = 0.4 \text{ kgm}^{-3}$, $\mu_m = 1 \text{ h}^{-1}$, $[S_0] = 28 \text{ kgm}^{-3}$, $Y_{X/S} = 0.5$.

Where conventional substrate inhibition kinetics is not applicable, a variety of empirical modifications of the Monod model have been proposed. For example, Eqn 8.11 includes an addition term in the denominator to account for a decrease in μ with increasing feed substrate concentration observed in an anaerobic digester (Pavlostathis & Giraldo-Gomez, 1991).

$$\mu = \frac{\mu_m [S]}{K_s + K_{s_0} [S_0] + [S]} \quad 8.11$$

Here, when the limiting substrate concentration in the feed increases the microbial growth rate decreases and μ_m is not achieved. This dependency of microbial growth rate on residual substrate concentration is shown in Figure 8.3 for three feed substrate concentrations.

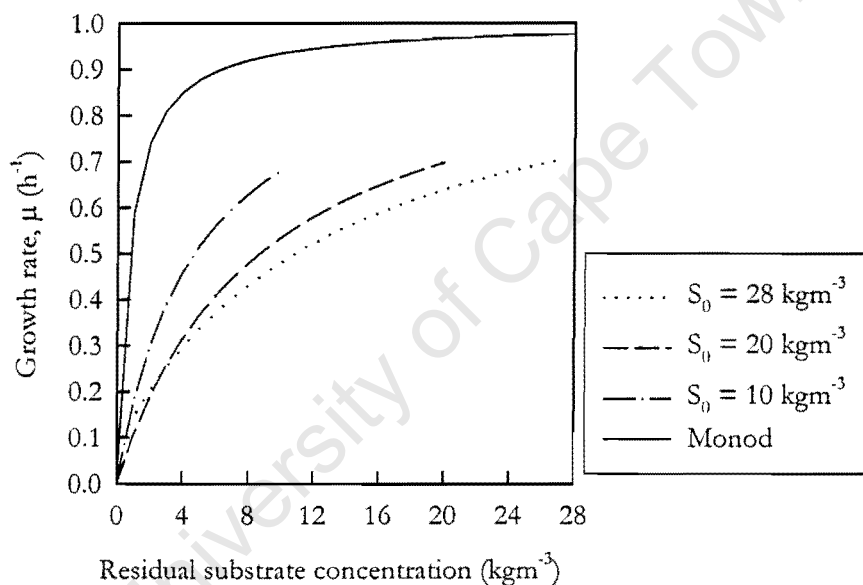


Figure 8.3. Prediction of microbial growth rate as a function of residual substrate concentration and the initial substrate concentration according to the Monod equation and Eqn 8.11.

Parameters: $K_s = 1 \text{ kgm}^{-3}$, $K_{s_0} = 1 \text{ kgm}^{-3}$, $K_{S_0} = 0.4 \text{ kgm}^{-3}$, $\mu_m = 1 \text{ h}^{-1}$, $[S_0] = 28 \text{ kgm}^{-3}$, $Y_{X/S} = 0.5$.

At high feed substrate concentrations, high biomass concentrations are supported. These high biomass concentrations increase suspension viscosity thereby influencing mass transfer. This mass transfer effect may be taken into account by modification of K_s , the bulk substrate concentration at which growth is supported at 50 % of μ_m . Examples of this modification include the Contois equation (8.12) and the Chen and Hashimoto equation (Eq 8.13) (Chen and Hashimoto, 1980).

$$\mu = \frac{\mu_m [S]}{K_s [X] + [S]} \quad 8.12$$

$$\mu = \frac{\mu_m [S]}{K_s [S_0] + (1 - K_s)[S]} \quad 8.13$$

The prediction of growth rate as a function of residual substrate concentration as described by the Contois model (Eqn 8.12) is illustrated in Figure 8.4. At high residual concentrations the model becomes unstable. The Contois model has also been derived by Fujimoto (1963, in Chen & Hashimoto, 1980) and confirmed on batch cultures of bakers' and alcohol yeasts and *Escherichia coli*.

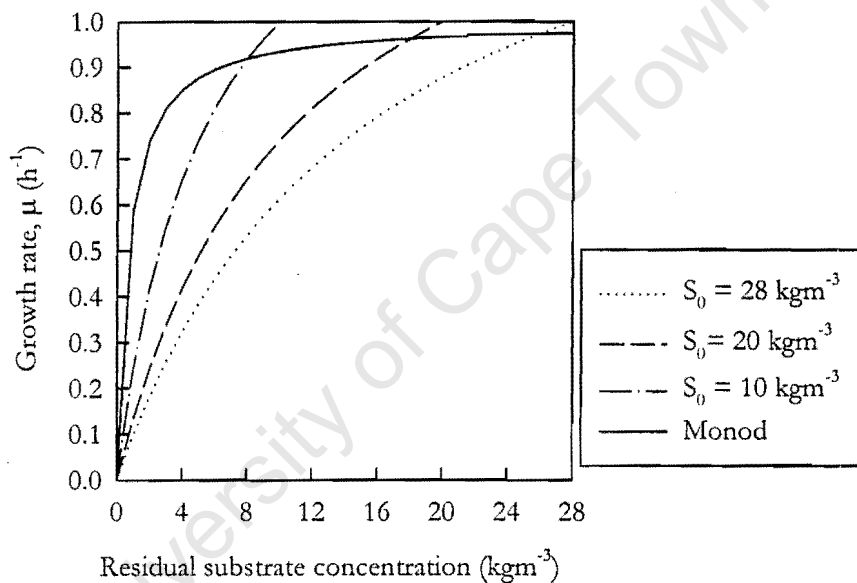


Figure 8.4. Prediction of microbial growth rate as a function of residual substrate concentration and initial substrate concentration according to the Monod equation and the Contois equation (Eqn 8.12).

Parameters: $K_s = 0.7 \text{ kgm}^{-3}$, $\mu_m = 1 \text{ h}^{-1}$, $Y_{X/S} = 0.5$.

The Chen and Hashimoto model is an adaptation of the Contois model in which K_s is redefined as the product of the yield coefficient and the K_s term in the Contois equation (Eqn 8.13). Figure 8.5 shows the prediction of microbial growth rate as a function of residual substrate concentration using Eqn 8.13 for three feed substrate concentrations. The growth rate observed is higher for the lower feed substrate concentration. At high residual substrate concentrations μ does not tend to μ_m .

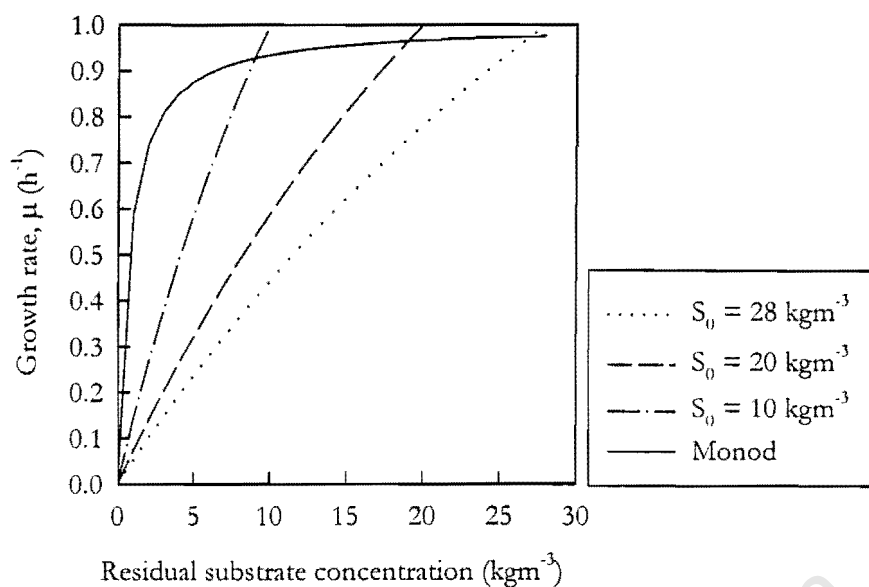


Figure 8.5. Prediction of microbial growth rate as a function of residual sulphate concentration and initial substrate concentration according to the Monod equation and the Chen and Hashimoto equation (Eqn 8.13).

Parameters: $K_s = 0.7 \text{ kgm}^{-3}$, $\mu_m = 1 \text{ h}^{-1}$, $[S_0] = 28 \text{ kgm}^{-3}$, $Y_{X/S} = 0.5$.

Table 8.1 summarizes the microbial growth models discussed above.

Table 8.1. Summary of the kinetic models of microbial growth.

Model	Equation	Reference
Monod	$\mu = \frac{\mu_m [S]}{K_s + [S]}$	Monod (1949)
Substrate inhibition	$\mu = \frac{\mu_m [S] K_i}{[S] K_i + K_s K_i + [S]^2}$	Pirt (1975)
Feed substrate inhibition	$\mu = \frac{\mu_m [S]}{K_s + K_{s_0} [S_0] + [S]}$	Pavlostathis and Giraldo-Gomez (1991)
Contois	$\mu = \frac{\mu_m [S]}{K_s X + [S]}$	Contois (1959)
Chen and Hashimoto	$\mu = \frac{\mu_m [S]}{K[S_0] + (1-K)[S]}$	Chen and Hashimoto (1980)

Product Inhibition

A product may inhibit microbial growth by competing with the growth limiting substrate for uptake (competitive inhibition), or by affecting growth through another mechanism (non-competitively). Competitive product inhibitors are usually substrate analogs. The net effect of this form of inhibition is an increase in the apparent K_s term. An example of a product which may cause competitive inhibition of growth is ferric ion obtained in the oxidation of ferrous ion by *Ferrobacillus* (Pirt, 1975). The competitive model is given as Eqn 8.14 and illustrated in Figure 8.6.

$$\mu = \frac{\mu_m [S]}{K_s \left(1 + \frac{[P]}{K_i}\right) + [S]} \quad 8.14$$

where: [P] = product concentration (kgm^{-3})

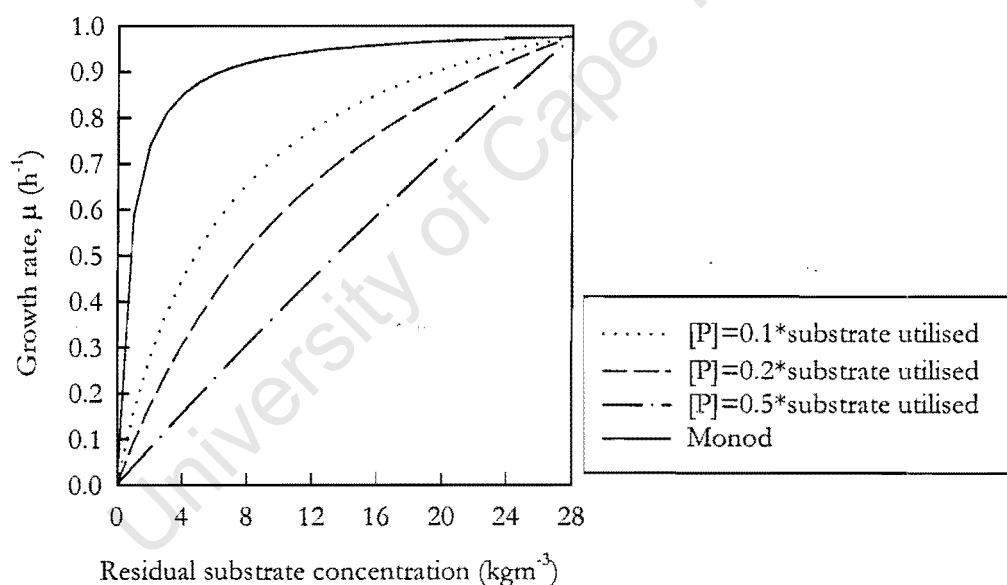


Figure 8.6. Prediction of microbial growth rate as a function of residual substrate concentration and product concentration according to the Monod equation and the competitive inhibition equation (Eqn 8.14)

Parameters: $K_s = 0.7 \text{ kgm}^{-3}$, $\mu_m = 1 \text{ h}^{-1}$, $[S_0] = 28 \text{ kgm}^{-3}$, $Y_{X/S} = 0.5$.

The non-competitive model for microbial growth is:

$$\mu = \frac{\mu_m [S]}{([S] + K_s) \left(1 + \frac{[P]}{K_i} \right)} \quad 8.15$$

Non-competitive inhibitors have no effect on the uptake of substrate by the microorganism and vice versa. The microorganism can take up the product inhibitor simultaneously and independently. To understand how it functions the enzymatic nature of the microbial reaction will be considered. The non-competitive substrate inhibits the formation of product by forming an enzyme-substrate-product complex from which product cannot be produced. Ethanol is an example of a product that causes non-competitive growth of yeast. The non-competitive model has been used to describe the inhibitory effect of sulphide on the sulphate reduction process. Reis *et al.* (1992) modelled the inhibitory effect of sulphide on a mixed SRB population growing in batch on lactate as the limiting nutrient by non-competitive inhibition. Later, Okabe *et al.* (1995) used the same approach to model the inhibition of sulphide on the growth of *Desulfovibrio desulfuricans* in a chemostat with lactate as the growth limiting substrate.

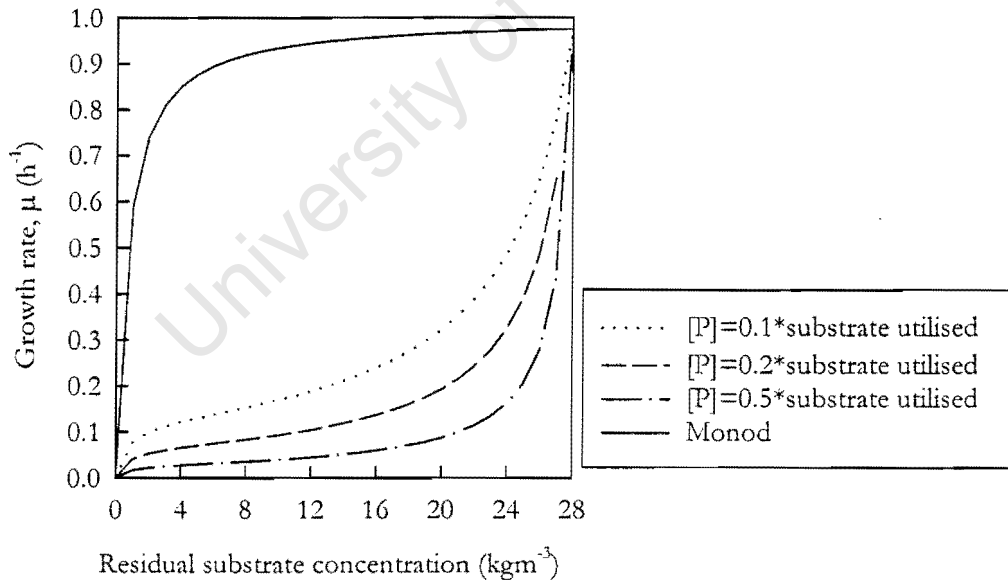


Figure 8.7. Prediction of microbial growth rate on residual substrate concentration and product concentration according to the Monod equation and the noncompetitive inhibition equation (Eqn 8.15).

Parameters: $K_s = 0.7 \text{ kgm}^{-3}$, $K_i = 0.4 \text{ kgm}^{-3}$, $\mu_m = 1 \text{ h}^{-1}$, $[S_0] = 28 \text{ kgm}^{-3}$, $Y_{X/S} = 0.5$.

Inhibition by toxic compounds

Inhibition of microbial growth by other toxic compounds can be described by the competitive and non-competitive models described. These are rewritten in terms of the inhibitor concentration in Eqn 8.16 and 8.17 respectively.

Competitive inhibition:

$$\mu = \frac{\mu_m [S]}{K_s \left(1 + \frac{[I]}{K_i} \right) + [S]} \quad 8.16$$

Non-competitive inhibition:

$$\mu = \frac{\mu_m [S]}{([S] + K_s) \left(1 + \frac{[I]}{K_i} \right)} \quad 8.17$$

where: [I] = inhibitor concentration (kgm^{-3})

Reis *et al.* (1990) examined the effect of undissociated acid over the pH range 5.8 to 7.0 on a sulphate reducing culture using lactate as the organic source. They concluded that a modified non-competitive model (Eqn 8.18) can be used to describe the effect of acetic acid inhibition on SRB population.

$$\mu = \frac{\mu_m}{1 + ([\text{Acetate}] / K_{i,\text{acetate}})^{1.08}} \quad 8.18$$

where: $K_{i,\text{acetate}}$ = Saturation constant for acetic acid

Un-competitive is another form of inhibition responsible for the inhibition of microbial growth. Adopting an enzymatic reaction approach to explain the mechanism of the inhibition on microbial growth, un-competitive inhibitors bind to the enzyme-substrate complex forming an enzyme-substrate-inhibitor complex. This inhibits the formation of product, which forms from the enzyme-substrate complex. Un-competitive inhibition is represented by Eqn 8.19.

Un-competitive inhibition:

$$\mu = \frac{\mu_m [S] / \left(1 + \frac{[I]}{K_i}\right)}{\left(1 + \frac{[I]}{K_i}\right) K_s + [S]} \quad 8.19$$

Maillacheruvu and Parkin (1996) showed that sulphide inhibition of the acetate utilizing MPB in a mixed anaerobic population containing SRB is un-competitive. The experiments were carried out in batch with propionate as the growth limiting substrate. Kalyuzhnyi and Fedorovich (1998), modelling the interactions between SRB, MPB, fermentative bacteria and acetogens proposed an improved model for each of the microbial groups (Eqn 8.20), which accounts for both dual substrate utilisation and sulphide inhibition.

$$\mu = \frac{\mu_m [S_1][S_2]}{(K_{s1} + [S_1])(K_{s2} + [S_2])} \left(1 - \frac{[I]}{K_i}\right) \quad 8.20$$

Table 8.2 provides a summary of the inhibition models presented.

Table 8.2. Kinetic models from literature for inhibition.

Model	Equation	Reference
Competitive inhibition	$\mu = \frac{\mu_m [S]}{K_s \left(1 + \frac{[I]}{K_i}\right) + [S]}$	Shuler & Kargi (1992)
Un-competitive inhibition	$\mu = \frac{\mu_m [S] / \left(1 + \frac{[I]}{K_i}\right)}{\left(1 + \frac{[I]}{K_i}\right) K_s + [S]}$	Shuler & Kargi (1992)
Non-competitive inhibition	$\mu = \frac{\mu_m [S]}{([S] + K_s) \left(1 + \frac{[I]}{K_i}\right)}$	Shuler & Kargi (1992)

8.1.2 CHEMOSTAT THEORY

The chemostat illustrated in Figure 8.8 is maintained at a constant culture volume, V , by the continuous addition of fresh medium and removal of culture at flowrate F (m^3h^{-1}). The feed enters the reactor with concentration $[S_0]$ (kgm^{-3}) with respect to the limiting substrate and bacterial feed concentration $[X_0]$, (kgm^{-3}). The residual concentration of limiting substrate, product and biomass in the reactor of volume V are $[S]$, $[P]$ and $[X]$ respectively.

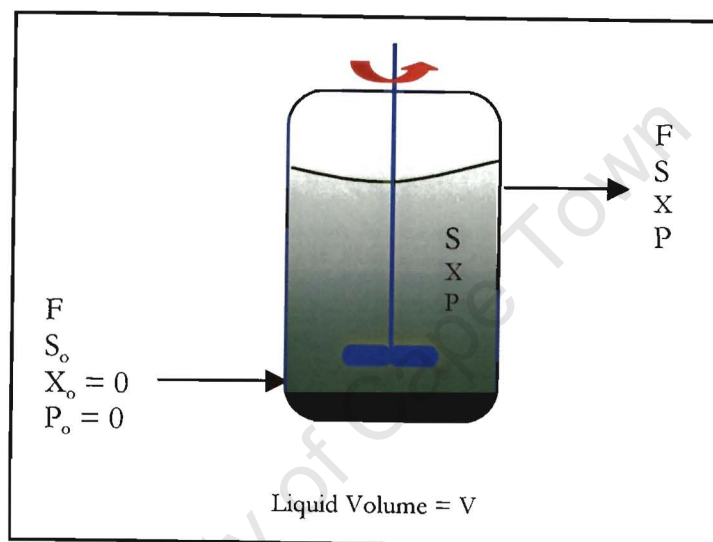


Figure 8.8. Diagrammatic representation of the chemostat. The biomass, growth limiting substrate concentration and product concentration in the reactor are represented by $[X]$, $[S]$ and $[P]$ respectively. F = flowrate and V = liquid volume in reactor.

The biomass material balance is given as:

$$F X_0 - F[X] + V\mu[X] = V \frac{dX}{dt} \quad 8.21$$

Assuming the feed is free of anaerobic microorganisms, $[X_0]$ is 0. The dilution rate, D , is defined as F/V .

Hence Eqn 8.21 is simplified to:

$$\frac{dX}{dt} = (\mu - D)[X] \quad 8.22$$

Furthermore at steady state, $dX/dt = 0$ and therefore:

$$\mu = D \quad 8.23$$

Kinetic models relating the specific growth rate μ to the residual substrate concentration, S have been presented in Section 8.1.2.

The material balance on the limiting substrate, S , is given by:

$$F[S_0] - F[S] - r_s V = V \frac{dS}{dt} \quad 8.24$$

where: r_s is the volumetric reduction rate of substrate S , and product accumulation and cell maintenance are negligible.

Hence at steady state (where $\frac{dS}{dt}$ is 0), Eqn 8.24 can be simplified to:

$$\frac{F}{V} (S_0 - S) = r_s \quad 8.25$$

The rate of substrate utilisation can be related to the rate of biomass formation via the yield coefficient ($Y_{x/s}$).

$$Y_{x/s} = \frac{dX/dt}{r_s} \quad 8.26$$

where:

$$\frac{dX}{dt} = \mu[X] \quad 8.27$$

resulting in:

$$r_s = \frac{\mu[X]}{Y_{x/s}} \quad 8.28$$

8.1.3 ENDOGENOUS METABOLISM

Since wastewater treatment systems (especially anaerobic systems) are operated at low specific growth rates attention is paid to endogenous respiration. Endogenous respiration is defined as the self-destruction of biomass, cell maintenance, predation, cell death and lysis, and other processes leading to a decrease in cell mass (Pirt, 1975). To account for the effect of these processes on the net growth rate, a decay term is

usually included in the growth rate expression (Lawrence and McCarty, 1970). The rate of bacterial growth (r_x) then takes the following form:

$$r_x = (\mu - k_d)[X] \quad 8.29$$

where: k_d = specific decay rate (h^{-1})

The decay rate, $(\frac{dX}{dt})_{decay}$, is proportional to the number of viable cells present (Pirt, 1979) and is independent of the residual limiting substrate concentration.

Pirt introduced the concept of 'maintenance energy' in the substrate balance to account for the energy used for maintenance of cellular structure (Pirt, 1975). The equation for the rate of substrate utilisation (Eqn 8.28) becomes:

$$r_s = \frac{1}{Y_{X/S}} \mu[X] + m_s[X] \quad 8.30$$

where: $Y_{X/S}$ = yield coefficient of limiting substrate (g bacteria/g substrate)

m_s = maintenance energy (h^{-1})

In this analysis, interpretation of the yield coefficient is important. The observed biomass yield coefficient, calculated on the basis of the measured biomass formation and the measured limiting substrate utilisation is a function of dilution rate, decreasing with decreasing dilution rate (Ginter, 1993). This is attributed to the greater fraction of substrate being consumed for cell maintenance at lower growth rates i.e. less biomass is produced at the longer retention times due to the higher maintenance requirement. The overall observed biomass yield is defined as shown:

$$Y_{X/S(obs)} = \frac{dX/dt}{dS/dt} \quad 8.31$$

where: $Y_{X/S(obs)}$ = observed yield coefficient of limiting substrate (g bacteria/g substrate)

The true or maximum yield coefficient represents the conversion of substrate to biomass only. The value is approached at high growth rate at which the contribution of cell maintenance is low. For continuous systems, the true bacterial yield calculated from the Pirt equation (Eqn 8.30) remains unchanged with changing retention time as the maintenance energy is taken into account during the calculation. Using the Pirt equation and, Eqn 8.31. the true yield coefficient ($Y_{X/S}$) can be related to $Y_{X/S(\text{obs})}$ as shown:

$$Y_{X/S(\text{obs})} = \frac{1}{m_s} + Y_{X/S} \quad 8.32$$

8.1.4 REVIEW OF KINETIC MODELLING OF ANAEROBIC SULPHATE REDUCTION

Several kinetic models have been proposed for anaerobic digesters operated as CSTR systems in which sulphate is present (Vasiliev *et al.*, 1993; Gupta *et al.*, 1994; Vavilin *et al.*, 1995, 1996; Kalyuzhnyi and Fedorovich, 1997, 1998). The processes modelled contain mixed microbial populations including SRB and MPB. Interactions between MPB and SRB have been considered.

Gupta *et al.* (1994) presented a model for anaerobic digestion in which sulphate is present. This model provides a full description of the chemistry of the system including acid-base equilibrium (carbonate), gas phase equilibrium (nitrogen, methane, carbon dioxide, ammonia, hydrogen sulphide and water vapour), charge balances and metal precipitation (sulphide and carbonate precipitates). The description of the biological subsystem is simplistic and accounted for by the Monod model with no inhibition of sulphide. The SRB and MPB may be accounted for as a single group or one of the two microbial groups can be used. The model was calibrated using data from batch spike tests with three organic substrates acetate, methanol and formate as limiting substrate. The model was shown to predict steady state and transient batch spike data for organic removal of the organic source by both MPB and SRB. The removal of sulphate was not included in the model.

A model focussing on ammonia and hydrogen sulphide inhibition during the anaerobic digestion of sulphate containing wastewater was developed by Vavilin *et al.* (1995). The model incorporates a physicochemical reaction subsystem similar to that developed by Gupta *et al.* (1994) consisting of ionic

equilibrium, acid-base equilibria and charge balances. The microbial process for MPB and SRB is described by a modification of the Monod equation to account for pH and sulphide inhibition:

$$\mu = \frac{\mu_m [S] f(\text{pH}) f(\text{H}_2\text{S})}{K_s + [S]} \quad 8.33$$

where: the inhibition terms used were of the following form:

$$f(I, K_2, K_{100}) = \frac{1}{1 + \left(\frac{1}{K}\right)^{\ln 99 / \ln(K_{100} / K_2)}} \quad 8.34$$

where: I = pH of H₂S

K₂ = concentration of I at which the growth rate is decreased twice (kgm⁻³)

K₁₀₀ = concentration of I at which the growth rate is decreased 100 times (kgm⁻³)

The model was calibrated using experimental data from Parkin *et al.* (1990). Parkin *et al.* (1990) investigated the interaction between SRB and MPB in 2l anaerobic continuous stirred tank reactor at feed COD to sulphate ratios ranging between 60:1 and 2:1 using either acetate or propionate as the limiting organic nutrient. The model of Vavilin *et al.* (1994) predicted the experimental data well, simulating the cessation of sulphate reduction and methanogenesis observed when the COD to sulphate ratio was less than 10:1.

The competition between MPB and SRB for acetate under sulphate limitation was studied by Omil *et al.* (1997) in a UASB. They simulated the long-term competition between SRB and MPB for acetate using Monod kinetic parameters. Simulations done by Omil *et al.* (1997) confirmed the experimental data. At pH 7.0 SRB required 600 d to outcompete MPB compared to 150 d when the pH was 8.0. The effect of the initial ratio of SRB to MPB in the seed sludge was also illustrated by the simulation. When the seed sludge contains 10 % SRB, 1000 d are required to degrade 90 % of the acetate compared to 270 d when the seed sludge consists of 90 % SRB.

The models discussed above are applicable to a mixed anaerobic population when a single limiting substrate is present. A model for the competition between SRB and MPB in anaerobic reactors with multiple substrates has been developed by Kalyuzhnyi and Federovich (1998). The approach adopted for

describing the physicochemical subprocess is similar to that of Gupta *et al.* (1994) and Vavilin *et al.* (1995). The physicochemical subprocess is described by liquid phase equilibria and gas phase equilibria. The microbial subprocess is divided into seven groups (fermentative bacteria, propionate degrading acid producers, acetate utilising SRB, acetate utilising MPB, acetate forming SRB, hydrogen utilising SRB and hydrogen utilising MPB) to account for the breakdown of multiple substrates. The growth rate of each microbial group proceeds according to Monod kinetics with inhibition by undissociated hydrogen sulphide. A dual substrate Monod equation is proposed for SRB to account for their growth limitation in sulphate deficient wastewaters. The data was calibrated with experimental data from Alphenaar *et al.* (1993). They investigated the competition between SRB and MPB in a UASB receiving feed containing sulphate, acetate, propionate and sucrose. The model successfully predicted the steady-state performance of the reactor and the increase in the COD converted by SRB relative to MPB from 25 to 80 % as the retention time was increased from 0.29 to 1.68 d.

The models discussed above have been developed to account for anaerobic organic compound removal in continuous bioreactors in the presence of sulphate. The models successfully predict the outcome of competition between MPB and SRB for various organic compounds. In the models discussed the sulphate reduction process was considered in competition to the methanogenic process. Consequently the effect of environmental factors on the growth of the SRB was not considered or modelled. Furthermore, the reactor performance with respect to sulphate conversion was not considered in detail. The subsequent section uses the data obtained from this work to model the effect of feed sulphate concentration and temperature on the performance of the sulphate reduction process.

8.2 MODELLING OF 'STABLE' STATE EXPERIMENTAL DATA

From the results presented in Chapters 6 and 7, it has become apparent that initial sulphate concentration and reaction temperature affect both the sulphate reduction rate and the bacterial growth (Figures 8.9 and 8.10). In order to investigate the effect of initial concentration of sulphate and temperature on the kinetics of bacterial sulphate reduction and microbial growth, the experimental data presented in Chapters 6 and 7, were analysed in terms of various kinetic models. Kinetic coefficients of each model were determined for growth at different conditions. The coefficients were then correlated with the variables of interest (sulphate concentration and temperature). These correlations were incorporated into an overall model

describing the kinetics of sulphate reduction in terms of initial and residual sulphate concentration, bacterial concentration and reaction temperature.

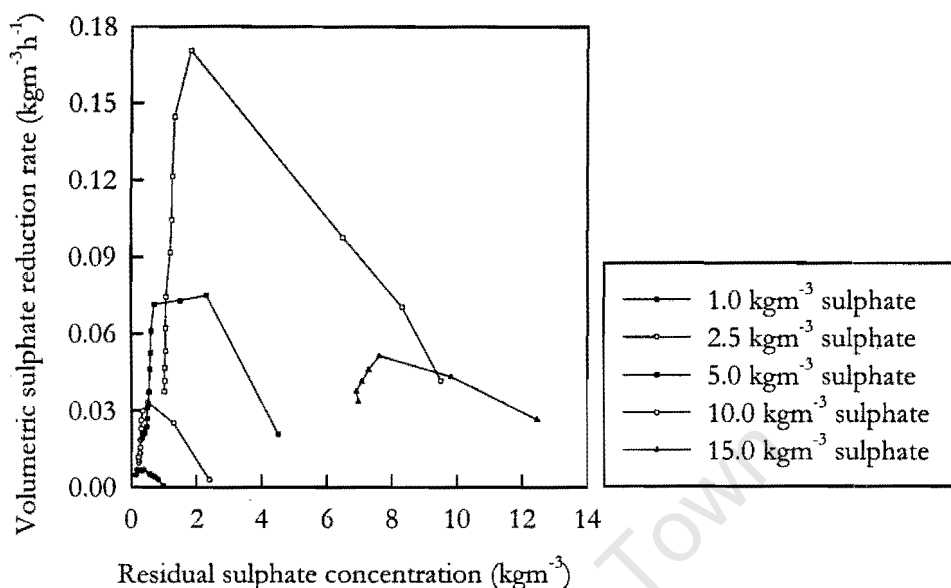


Figure 8.9. Dependency of volumetric sulphate reduction rate on residual sulphate concentration and feed sulphate concentration for feed sulphate concentrations of 1.0 (●), 2.5 (○), 5.0 (■), 10.0 (□) and 15.0 (▲) kgm^{-3} . Data obtained from continuous bioreactors operated at a temperature of 35°C and pH 7.8.

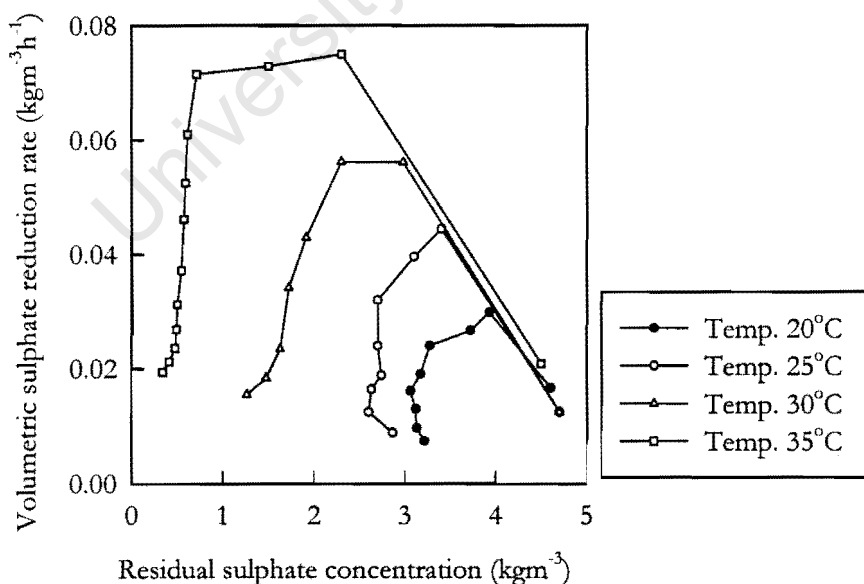


Figure 8.10. Dependency of volumetric sulphate reduction rate on residual sulphate concentration and temperature for temperatures of 20 (●), 25 (○), 30 (■) and 35 (□) °C. Data obtained from continuous bioreactors operated pH 7.8 with feed sulphate and sulphate concentrations of 5.0 and 17.5 kgm^{-3} respectively.

8.2.1 DEVELOPMENT OF A KINETIC MODEL TO DESCRIBE THE DEPENDENCY OF BACTERIAL KINETICS ON INITIAL SULPHATE CONCENTRATION

8.2.1.1 MODELLING r_s AND μ AS A FUNCTION OF RESIDUAL SULPHATE CONCENTRATION

The Pirt equation (Eqn 8.30) forms the basis for the development of a chemostat model to describe the kinetics of biological sulphate reduction and microbial growth.

$$r_s = \frac{1}{Y_{X/S}} \mu[X] + m_s [X] \quad 8.30$$

In Eqn 8.30 the dependency of specific bacterial growth rate, μ , on residual limiting substrate concentration can further be described by a variety of existing models (Tables 8.1 and 8.2). For this work the decay term was incorporated into the definition of μ , the maintenance coefficient was insignificant consequently the calculation of the reduction rate of limiting substrate excludes the maintenance term and becomes:

$$r_s = \frac{1}{Y_{X/S}} (\mu - k_d)[X] \quad 8.35$$

Results presented in Chapter 6 indicate that the anaerobic sulphate reduction process was inhibited when the feed sulphate concentration was 15.0 kgm^{-3} , as a result the 'stable' state data obtained for this experiment will not be used for the model development. The kinetic constants will be calculated to allow for comparison. The 'stable' state data and calculated kinetic constants for the continuous bioreactors receiving 1.0 , 5.0 and 10.0 kgm^{-3} is used to calibrate the model and the 'stable' data obtained for the continuous bioreactor receiving 2.5 kgm^{-3} is used to validate the proposed reaction rate model.

The models tested to describe the dependency of microbial growth on residual limiting substrate concentration include those of Monod (Eqn 8.36), Contois (Eqn 8.37), Chen & Hashimoto (Eqn 8.38).

$$\mu = \frac{\mu_m [S]}{K_s + [S]} - k_d \quad 8.36$$

$$\mu = \frac{\mu_m [S]}{K_s X + [S]} - k_d \quad 8.37$$

$$\mu = \frac{\mu_m [S]}{K[S_0] + (1-K)[S]} - k_d \quad 8.38$$

Using the 'stable' state experimental data obtained for initial sulphate concentrations of 1.0, 2.5, 5.0, 10.0 and 15.0 kgm⁻³ sulphate and a non-linear regression program (Sigmaplot), the values of the kinetic microbial growth coefficients (μ_m , K_s , and k_d) for Monod, Chen and Hashimoto and Contois models with a decay term were determined. The Monod model since it has been used previously to describe the specific microbial growth rate as a function of residual substrate concentration in anaerobic systems (Bailey & Ollis, 1986, Shuler & Kargi, 1987). The Chen and Hashimoto and Contois models were chosen because they have been used to describe specific microbial growth rate as a function of residual substrate concentration at high feed substrate and high biomass concentrations (Chen & Hashimoto, 1980; Contois, 1959).

The kinetic constants for the three models are detailed in Table 8.3. The decay term was independent of feed sulphate concentration hence the average value of 0.035 h⁻¹ was used. The resultant parity charts for the Monod, Chen & Hashimoto and Contois models are shown in Figures 8.11 to 8.13. A parity chart providing comparison of the three models at a feed sulphate concentration of 5.0 kgm⁻³ is shown as Figure 8.14. In all cases the data points are scattered either side of the parity line suggesting fluctuations in data due to experimental error. The parity chart gives a visual indication of the goodness-of-fit of a model by plotting the actual versus the predicted data. A further test of the goodness-of-fit of a chosen model is the coefficient of variance. This is the ratio of the standard deviation to the mean for each particular model. The coefficient of variance (on a percentage basis) obtained for the three models are given in Table 8.4. On examination of the coefficients obtained it is evident that the Contois model fits the data with a higher consistency than either Monod or Chen and Hashimoto models. Therefore the Contois model was used to describe specific microbial growth rate as a function of residual sulphate concentration for further model development. The Contois model has been applied extensively to the substrate utilisation kinetics of biological treatment systems (Chen & Hashimoto, 1980).

Table 8.3. Kinetic constants obtained using Monod, Contois and Chen and Hashimoto kinetic models.

The constants were obtained using a non-linear regression in Sigmaplot. The data was obtained from continuous bioreactors operating at 35°C and pH 7.8.

Feed sulphate concentration (kgm ⁻³)	Monod			Contois			Chen & Hashimoto		
	μ_m	K_s	k_d	μ_m	K_s	k_d	μ_m	K_s	k_d
1.0	0.052	0.043	0.035	0.058	0.027	0.035	0.049	0.041	0.035
2.5	0.063	0.132	0.035	0.061	0.038	0.035	0.061	0.050	0.035
5.0	0.071	0.327	0.035	0.063	0.071	0.035	0.063	0.061	0.035
10.0	0.077	0.853	0.035	0.065	0.125	0.035	0.071	0.079	0.035
15.0	0.051	2.621	0.035	0.050	0.231	0.035	0.049	0.148	0.035

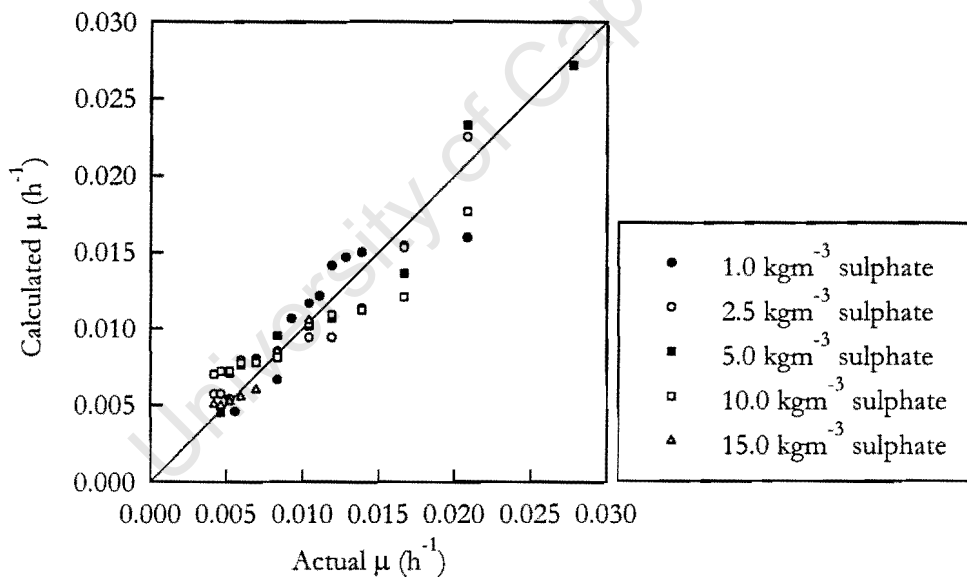


Figure 8.11. Parity chart for the Monod bacterial kinetic model for the continuous bioreactors reducing 1.0 (●), 2.5 (○), 5.0 (■), 10.0 (□) and 15.0 (△) kgm⁻³ sulphate. Data obtained from continuous bioreactors operated at a temperature of 35°C and pH 7.8.

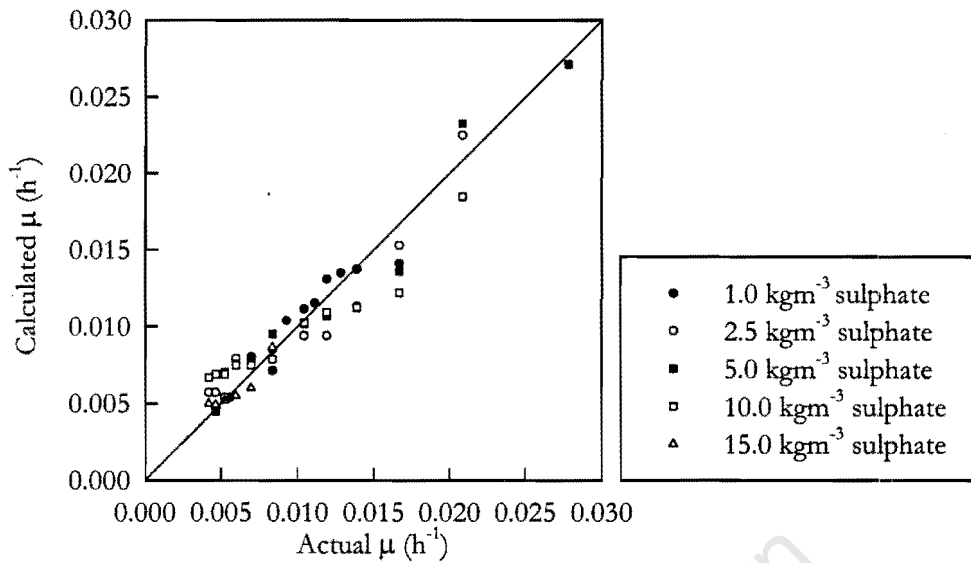


Figure 8.12. Parity chart for the Chen and Hashimoto bacterial kinetic model for continuous bioreactors reducing 1.0 (●), 2.5 (○), 5.0 (■), 10.0 (□) and 15.0 (Δ) kgm^{-3} . Data obtained from continuous bioreactors operated at a temperature of 35°C and pH 7.8.

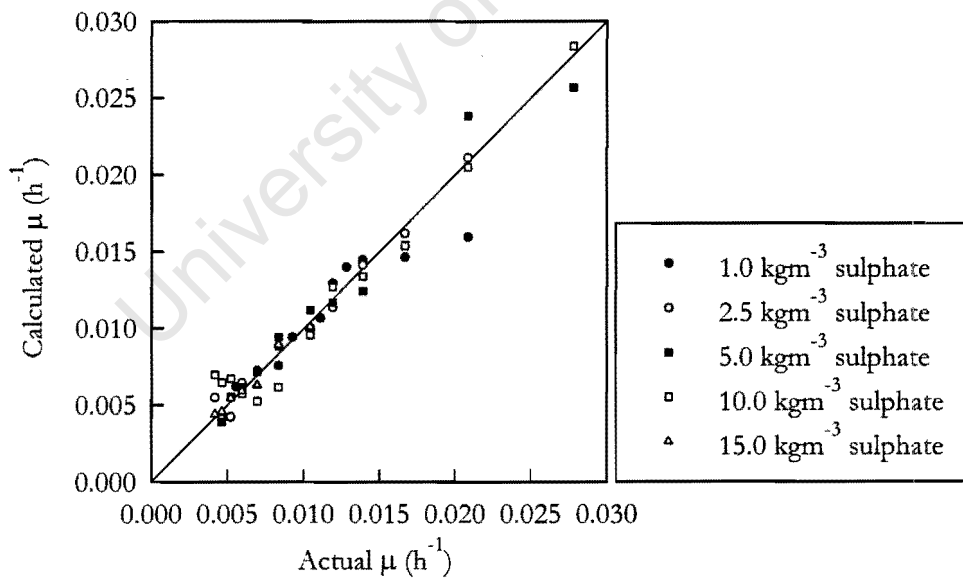


Figure 8.13. Parity chart for the Contois bacterial kinetic model for continuous bioreactors reducing 1.0 (●), 2.5 (○), 5.0 (■), 10.0 (□) and 15.0 (Δ) kgm^{-3} . Data obtained from continuous bioreactors operated at a temperature of 35°C and pH 7.8.

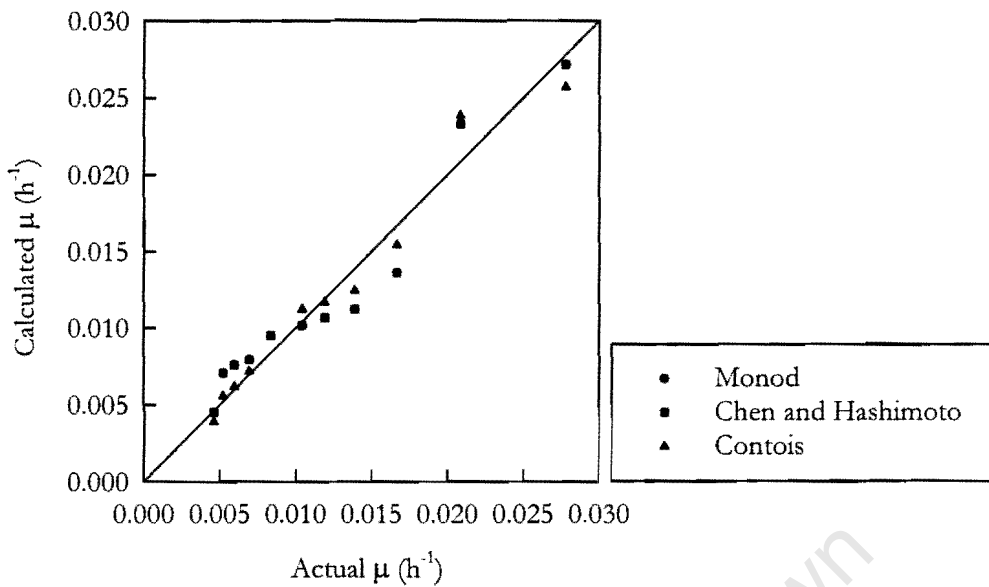


Figure 8.14. Comparison of the Monod (●), Chen and Hashimoto (■) and Contois (▲), and model when the initial sulphate concentration was 5.0 kgm^{-3} . Data obtained from the continuous bioreactor operated at a temperature of 35°C and pH 7.8 with 17.5 kgm^{-3} acetate in the feed

Table 8.4. Coefficient of variance (CV) for the Monod, Chen and Hashimoto and Contois fits for feed sulphate concentrations of 1.0, 2.5, 5.0, 10.0 and 15.0 kgm^{-3} . Data obtained from continuous bioreactors operating at 35°C and pH 7.8.

Feed sulphate concentration (kgm^{-3})	Monod model (CV %)	Chen & Hashimoto model (CV %)	Contois model (CV %)
1.0	18.20	17.79	15.75
2.5	13.85	12.71	7.20
5.0	11.90	11.05	9.03
10.0	11.98	11.52	10.84
15.0	8.35	25.18	19.06

8.2.1.2 DEPENDENCE OF K_s ON FEED SULPHATE CONCENTRATION

From Table 8.3 it is evident that the K_s term in all three models is an increasing function of the inlet sulphate concentration. For the Contois model increasing the initial sulphate concentration from 1.0 to 15 kgm^{-3} resulted in an increase of 83 % in the saturation constant, K_s . The higher K_s values observed at

the higher feed sulphate concentrations indicate that the affinity of the microbes for sulphate is decreased as the feed sulphate concentration is increased. From the definition of K_s , the residual sulphate concentration at which the specific microbial growth rate is half the maximum specific growth rate is an increasing function of feed sulphate concentration. This is in agreement with Chen & Hashimoto (1980). Studying the fermentation of methane using livestock waste they found that as the COD was increased from 0.50 to 1.50 kgm^{-3} , K_s was constant while above a COD of 1.50 kgm^{-3} K_s increased with increasing COD. They attribute the increase in K_s to the system becoming saturated with substrate. In Table 8.5 kinetic parameters reported in the literature are compared with those of this work. It can be seen that the K_s values obtained for this work compare well with those reported in the open literature for the anaerobic reduction of sulphate with acetate as the organic source. Correlation of the K_s values determined as a function the feed sulphate concentration (Eqn 8.39), for feed sulphate concentrations of 1.0, 5.0 and 10 kgm^{-3} , shows a linear dependence (Figure 8.15).

$$K_s = K'_s \cdot [S_0] \quad 8.39$$

where: K'_s = apparent saturation constant (kgm^{-3})

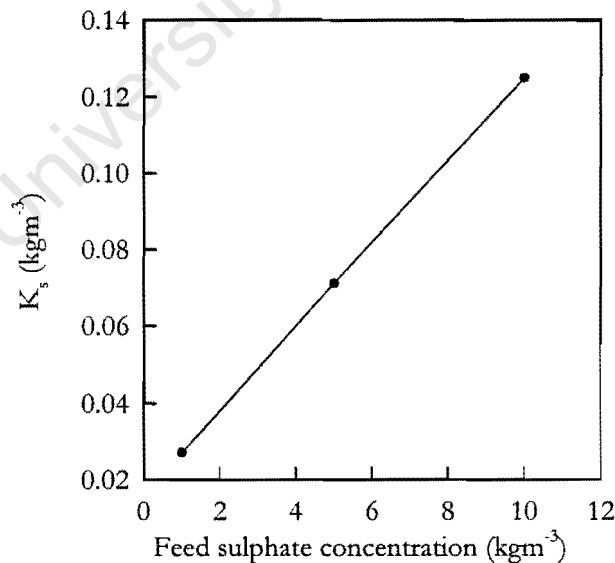


Figure 8.15. Linear dependency of K_s on feed sulphate concentration. Data obtained from continuous bioreactors operating at 35°C and pH 7.8 with feed sulphate concentrations of 1.0, 5.0 and 10.0 kgm^{-3}

Including the dependency of K_s on feed sulphate concentration, the Contois model is modified to the following form:

$$\mu = \frac{\mu_m [S]}{K'_s [S_0] X + [S]} - k_d \quad 8.40$$

8.2.1.3 DEPENDENCE OF μ_m ON FEED SULPHATE CONCENTRATION

The dependence of μ_m of feed sulphate concentration is given in Table 8.3 in terms of the Monod, Contois and Chen and Hashimoto models. For all three kinetic models, μ_m displays a slight increase with inlet sulphate concentration. Using a t-test it was shown that μ_m does not vary significantly as a function of feed sulphate concentration across the range 1.0 to 10.0 kgm⁻³ at the 95% confidence interval. A mean μ_m of 0.061 h⁻¹ with a variance of 4 % was obtained. The constant value for μ_m for feed sulphate concentrations between 1.0 and 10.0 kgm⁻³ is supported by the constant dilution rate (D of 0.005 h⁻¹) at which the maximum biomass concentration occurs (Section 6.5.4). A constant μ_m with increasing feed substrate was also observed by Grady *et al.* (1972), Grady and Williams (1975) and Morris (1976). Grady *et al.* (1972) observed a constant μ_m of 0.512 h⁻¹ with increasing feed COD concentrations from 0.50 to 1.50 kgm⁻³ for a pure batch culture (*Aerobacter aerogenes*) aerobically degrading glucose. When using a mixed culture to degrade glucose in batch, Grady *et al.* (1972) found a constant μ_m of 0.455 h⁻¹ as the feed COD concentration was increased in the range 0.5 to 2.0 kgm⁻³. Studying the degradation of a mixed feed substrate (glucose, fructose, sorbital and lysine) in batch Grady and Williams (1975) found that μ_m remained constant at 0.274 h⁻¹ as the feed COD was increased from 0.50 to 2.00 kgm⁻³. A constant μ_m of 0.467 h⁻¹ was observed by Morris (1976) studying the anaerobic degradation of dairy manure with increasing feed volatile solids concentration in the range 0.035 to 0.088 kgm⁻³. The constant μ_m with increasing feed sulphate concentration in the range 1.0 to 10.0 kgm⁻³ indicates that increasing the feed sulphate in this range has no significant effect on the maximum specific growth rate of the microbial population. This is consistent with Bailey and Ollis (1986) who report that μ_m is not expected to change with increasing substrate concentrations to a certain maximum value after which the system will react

unfavourably due to substrate inhibition. The decline of μ_m by 23 % when the initial sulphate concentration was increased from 10 kgm⁻³ to 15.0 kgm⁻³ can be attributed to sulphate inhibition (Section 6.5.1), which is not accounted for explicitly in these models. The μ_m obtained for this work (Table 8.5) compares favourably with similar constants from the literature for the reduction of sulphate by SRB using acetate as the organic nutrient (Table 8.6). The average μ_m value obtained for this work (0.061 h⁻¹) is similar to the value obtained for a pure culture of *Desulfotomaculum acetoxidans* (Widdel & Pfennig, 1986; O' Flaherty *et al.* 1998).

Substituting for the constant value of μ_m , K_s 's and k_d , shown in Table 8.5, Eqn 8.40 can be rewritten as:

$$\mu = \frac{0.061_m [S]}{0.015[S_o] + [X] + [S]} - 0.035 \quad 8.41$$

Table 8.5. Kinetic constants obtained for the continuous conversion of sulphide used in proposed Contois model. Data obtained from continuous bioreactors operating at 35°C and pH 7.8 with feed sulphate concentrations of 1.0, 5.0 and 10.0 kgm⁻³.

μ_m (h ⁻¹)	0.061
K_s 's (kgm ⁻³)	0.015
k_d (h ⁻¹)	0.035

Table 8.6. Comparison of kinetic parameters for sulphate reduction, using acetate as the organic substrate.

Reference	Culture	Temp (°C)	Reactor	Conditions	μ_m (h ⁻¹)	K_s (kgm ⁻³)
Middleton & Lawrence (1977) ¹	Mixed SRB	31	STR	C, CL	0.013	-
Maillacheruvu & Parkin (1996)	Mixed SRB		SF	B, CL	0.024	-
Widdel & Pfennig (1977) ¹	<i>Desulfotomaculum</i> <i>acetoxidans</i>	30	SF	B, CL	0.027 - 0.068	-
Widdel (1987) ¹	<i>Desulfobacter</i> <i>hydrogenophilus</i>	30	SF	B, CL	0.038	-
Widdel (1987) ¹	<i>Desulfobacter</i> <i>curbatus</i>				0.033	-
Visser (1995) ¹	Mixed SRB and MPB (granular)	30	UASB	C	0.005	0.033
	Mixed SRB and MPB (suspended)	30	UASB	C	0.002	0.018
Ingvorsen <i>et al.</i> (1984) ¹	<i>Desulfobacter</i> <i>postgatei</i>	30	STR	C, SL	0.013	0.024
	<i>Desulfobacter</i> <i>postgatei</i>	30	STR	C, CL	0.013	
O' Flaherty <i>et al.</i> (1998) ¹	<i>Desulfotomaculum</i> <i>acetoxidans</i>	35	STR	B, CL	0.063	
This work 1.0 kg m ⁻³ SO ₄ ²⁻	Mixed SRB	35	STR	C, SL	0.058	0.027
This work 2.5 kg m ⁻³ SO ₄ ²⁻	Mixed SRB	35	STR	C, SL	0.061	0.038
This work 5.0 kg m ⁻³ SO ₄ ²⁻	Mixed SRB	35	STR	C, SL	0.063	0.071
This work 10.0 kg m ⁻³ SO ₄ ²⁻	Mixed SRB	35	STR	C, SL	0.065	0.125
This work 15.0 kg m ⁻³ SO ₄ ²⁻	Mixed SRB	35	STR	C, SL	0.050	0.231

Reactor types are: STR stirred tank reactor, SF shake flask UASB upflow anaerobic sludge bed

Conditions are: B batch, C continuous, CL carbon limited, SL sulphate limited

¹ The Monod equation was used to determine the kinetic parameters.

For this work the Contois equation was used to determine the kinetic parameters.

8.2.1.4 DEPENDENCE OF YIELD COEFFICIENT ON FEED SUBSTRATE CONCENTRATION

Since endogenous metabolism is accounted for in the specific growth rate expression, the rate of sulphate utilisation can be represented by the following equation where $Y_{x/s}$ is a constant value ($Y_{x/s}^{\max}$):

$$\frac{r_s}{[X]} = \frac{1}{Y_{x/s}} \mu \quad 8.42$$

The reciprocal of the slope of specific sulphate reduction rate as a function of specific microbial growth rate represents $Y_{x/s}$. $Y_{x/acetate}$ was calculated in a similar manner based on the acetate utilised. The r^2 value for the determination of $Y_{x/s}$ ranged between 0.87 and 0.92 and for the determination of $Y_{x/acetate}$ between 0.89 and 0.93. The values of $Y_{x/s}$ and $Y_{x/acetate}$ are presented in Table 8.7. Both $Y_{x/s}$ and $Y_{x/acetate}$ did not differ significantly as a function of sulphate concentration in the range 1.0 to 10.0 kgm⁻³ at the 99 % confidence interval (t test, calculated variance of 1 % for $Y_{x/s}$ and 1.3 % for $Y_{x/acetate}$)

Data for cell yield based on sulphate are not readily available in the literature (Table 8.8). Ingvorsen *et al.* (1984) determined $Y_{x/s}$ of *Desulfobacter postgatei* grown in batch to be 0.158 kg bacteria (kg sulphate)⁻¹. There is a large variation in yield coefficients based on acetate, $Y_{x/acetate}$, reported in the literature (Table 8.8). Middleton and Lawrence (1977) observed that the overall yield for a mixed SRB culture grown on acetate-sulphate medium in a batch system was 0.065 kg bacteria (kg acetate)⁻¹. Widdel and Pfennig (1981) reported yield coefficients for *Desulfobacter postgatei* and *Desulfotomaculum acetoxidans* to be 0.074 and 0.095 kg bacteria (kg acetate)⁻¹ respectively. Employing a mixed anaerobic culture in a continuous system Visser (1995) calculated a yield coefficient of 0.043 kg bacteria (kg acetate)⁻¹. Growing *Desulfotomaculum acetoxidans* and *Desulfonema magnum* in a batch system, O'Flaherty *et al.*, (1998) reported the yield coefficients to be 0.114 kg and 0.139 bacteria (kg acetate)⁻¹ respectively. The yield coefficient determined in this work are 4 times greater than $Y_{x/s}$ reported in literature (Ingvorsen *et al.* (1984) and between 4 and 13 times higher than $Y_{x/acetate}$ reported. It can be hypothesised that the dry weight method used for biomass determination in this work may overestimate the biomass phase and $Y_{x/s}$ and $Y_{x/acetate}$.

Tables 8.7. Dependency of yield coefficients of microbial growth based on sulphate ($Y_{X/S}$) and acetate ($Y_{X/acetate}$) based on feed sulphate concentration. Data obtained from continuous bioreactors operating at 35°C and pH 7.8.

Inlet sulphate (kg m ⁻³)	$Y_{X/S}$ (kg bacteria (kg sulphate) ⁻¹)	$Y_{X/acetate}$ (kg bacteria (kg acetate) ⁻¹)
1.0	0.584	0.575
2.5	0.573	0.561
5.0	0.567	0.580
10.0	0.569	0.572
15.0	0.325	0.331

Table 8.8. Comparison of yield coefficients for sulphate reduction, using acetate as the organic substrate.

Reference	Culture	Temp (°C)	Reactor	Conditions	$Y_{X/Acetate}$ kg bacteria (kg acetate) ⁻¹	$Y_{X/S}$ kg bacteria (kg sulphate) ⁻¹
Middleton & Lawrence (1977)	Mixed SRB	30	STR	B, CL	0.065	-
Widdel and Pfennig (1981)	<i>Desulfobacter postgatei</i>		SF	B, CL	0.074	-
	<i>Desulfotomaculum acetoxidans</i>		SF	B, CL	0.095	-
Ingvorsen <i>et al.</i> , (1984)	<i>Desulfobacter postgatei</i>		SF	B, SL	-	0.158
Visser (1995)	Mixed SRB and MPB (granular)	30	UASB	C, CL	0.043	-
O'Flaherty <i>et al.</i> (1998)	<i>Desulfotomaculum acetoxidans</i>	35	SF	B, CL	0.114	
	<i>Desulfonema magnum</i>	35	SF	B, CL	0.139	
This work	Mixed SRB	35	STR	C, SL	0.572	0.573

Reactor types are: STR stirred tank reactor, SF shake flask UASB upflow anaerobic sludge bed

Conditions are: B batch, C continuous, CL carbon limited, SL sulphate limited

8.2.1.5 DERIVED MODEL

Using the modified Contois growth rate model and the value obtained for $Y_{x/s}$, the rate of sulphate reduction can be represented by Eqn 8.43. The rate of biological sulphate reduction is expressed as a function of the inlet sulphate concentration, residual sulphate concentration and the bacterial concentration in the reactor.

$$r_s = \left(\frac{0.061[S]}{0.015[S_0 - [X]] + [S]} - 0.035 \right) \frac{[X]}{0.574} \quad 8.43$$

8.2.1.6 ERROR ESTIMATION

The overall error of the model prediction can be determined for individual values of the rate of sulphate utilisation by applying the propagation of error concept. This involves taking the differential of r_s with respect to each variable or constant that contributes to the error. It is applicable where the variables are independent. Considering that:

$$r_s = \frac{\mu[X]}{Y_{x/s}}$$

the contribution to the overall error arises from the error in regressing the experimental data to ascertain $Y_{x/s}$ and μ and the experimental error arising from the determination of $[X]$ and $[S]$

Considering all the contributory factors, the overall error can be estimated by the following expression:

$$\sigma_{r_s}^2 = \left(\frac{[X]}{Y_{x/s}} \right)^2 \sigma_{\mu}^2 + \left(\frac{\mu}{Y_{x/s}} \right)^2 \sigma_x^2 + \left(\frac{-\mu[X]}{Y_{x/s}^2} \right) \sigma_{Y_{x/s}}^2 \quad 8.44$$

where: σ = error

Using the equation for r_s shown above the percentage overall error is given by:

$$\frac{\sigma_{r_s}}{\bar{r}_s} = \sqrt{\frac{\sigma_{\mu}^2}{\bar{\mu}} + \frac{\sigma_x^2}{[\bar{X}]} + \frac{\sigma_{Y_{x/s}}^2}{\bar{Y}_{x/s}}} \quad 8.45$$

Table 8.9 summarises the percentage errors obtained for the variables that occur in the rate equation (Eqn 8.35). The percentage error in μ and $Y_{x/s}$ results from error fitting the experimental data to the Contois model and Pirt equation respectively.

Table 8.9. Percentage errors for the model prediction of r_s . Data obtained from chemostats operating at 35°C and pH 7.8 with feed sulphate concentrations of 1.0, 2.5, 5.0 and 10.0 kg m⁻³.

Sulphate (kgm ⁻³)	Percentage error				
	μ	$Y_{x/s}$	[X]	[S]	r_s
1.0	16	9	15	12	24
2.5	7	9	15	12	19
5.0	9	9	15	12	20
10.0	11	9	15	12	21

To validate the propagation of error used for the model prediction of r_s , the error estimation in the model is included in the parity chart. If the experimental data lie within the error lines, the model predicts the data to within the defined error. The parity chart for experiment conducted at an initial sulphate concentration of 2.5 kg m⁻³ (data used for model validation) and is shown as Figure 8.13. A parity chart for experiments conducted at initial sulphate concentrations of 1.0, 2.5, 5.0 and 10.0 kg m⁻³ is shown as Figure 8.14. Based on the errors reported in table 8.9, a 24 % error was used. It can be seen that the calculated reduction rate points (y axis) lie within the error defined on the parity line. Hence the model predicts the data well, to within the limits allowed by the error propagation. Additionally, from χ^2 tests which are used to compare two set of data for significant differences or similarities depending on the hypothesis (χ^2 is the sum of the ratio of the squares of the difference between the actual and predicted value and the predicted value, $\sum \frac{(\text{Actual}-\text{Predicted})^2}{\text{Predicted}}$) for the four sulphate concentrations it was found that the model was significant within the 95% confidence level (χ^2 less than 1.43).

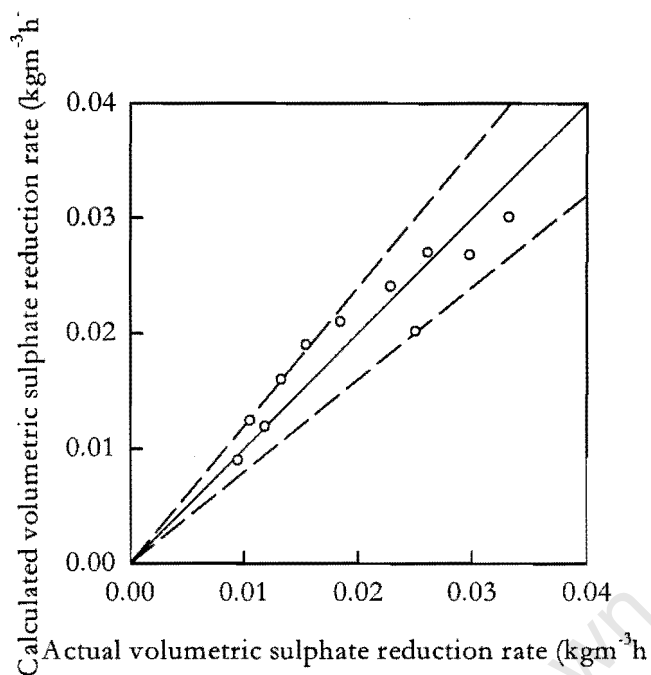


Figure 8.16. Parity chart for volumetric sulphate reduction rates for the chemostat reducing 2.5 kgm^{-3} sulphate. (—) represents parity line and (---) represents 20 % error line. Data obtained from chemostat operating at 35°C and pH 7.8.

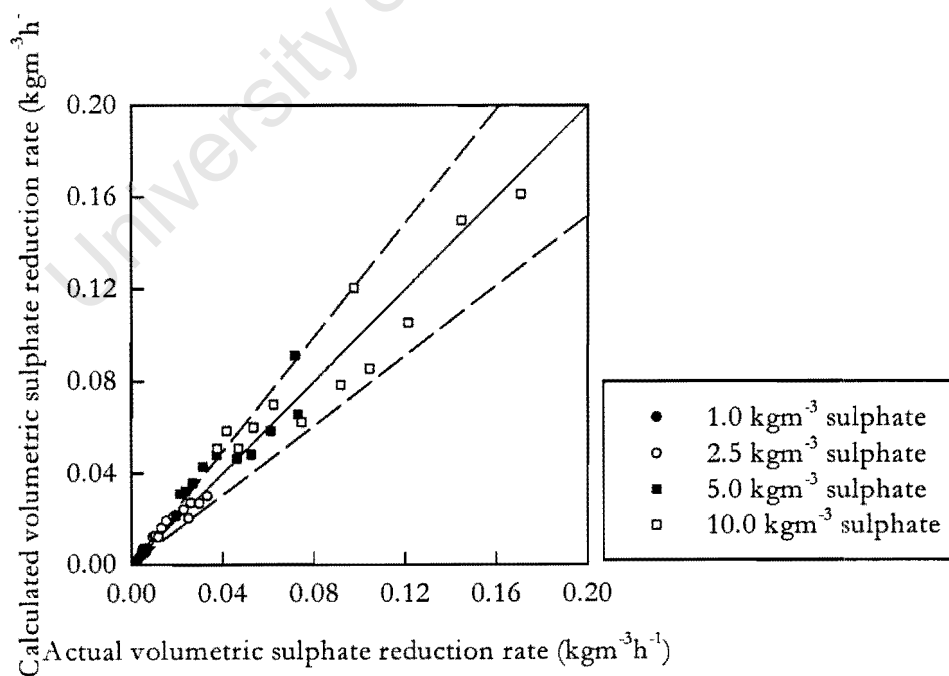


Figure 8.17. Parity chart for volumetric sulphate reduction rates for the chemostat reducing 1.0 (\bullet), 2.5 (\circ), 5.0 (\blacksquare) and 10.0 (\square) kgm^{-3} sulphate. (—) represents parity line and (---) represents 24 % error line. Data obtained from chemostat operating at 35°C and pH 7.8.

8.2.2 EXTENSION OF THE KINETIC MODEL TO DESCRIBE THE DEPENDENCY OF BACTERIAL KINETICS ON INITIAL SULPHATE CONCENTRATION AND REACTION TEMPERATURE

In order to incorporate the effect of temperature into the derived model (Eqn 8.43) the experimental data were fitted to Eqn 8.12, the Contois equation, and the values of maximum specific growth rate (μ_{max}), the saturation constant (K_s) and the decay coefficient (k_d) were calculated for the four temperatures investigated in this work. These are given in Table 8.10. The coefficient of variance obtained is given in Table 8.11 and the parity charts is presented in Figure 8.15.

Table 8.10. Dependency of kinetic constants on temperature in the range 20 to 35°C. Data obtained from continuous bioreactors operating at pH 7.8 with feed sulphate and acetate concentrations of 5.0 and 17.5 gm⁻³ respectively.

Temperature (°C)	μ_m (h ⁻¹)	K_s (kgm ⁻³)	k_d (h ⁻¹)
20	0.062	4.746	0.008
25	0.060	1.467	0.022
30	0.059	0.613	0.025
35	0.063	0.071	0.035

Table 8.11. Dependency of coefficient of variance (CV) on temperature in the range 20 to 35°C for the prediction of μ using Eqn 8.12 .

Temperature (°C)	20	25	30	35
CV (%)	14.578	13.269	9.153	10.840

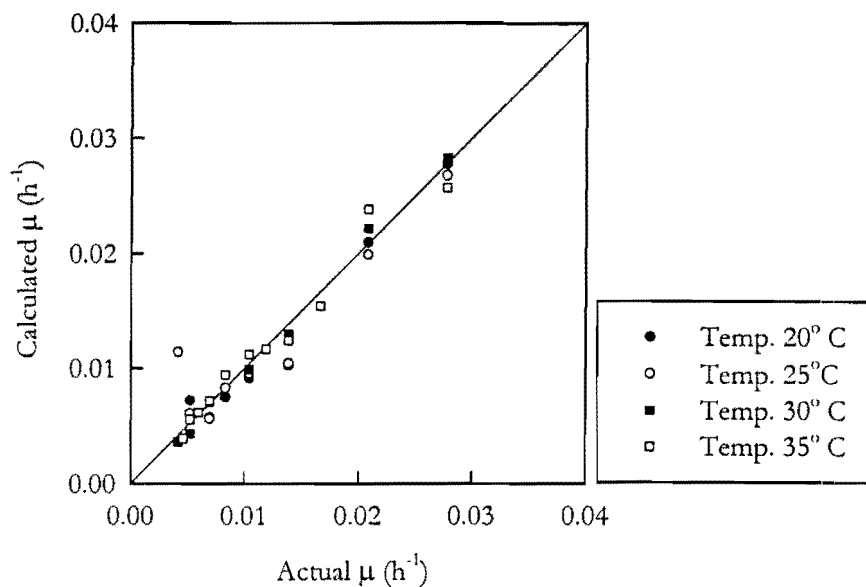


Figure 8.18. Parity chart for the Contois model for the continuous reduction of sulphate at reaction temperatures of 20 (\bullet), 25 (\circ), 30 (\blacksquare) and 35 (\square) $^{\circ}\text{C}$. Data obtained from the continuous bioreactor operated at pH 7.8 with feed sulphate and acetate concentrations of 5.0 and 17.5 kgm^{-3} respectively.

As can be seen in Table 8.11, an increase in temperature results in a decrease in K_s . While a decrease in K_s represents enhanced affinity of the bacterial enzymatic system for the substrate (sulphate), the decrease in K_s may also be explained by enhanced mass transfer with increasing temperature. Information in the literature regarding the effect of temperature on K_s for SRB is inconsistent. A decrease in K_s (acetate) from 0.250 to 0.006 kgm^{-3} with increasing temperature in the range 20 to 31 $^{\circ}\text{C}$ was observed by Middleton and Lawrence (1977) while studying the continuous reduction of sulphate in a chemostat with acetate as the limiting organic nutrient. Using data from a batch system, Characklis *et al.* (1989) reported an increase in K_s with an increase in temperature. However, analysing data from a continuous system, they report a decrease K_s with increasing temperature. Okabe and Characklis (1992) conducting chemostat studies with lactate as the limiting carbon source noted that K_s for *Desulfobrio desulfuricans* remained relatively constant as the temperature was increased from 25 to 35 $^{\circ}\text{C}$. Morris (1976), studying the batch anaerobic degradation of dairy manure, showed that K_s decreased from 1.02 to 2.03 kgm^{-3} as the temperature was increased from 20 to 32.5 $^{\circ}\text{C}$. For the methanogenic process using acetate, K_s has been shown to be sensitive to temperature. Lawrence and McCarty (1969) developed the following Arrhenius

equation to describe the relation between temperature and K_s for general anaerobic digestion (Speece, 1996):

$$\log \frac{(K_s)_2}{(K_s)_1} = 6980 \left(\frac{1}{T_2} - \frac{1}{T_1} \right) \quad 8.46$$

where:

$(K_s)_1$	=	Saturation constant at temperature 1 (kgm^{-3})
$(K_s)_2$	=	Saturation constant at temperature 2 (kgm^{-3})
T_1	=	Temperature 1 (K)
T_2	=	Temperature 2 (K)

Lin *et al.* (1987) proposed the following equation for the temperature dependence of K_s for the methanogenesis of volatile fatty acids across the range 15 to 35°C (Speece, 1996):

$$K_s = 230(0.939)^{(T-25)} \quad 8.47$$

For this study the dependency of K_s on temperature can be described by an Arrhenius type equation:

$$K_s = K_0 e^{-E_a/RT} \quad 8.48$$

where:

E_a	=	psuedo-activation energy, kJ mol^{-1}
R	=	universal gas constant, $\text{kJ K}^{-1} \text{mol}^{-1}$
K_0	=	constant, $\text{kg substrate m}^{-3}$
T	=	absolute temperature, K

The 'Arrhenius type' dependence of the saturation constant on temperature is shown in Figure 8.19 for temperatures of 20, 30 and 35°C (r^2 of 0.96). The values of K_0 and E_a determined were $3.26 \times 10^{-35} \text{ kgm}^{-3}$ and $-198 \text{ kJ K}^{-1} \text{mol}^{-1}$ respectively. It should be noted that the calculation of the coefficients was performed using three sets of experimental data (20, 30 and 35°C). The experimental data obtained at 25°C was used to verify the validity of the model.

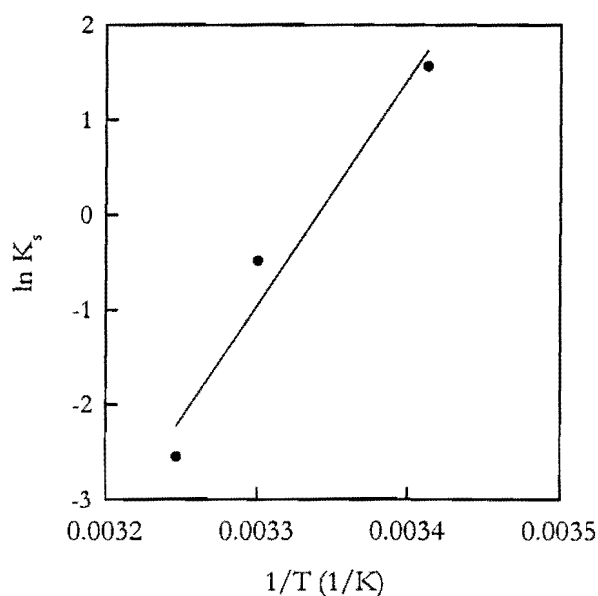


Figure 8.19. Arrhenius plot for the dependency of K_s on temperature for the continuous reduction of sulphate at reaction temperatures of 20, 30 and 35°C. Data obtained from the continuous bioreactor operated at pH 7.8 with feed sulphate and acetate concentrations of 5.0 and 17.5 kgm⁻³ respectively.

μ_m , $0.061 \pm 0.001 \text{ h}^{-1}$, was found to be constant at the 99 % significance level from a t-test. This indicates that the maximum specific growth rate of SRB was not influenced by temperature in the range 20 to 35°C. The values obtained are in agreement with those obtained for the feed sulphate concentration experiments in this study and with those reported in literature (Table 8.5). The maximum specific growth rate of *Desulfotomaculum acetoxidans* and *Desulfobacter postgatei* are reported as 0.058 h^{-1} and 0.038 h^{-1} respectively (Ingvorsen *et. al.*, 1984, Widdel and Pfennig, 1981). Middleton and Lawrence (1977) a μ_m of 0.022 h^{-1} for a mixed continuous culture of SRB, grown in acetate-sulphate medium.

The denaturation of proteins and enzymes in a microbial system is highly temperature dependent (Roëls, 1983). As can be seen in Table 8.10, the decay coefficient, k_d , increased with increasing in temperature. Lawrence and Middleton (1977) observed a similar trend for the reduction of sulphate using a mixed anaerobic culture. In a review presented by Speece (1996) k_d has been shown to increase with increasing temperatures for methanogenesis. The dependency of k_d on temperature can also be described by an

Arrhenius function. In Figure 8.20, the Arrhenius behaviour is illustrated for 20, 30 and 35°C (r^2 of 0.99). An Arrhenius constant and activation energy of $8.8 \times 10^{11} \text{ kgm}^{-3}$ and $78.7 \text{ kJ K}^{-1} \text{ mol}^{-1}$ were determined.

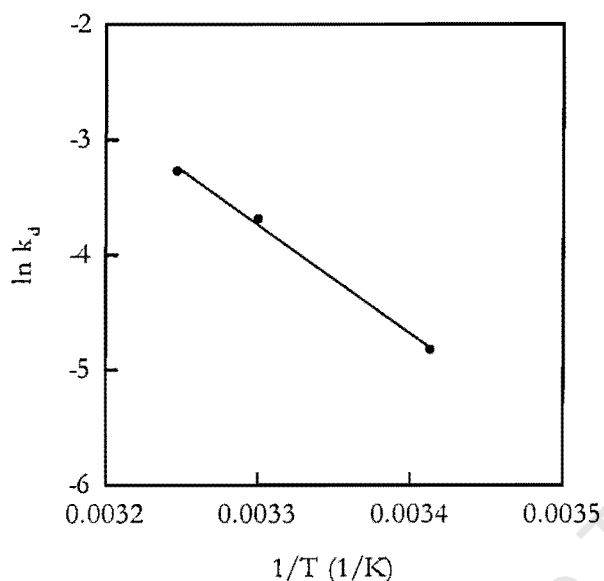


Figure 8.20. Arrhenius plot for the dependency of k_d on temperature for the continuous reduction of sulphate at reaction temperatures of 20, 30 and 35°C. Data obtained from the continuous bioreactor operated at pH 7.8 with feed sulphate and acetate concentrations of 5.0 and 17.5 kgm^{-3} respectively.

The yield coefficients were calculated according to Section 8.2.1.4 and are presented in Table 8.12. $Y_{x/s}$ and $Y_{x/\text{acetate}}$ remained constant at the 99 % significance level (t-test, $Y_{x/s}$ had a variance of 1.2 % and $Y_{x/\text{acetate}}$ a variance of 1.4 %) over the range of applied temperatures. The $Y_{x/s}$ for the temperature experiments did not differ at the 97 % significance level (t-test) at to that obtained for the sulphate experiments. Consequently, an average $Y_{x/s}$ of 0.568 ± 0.0087 was used in the model. Using a pure culture of *Desulfobacter desulfuricans*, Sanez (1962 in Okabe and Characklis, 1992) and Okabe and Characklis (1992) concluded that temperature had no effect on yield coefficient calculated in terms of organic substrate. The values reported in literature for $Y_{x/\text{acetate}}$ range between 0.065 and 0.141 $\text{kg bacteria (kg acetate)}^{-1}$. For $Y_{x/s}$ a value of 0.158 $\text{kg bacteria (kg sulphate)}^{-1}$ is reported (Middelton & Lawrence, 1977; Widdel & Pfennig, 1981; Visser, 1995; O' Flaherty *et al.*, 1998; Ingvorsen *et al.*, 1984).

Table 8.12. Dependency of yield coefficients on temperatures in the range 20 to 35°C. Data obtained from continuous bioreactors operating at pH 7.8 with feed sulphate and acetate concentrations of 5.0 and 17.5 kgm⁻³ respectively.

Temperature (°C)	$Y_{x/s}$ (kg bacteria (kg sulphate) ⁻¹)	$Y_{x/acetate}$ (kg bacteria (kg acetate) ⁻¹)
20	0.554	0.620
25	0.561	0.600
30	0.570	0.600
35	0.567	0.580

8.2.3 MODEL DEVELOPMENT WITH RESPECT TO TEMPERATURE

The dependency of volumetric reduction rate of sulphate on bacterial concentration and residual sulphate concentration can be represented by Eqn 8.20. Dependence of the parameters K_s and k_d on temperature has been illustrated, while μ_m and $Y_{x/s}$ appear independent of temperature.

K_s can be substituted according to Eqn 8.49 to describe dependency on both temperature (T) and initial sulphate concentration (S_0).

$$K_s = 6.52 \times 10^{-36} e^{198/RT} [S_0] \quad 8.49$$

Similarly k_d can be substituted according to Eqn 8.50 to include the Arrhenius dependence on T .

$$k_d = 8.8 \times 10^{11} e^{-787/RT} \quad 8.50$$

The resultant model for the volumetric sulphate reduction rate is:

$$r_s = \left(\frac{0.061[S]}{6.52 \times 10^{-35} e^{198/RT} [S_0][X] + [S]} - 8.8 \times 10^{11} e^{-787/RT} \right) \frac{[X]}{0.568} \quad 8.51$$

Simplification of this equation yields:

$$r_s = \frac{[S][X](0.107 - 1.530 \times 10^{12} e^{-9467/T}) - [X]^2 [S_0](9.97 \times 10^{-24} e^{14305/T})}{(6.52 \times 10^{-36} e^{23772/T} [X][S_0] + [S])} \quad 8.52$$

Using Eqn 8.52 as the governing expression for the kinetics of anaerobic reduction of sulphate and writing a mass balance, the performance of the continuous bioreactor, operating at different temperatures was assessed. The experimental error in the model prediction was calculated as outlined in Section 8.3.1.1 and is presented as Table 8.13. It should be noted that data from experiments at 20, 30 and 35°C were used to calculate the coefficients of model and data at 25°C was used to evaluate the model. The parity chart for the validation of the model (25°C) is given as Figure 8.21. The parity chart for the range of reaction temperatures (20, 25, 30 and 35°C) and a feed sulphate concentration of 5.0 kgm⁻³ is shown as Figure 8.22. For this plot an overall error of 24 % (Table 8.15) was used. The resultant parity chart, when using the overall model Eqn 8.46 to predict the volumetric reduction rate as a function of initial sulphate concentration, at a temperature of 35°C is shown as Figure 8.23. Visually there is good agreement between the actual and calculated reduction rate. The model is significant at the 95 % confidence limit (chi² test).

Table 8.13. Percentage error for the prediction of r_s . Data obtained from continuous bioreactors operating at pH 7.8 with feed sulphate and acetate concentrations of 5.0 and 17.5 kgm⁻³ respectively.

Temperature (°C)	Percentage Error				
	μ	$Y_{x/s}$	[X]	[S]	r_s
20	14	9	15	12	25
25	13	9	15	12	24
30	9	9	15	12	23
35	9	9	15	12	23

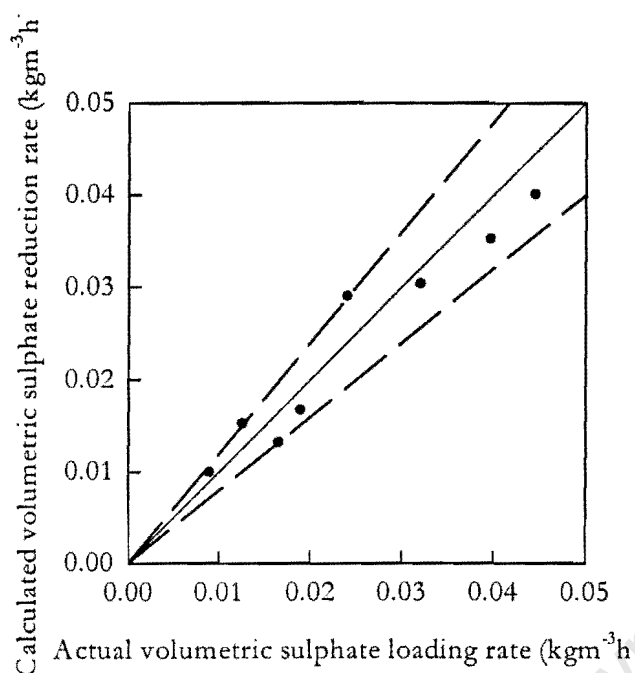


Figure 8.21. Parity chart for volumetric reduction rate for continuous reduction of sulphate at a reaction temperature of 25°C. (—) represents parity line and (---) represents 24 % error line. Data obtained from chemostat operating at pH 7.8 with feed sulphate and acetate concentrations of 5.0 and 17.5 kgm⁻³ respectively.

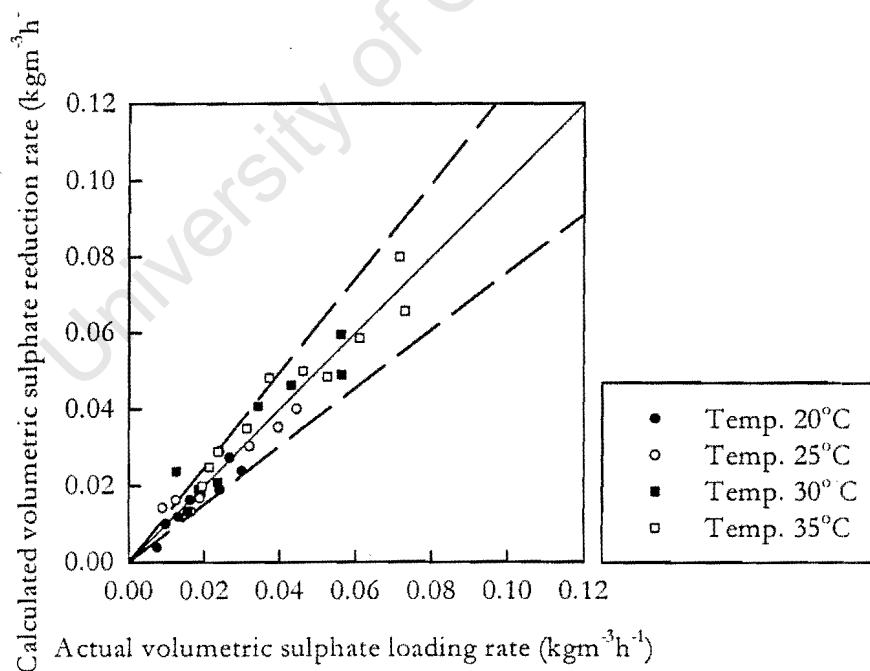


Figure 8.22. Parity chart for volumetric reduction rate for continuous reduction of sulphate at reaction temperatures of 20 (●), 25 (○), 30 (■) and 35 (□)°C. (—) represents parity line and (---) represents 24 % error line. Data obtained from chemostat operating at pH 7.8 with feed sulphate and acetate concentrations of 5.0 and 17.5 kgm⁻³ respectively.

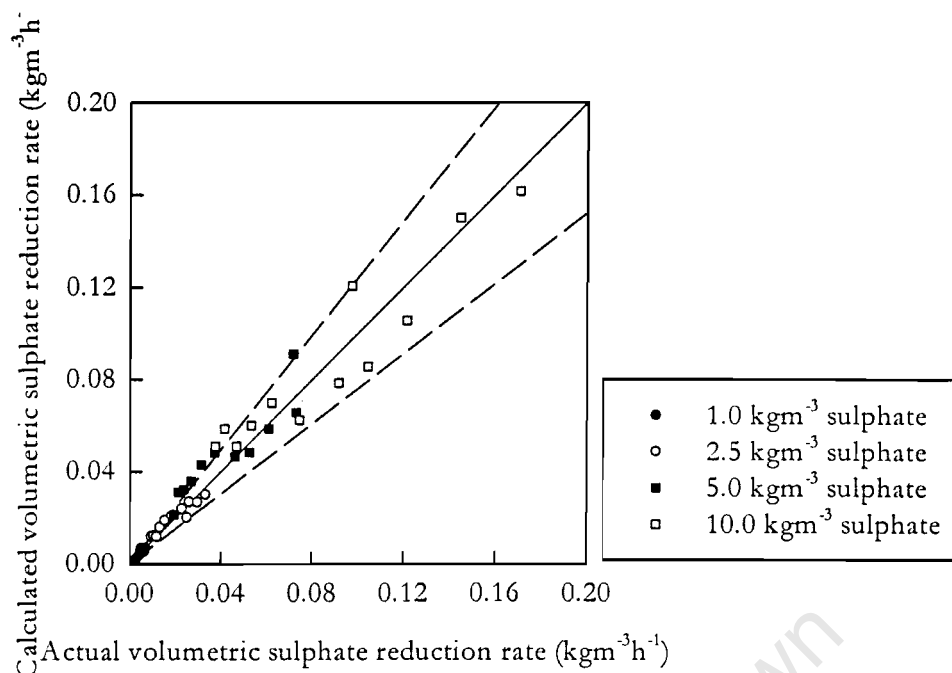


Figure 8.23. Parity chart for volumetric reduction rate for continuous reduction of 1.0 (●), 2.5 (○), 5.0 (■) and 10.0 (□) kg m⁻³ sulphate. (—) represents parity line and (---) represents 20 % error line. Data obtained from chemostat operating at 35°C and pH 7.8.

8.3 CHAPTER SUMMARY

In this chapter, it has been shown that the initial sulphate concentration and reaction temperature has a significant effect on the kinetics of sulphate reduction and microbial growth. This effect was manifested by enhanced reduction rates as the sulphate concentration was increased from 1.0 to 10.0 kg m⁻³ and as the temperature was increased from 20 to 35°C. From batch studies it was shown that further increase of the feed sulphate and reaction temperature had an inhibitory effect on the microorganisms.

The effect of sulphate concentration and reaction temperature on the bacterial growth rate was modelled using a modified Contois model using a decay coefficient to account for endogenous respiration. The maximum specific growth rate remained constant at 0.061 h⁻¹ across the temperature and initial sulphate concentration range studied. The decay coefficient was a function of an Arrhenius function of temperature according to:

$$k_d = 8.8 \times 10^{11} e^{-787/RT}$$

The saturation constant displayed a linear increase with increasing inlet sulphate concentration and an Arrhenius dependence on temperature. This could be represented by the following relation:

$$K_s = 6.52 \times 10^{-36} e^{198/RT} [S_o]$$

Using experimental data a model for the rate of sulphate reduction as a function of inlet sulphate concentration, temperature, bacterial concentration and residual sulphate concentration was developed. The model was shown to represent independent data sets to within the defined experimental error of 20 to 24 %.

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CHAPTER 9

CONCLUSIONS AND RECOMMENDATIONS

The anaerobic sulphate reduction process has shown potential for the treatment of sulphate containing effluents. An example pertinent in the South African context is acid mine drainage (AMD). In this thesis, the kinetics of anaerobic sulphate reduction has been studied with particular reference to the effect of feed sulphate concentration and temperature on the kinetics of the biological process. A mixed anaerobic microbial population enriched for sulphate reducing bacteria (SRB) was studied. Acetate was used as the carbon source and electron donor to support growth in continuous bioreactors under various conditions and the kinetics of the process was ascertained from the 'stable' state data. The results from this study find application in the design of anaerobic sulphate reduction treatment systems. While the treatment of various sulphate and organic containing streams has been studied extensively, the availability of rigorous kinetic data on the biological sulphate reduction process is limited. This work provides rigorous kinetic data for anaerobic sulphate reduction, including the effect of feed sulphate concentration and temperature. A kinetic model of the process is presented. The major findings from this investigation are

summarised in this chapter. Thereafter areas in which additional research may yield fruitful results on the improvement of anaerobic sulphate reduction process to ultimately inform the design of a full-scale plant are discussed.

9.1 CONCLUSIONS

9.1.1 EFFECTS OF ENVIRONMENTAL CONDITIONS ON ANAEROBIC SULPHATE REDUCTION

The effects of three physical parameters: pH, temperature and initial sulphate concentration on the anaerobic reduction of sulphate was studied in a batch system. A mixed anaerobic population comprising SRB, MPB and APB was employed. Glucose was used as the organic source. The results confirmed the importance of reaction pH, temperature and feed sulphate concentration on the sulphate removal and glucose utilisation capacity of the microbial culture. Based on the results of this study, the optimum environmental conditions for sulphate removal were a pH of 8.0, temperature of 35°C and a feed sulphate concentration in the range 1.0 to 2.5 kgm⁻³. The APB, utilising glucose to provide the carbon source for the SRB and MPB showed a pH optimum of 7.5 to 8.0, a temperature optimum of 25 to 35°C and sulphate inhibition at a feed sulphate concentration of 5.0 kgm⁻³. These results served to inform further experiments to ascertain the effect of feed sulphate concentration and temperature on the kinetics of microbial sulphate reduction.

9.1.2 EFFECT OF FEED SULPHATE CONCENTRATION ON THE KINETICS OF ANAEROBIC SULPHATE REDUCTION

The effect of feed sulphate concentration on the kinetics of the anaerobic sulphate reduction process was studied in one litre continuous bioreactors. A microbial population enriched for SRBs was employed using acetate as the organic and electron source. The choice of acetate as the organic and electron source was motivated by the need to study the kinetics of SRB exclusively i.e. the presence of MPB and APB was largely supported. The kinetics of the anaerobic sulphate reducing system was determined at 'stable' state conditions. 'Stable' state was considered to have been achieved when the residual sulphate concentration

and bacterial concentration did not vary by more than between 10 and 12 % respectively for a period of at least one retention time. The results indicated that feed sulphate concentration had an effect on the biological sulphate reduction process. As the feed sulphate concentration was increased in the range 1.0 to 10.0 kgm⁻³, an enhancement of the volumetric sulphate reduction was observed. The maximum volumetric sulphate reduction rates were 0.007 and 0.170 kgm⁻³h⁻¹ for feed sulphate concentrations of 1.0 and 10.0 kgm⁻³ respectively. When the initial sulphate concentration was 15.0 kgm⁻³ the volumetric sulphate reduction rate and sulphate conversion decreased. The maximum volumetric sulphate reduction rate when the feed sulphate concentration was 15.0 kgm⁻³ was 0.051 kgm⁻³h⁻¹. The specific metabolic activity displayed a decrease as the feed sulphate concentration was increased from 1.0 to 15.0 kgm⁻³. This decrease was manifest by a decrease in specific volumetric sulphate reduction rate with increasing feed sulphate concentration. Despite the decrease in specific metabolic activity an enhancement of the volumetric sulphate reduction rate occurred with increasing feed sulphate concentration in the range 1.0 to 10.0 kgm⁻³ because of the increase in bacterial concentration. The maximum bacterial concentration increased from 0.98 gL⁻¹ at a feed sulphate concentration of 1.0 kgm⁻³ to 5.10 gL⁻¹ for a feed sulphate concentration of 10.0 kgm⁻³. The lower volumetric sulphate reduction rates and sulphate conversion observed for a feed sulphate concentration of 15.0 kgm⁻³ resulted in indication of the that a feed sulphate concentration of 15.0 kgm⁻³ inhibited the activity of sulphate reducers.

9.1.3 EFFECT OF TEMPERATURE ON THE KINETICS OF ANAEROBIC SULPHATE REDUCTION

The effect of temperature on the kinetics of anaerobic sulphate reduction was studied in one litre continuous bioreactors. A mixed sulphate reducing population grown with acetate as the carbon and electron source was employed. The continuous bioreactors were operated at temperatures of 20, 25, 30 and 35°C and the feed sulphate and acetate concentration were 5.0 and 17.5 kgm⁻³ respectively. The kinetics of the continuous system was determined from 'stable' state data. The results illustrated that increasing the temperature in the range 20 to 35°C resulted in an increase in sulphate conversion. The trends observed in terms of residual sulphate concentration, sulphate conversion, residual acetate concentration, volumetric sulphate reduction rate and bacterial concentration as a function of volumetric

sulphate loading rate and dilution rate were similar for all four temperatures employed. However, there was an enhancement of sulphate conversion, volumetric sulphate reduction rate and bacterial concentration with increasing temperature in the range 20 to 35°C. The maximum sulphate conversion increased from 39 % at 20°C to 93 % at 35°C. Similarly, the maximum volumetric sulphate reduction rate displayed an increase from 0.03 to 0.075 kgm⁻³h⁻¹ as the temperature was increased from 20 to 35°C. The specific volumetric sulphate reduction rate increased with increasing temperature indicating the enhancement of microbial activity with increasing temperature in the range 20 to 35°C. The enhancement of the sulphate reducing capacity of the system in terms of sulphate conversion and volumetric sulphate reduction rate was attributed to the enzymatic nature of the sulphate reduction reaction which is enhanced with increasing temperature, the reduced H₂S solubility at increased temperature and the decreased amount of H₂S relative to HS⁻ with increasing temperature in the range 20 to 35°C. The optimum temperature (in the range 20 to 35°C) for sulphate reduction was 35°C.

9.1.4 MODELLING OF EXPERIMENTAL DATA

From continuous experiments performed, it was shown that the initial sulphate concentration and reaction temperature has a significant effect on the kinetics of anaerobic sulphate reduction and microbial growth. This effect was manifest by enhanced reduction rates as the feed sulphate concentration was increased from 1.0 to 10.0 kgm⁻³ and as the temperature was increased from 20 to 35°C. Batch studies showed that further increasing of the temperature above 35°C had an inhibitory effect on the microorganisms. The effect of feed sulphate concentration and reaction temperature on the bacterial growth rate was modelled using the Contois model

$$\mu = \frac{\mu_m [S]}{K_s [X] + [S]}$$

The Contois model has previously been used to describe the dependency of microbial growth rate on substrate concentration when mass transfer limitations occur due to high bacterial concentrations as a consequence of high feed substrate concentrations (Chen and Hashimoto, 1980). The maximum specific

growth rate (μ_m) remained constant at 0.061 h^{-1} regardless of temperature or initial sulphate concentration.

The decay coefficient displayed an Arrhenius dependence on temperature according to:

$$k_d = 8.8 \times 10^{11} e^{-78.7/RT}$$

The saturation constant (K_s) displayed a linear increase with increasing inlet sulphate concentration and an Arrhenius dependence on temperature. The following relation explained this dependency:

$$K_s = 6.52 \times 10^{-35} e^{198/RT} [S_0].$$

Using the 'stable' state experimental data obtained from this study a model for the rate of sulphate reduction as a function of inlet sulphate concentration, temperature, bacterial concentration and residual sulphate concentration was developed. The derived model is:

$$r_s = \left(\frac{0.061[S]}{6.52 \times 10^{-35} e^{198/RT} [S_0] + [S]} - 8.8 \times 10^{11} e^{-78.7/RT} \right) \frac{[X]}{0.568}$$

The model was calibrated with 'stable' state data from the experiments operated at a feed sulphate concentration of 1.0, 5.0, 10.0 kgm^{-3} and 35°C and experiments operated at a feed sulphate concentration of 5.0 kgm^{-3} and reaction temperatures of 20, 30 and 35°C. Validation of the model was performed using data from the bioreactor operated at a feed sulphate concentration of 2.5 kgm^{-3} at 35°C and the bioreactor operated at a feed sulphate concentration of 5.0 kgm^{-3} and a temperature of 25°C. Statistically, the model was shown to represent the experimental data to within a 20 to 24 % error limit, defined by error propagation.

9.2 RECOMMENDATIONS FOR FUTURE WORK

The following section presents recommendations for future studies in this area. These provide a context for future work with respect to the application of the anaerobic sulphate reduction process for the treatment of sulphate containing effluents.

The volumetric sulphate reduction rates obtained for this work were low (a maximum of $0.170 \text{ kgm}^{-3}\text{h}^{-1}$ was observed when the feed sulphate concentration was 10.0 kgm^{-3}). For full-scale implementation of the SRB process, enhancement of the volumetric sulphate reduction rate is required. Enhancement of the

volumetric sulphate reduction rate can be achieved by using reactor systems in which high cell density is favoured. Typically, these are immobilised cell systems or reactor designs that allow cell recirculation. Immobilised cell systems have been used successfully in other applications for the improvement of reduction rates (Nemati *et al.*, 1996) and show potential for application in the sulphate reduction systems. Consequently it is recommended that sulphate reduction be studied in immobilised cell systems. This study should include the optimisation of sulphate reduction by a sulphate reducing consortia with respect to support media and reactor configuration.

The anaerobic sulphate reduction process was inhibited at a feed sulphate concentration of 15.0 kgm^{-3} . This inhibitory effect may result from the toxicity of sulphate, sulphide or sodium on SRB. Data presented allowed the hypothesis of sulphate toxicity, however this was not incorporated into the kinetic model. To include the inhibitory effect of sulphate in the kinetic model further experiments are required across a range of sulphate concentrations that are inhibitory to the biological sulphate reduction process. This will allow for rigorous data collection so that a value for K_I (where I is the substrate) to be determined and used to extend the model. Typically the study should be carried out in continuous bioreactor experiments receiving sulphate at concentrations between 10.0 and 15.0 kgm^{-3} and above 15.0 kgm^{-3} . Using sulphate concentrations between 10.0 and 15.0 kgm^{-3} will allow determination of the exact concentration at which the onset of sulphate inhibition occurs.

To eliminate the inhibitory effect of sulphide, experiments were carried out at a pH of 7.8 at which sulphide speciation is biased towards the non-toxic HS^- species. Most sulphate containing effluent streams (including AMD) are below this pH. It is therefore recommended that experimental studies be carried out across a range of pH values. The proposed study should be extended to ascertain the effect of sulphide on the kinetics of anaerobic sulphate reduction, since the toxicity of sulphide is pH dependent owing to the variation in sulphide speciation with pH. Using the results from the proposed study the kinetic model presented in this study can be extended to include the effect of pH and sulphide concentration. Furthermore, the combination of the pH and sulphide concentration studies will provide conclusive

information regarding the relative toxicity of the sulphide species (H_2S , HS^- or S^{2-}) on sulphate reducing microorganisms.

For this study, acetate was used as the carbon and electron donor for a mixed anaerobic sulphate reducing microbial group. The choice of acetate was motivated by the requirement to establish the effect of feed sulphate concentration and temperature on the kinetics of a mixed sulphate reducing consortium in the absence of interacting effects on MPB and APB. On an industrial scale acetate will not necessarily be the organic source of choice since the choice of organic source is motivated by availability and cost. Consequently readily available and cost effective carbon and electron sources for anaerobic sulphate reduction need to be studied. Where long chain organic compounds are used, the involvement of acid producers and methanogens will occur. The kinetic results obtained can be used to extend the kinetic model developed for this study to include the methanogens and acid producer populations. Furthermore kinetic data on complete and incomplete oxidising SRB require comparison.

It is envisaged that this study together with the recommended work will provide data to inform the extension of the kinetic model developed to include the inhibitory effect of sulphate and sulphide, the pH effect and to account for all the microbial groups involved in the conversion of sulphate to sulphide using long chain organic compounds. This model can be used to advise the design of full-scale processes for the biological treatment of sulphate containing effluents.

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APPENDIX A

TRANSIENT DATA SHOWING ESTABLISHMENT OF
'STABLE'STATE

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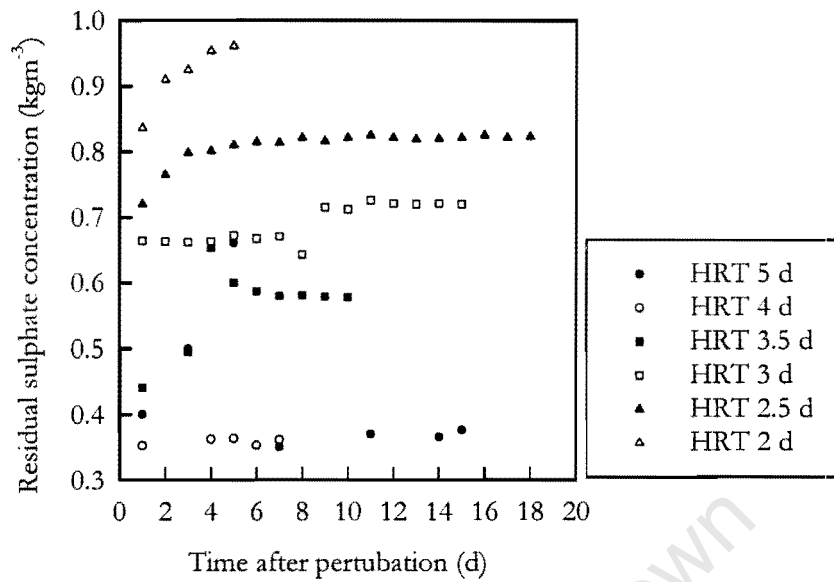


Figure B1. Residual sulphate concentration profiles as a function of time after perturbation for a feed sulphate concentration of 1.0 kgm⁻³ sulphate at retention times between 5 and 2 d. Data taken from a continuous bioreactor operating at 35°C, pH 7.8 with feed acetate concentration of 2.5 kgm⁻¹.

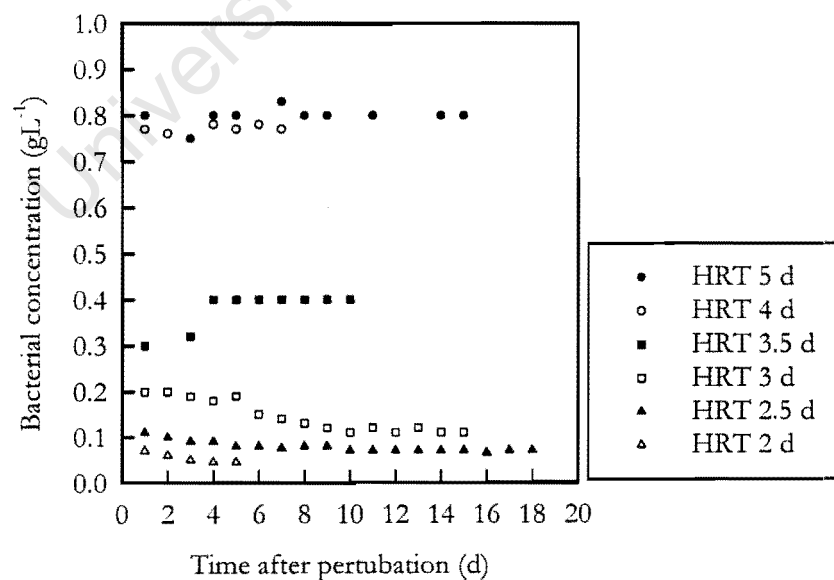


Figure B2. Bacterial concentration profiles as a function of time after perturbation for a feed sulphate concentration of 1.0 kgm⁻³ sulphate at retention times between 5 and 2 d. Data taken from a continuous bioreactor operating at 35°C, pH 7.8 with feed acetate concentration of 2.5 kgm⁻¹.

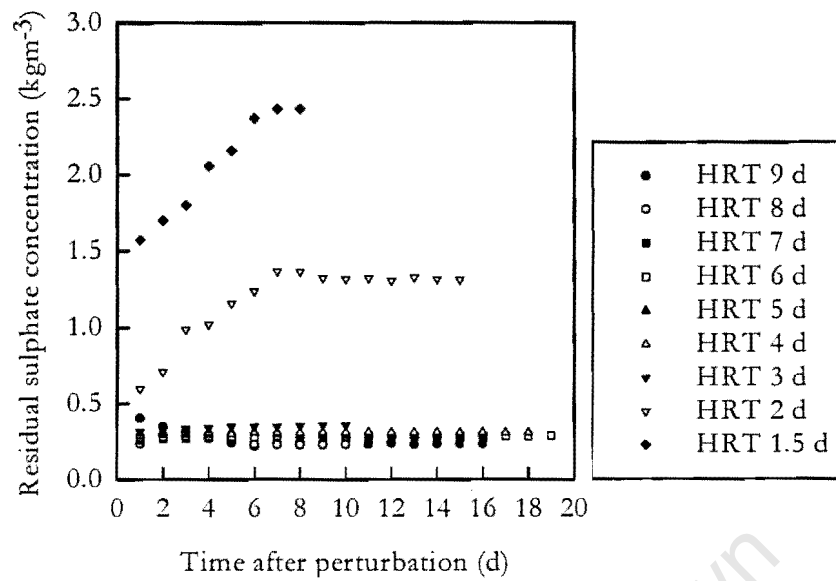


Figure B3. Residual sulphate concentration profiles as a function of time after perturbation for a feed sulphate concentration of 2.5 kgm^{-3} at retention times between 9 and 1.5 d. Data taken from a continuous bioreactor operating at 35°C , pH 7.8 with feed acetate concentration of 17.5 kgm^{-1} .

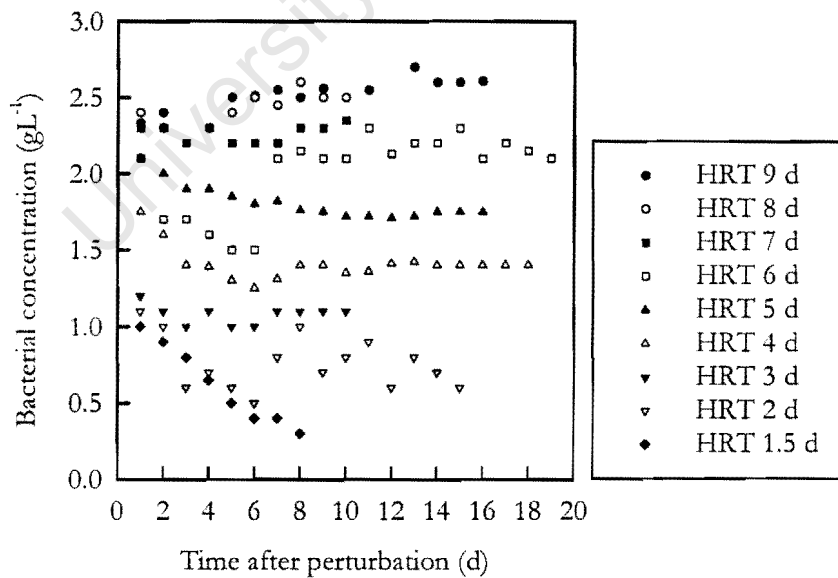


Figure B4. Bacterial concentration profiles as a function of time after perturbation for a feed sulphate concentration of 2.5 kgm^{-3} at retention times between 9 and 1.5 d. Data taken from a continuous bioreactor operating at 35°C , pH 7.8 with feed acetate concentration of 17.5 kgm^{-1} .

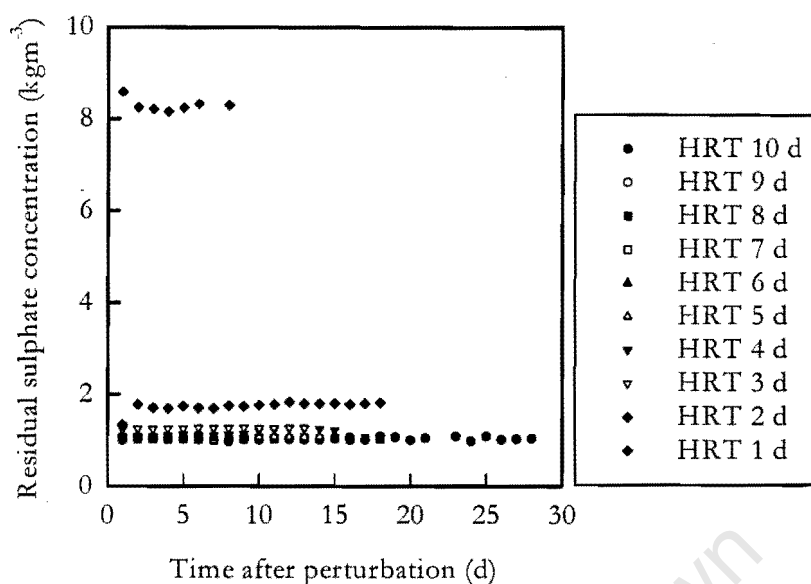


Figure B5. Residual sulphate concentration profiles as a function of time after perturbation for a feed sulphate concentration of 10.0 kgm^{-3} sulphate at retention times between 10 and 1 d. Data taken from a continuous bioreactor operating at 35°C , pH 7.8 with feed acetate concentration of 17.5 kgm^{-1} .

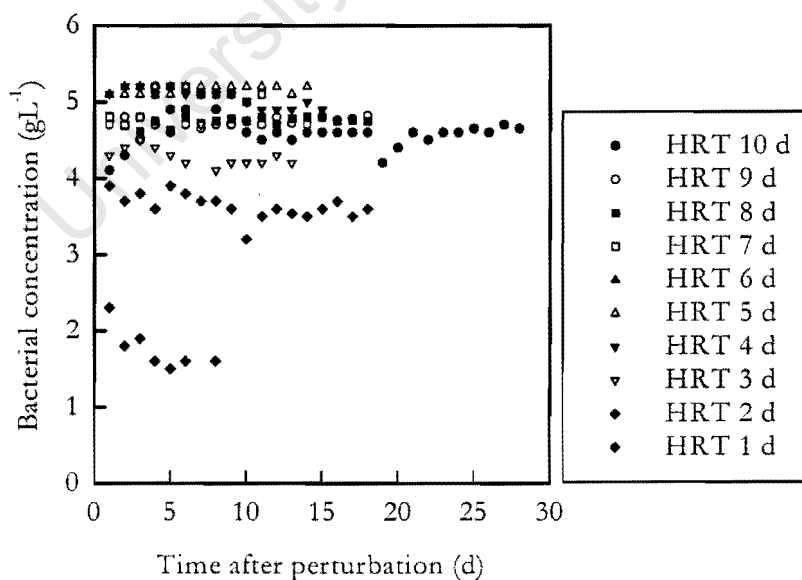


Figure B6. Bacterial concentration profiles as a function of time after perturbation for a feed sulphate concentration of 10.0 kgm^{-3} sulphate at retention times between 10 and 1 d. Data taken from a continuous bioreactor operating at 35°C , pH 7.8 with feed acetate concentration of 17.5 kgm^{-1} .

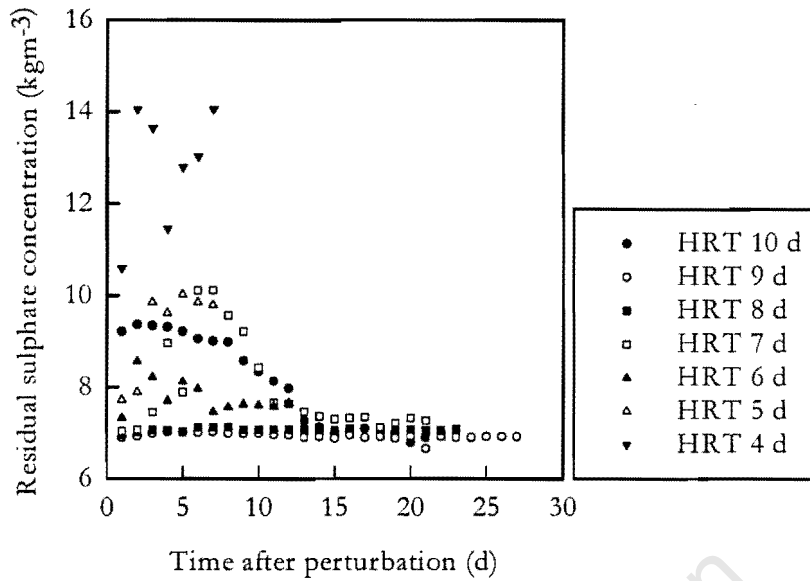


Figure B7. Residual sulphate concentration profiles as a function of time after perturbation for a feed sulphate concentration of 15.0 kgm^{-3} sulphate at retention times between 10 and 4 d. Data taken from a continuous bioreactor operating at 35°C , pH 7.8 with feed acetate concentration of 17.5 kgm^{-1} .

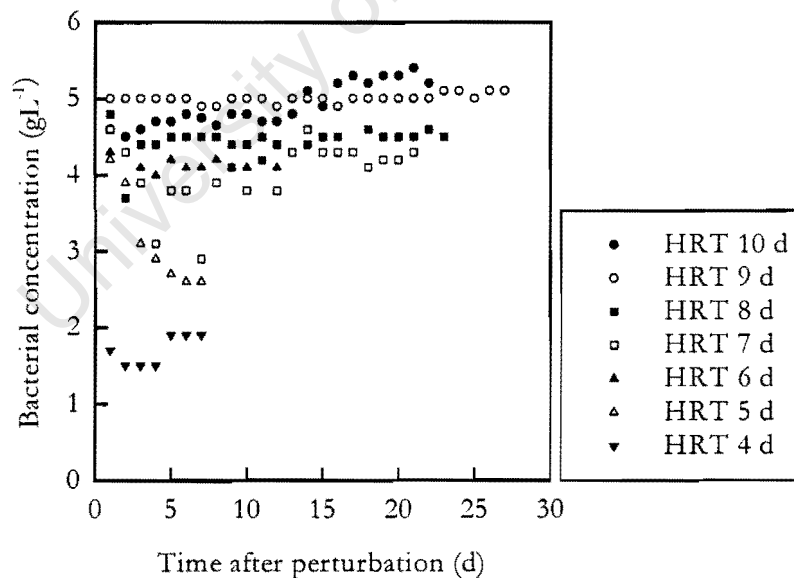


Figure B8. Bacterial concentration profiles as a function of time after perturbation for a feed sulphate concentration of 15.0 kgm^{-3} sulphate at retention times between 10 and 4 d. Data taken from a continuous bioreactor operating at 35°C , pH 7.8 with feed acetate concentration of 17.5 kgm^{-1} .

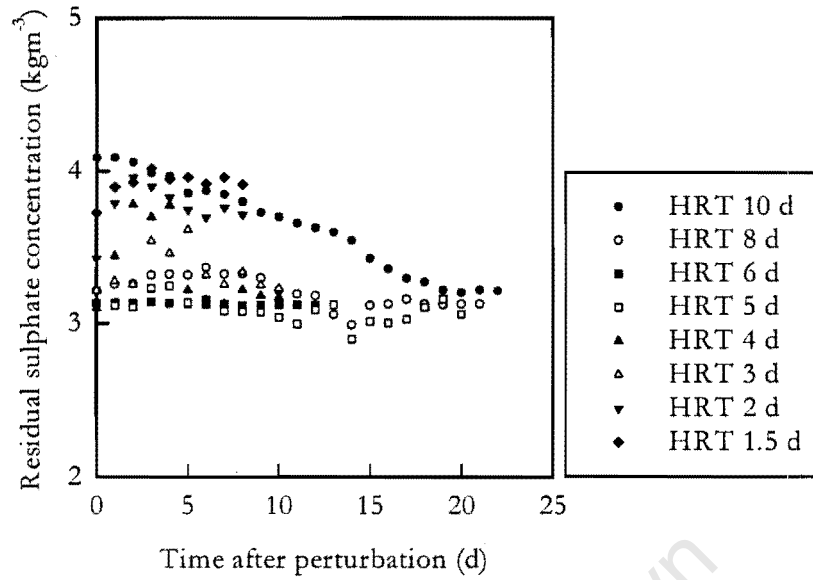


Figure B7. Residual sulphate concentration profiles as a function of time after perturbation at 20°C at retention times between 10 and 4 d. Data taken from a continuous bioreactor operating at pH 7.8 with feed sulphate and acetate concentrations of 5.0 and 17.5 kgm⁻¹ respectively.

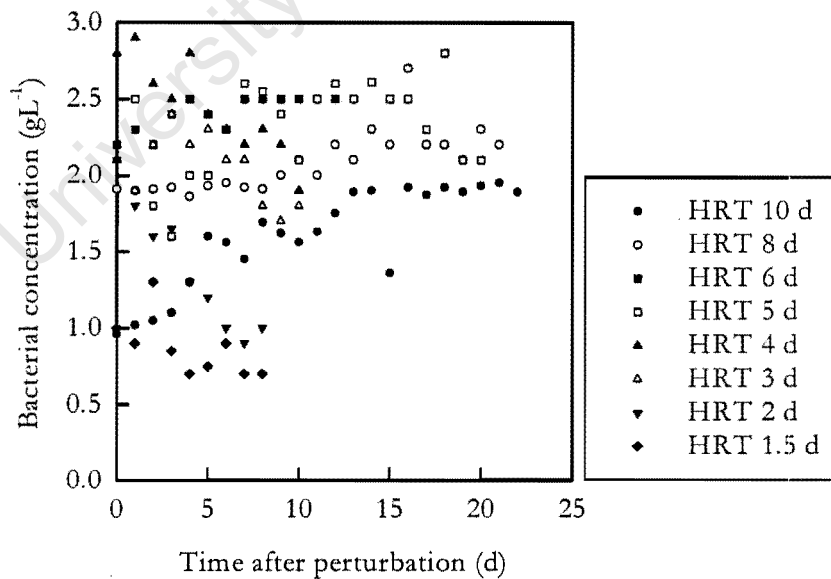


Figure B8. Bacterial concentration profiles as a function of time after perturbation at 20°C at retention times between 10 and 4 d. Data taken from a continuous bioreactor operating at pH 7.8 with feed sulphate and acetate concentrations of 5.0 and 17.5 kgm⁻¹ respectively.

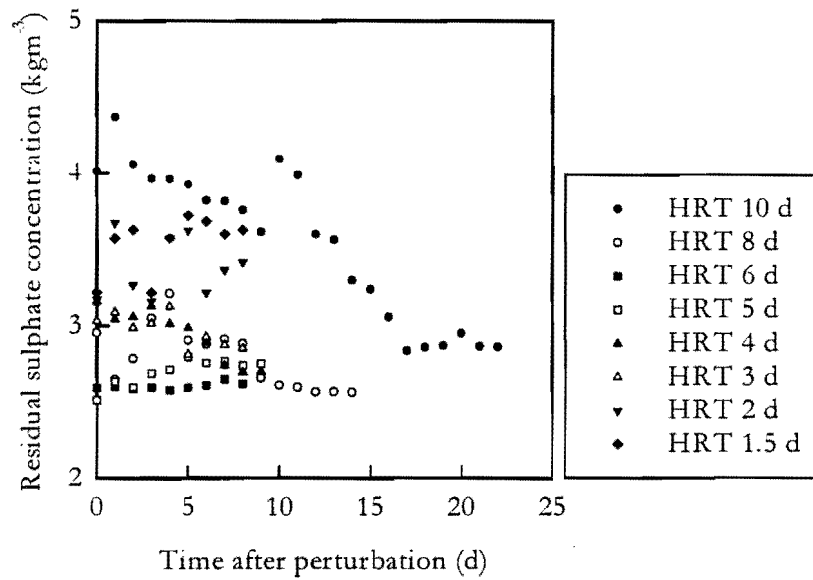


Figure B9. Residual sulphate concentration profiles as a function of time after perturbation at 25°C at retention times between 10 and 4 d. Data taken from a continuous bioreactor operating pH 7.8 with feed sulphate and acetate concentrations of 5.0 and 17.5 kgm^{-1} respectively.

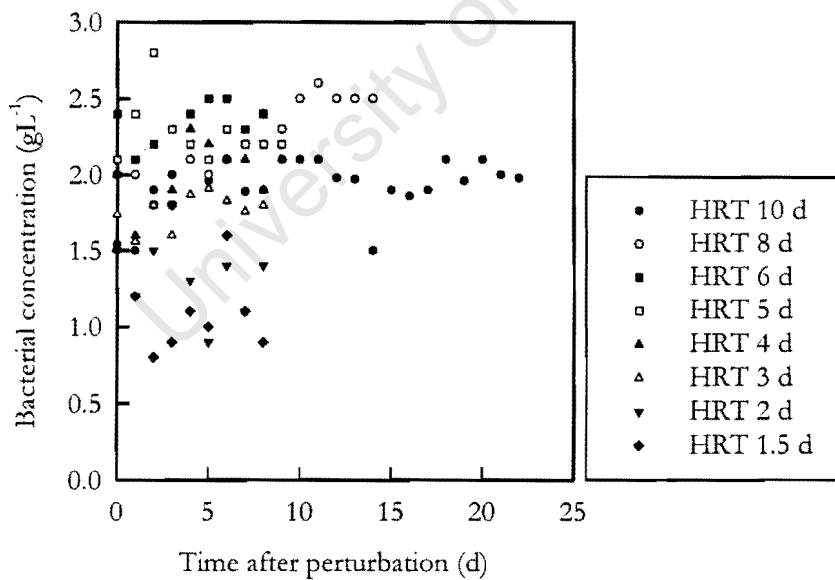


Figure B10. Residual sulphate concentration profiles as a function of time after perturbation at 25°C at retention times between 10 and 4 d. Data taken from a continuous bioreactor operating at pH 7.8 with feed sulphate and acetate concentrations of 5.0 and 17.5 kgm^{-1} respectively.

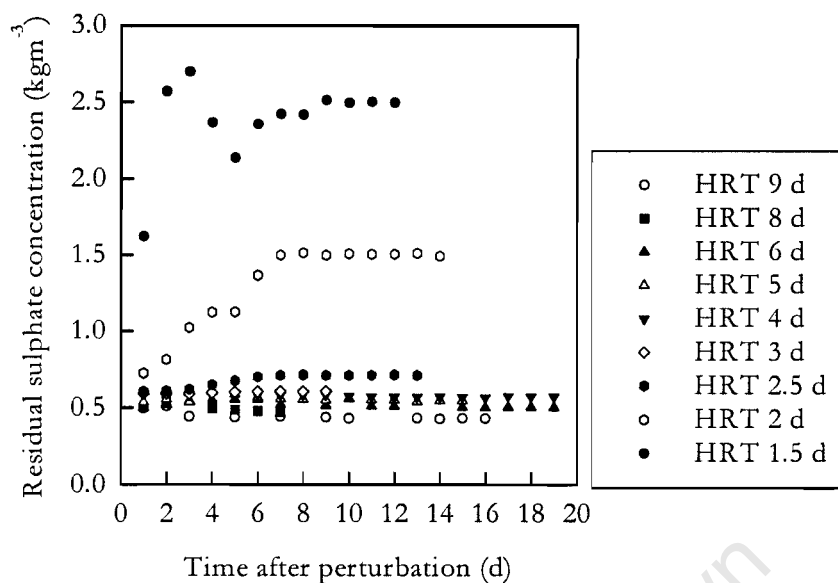


Figure B11. Residual sulphate concentration profiles as a function of time after perturbation at 35°C at retention times between 10 and 4 d. Data taken from a continuous bioreactor operating at pH 7.8 with feed sulphate and acetate concentrations of 5.0 and 17.5 kgm^{-1} respectively.

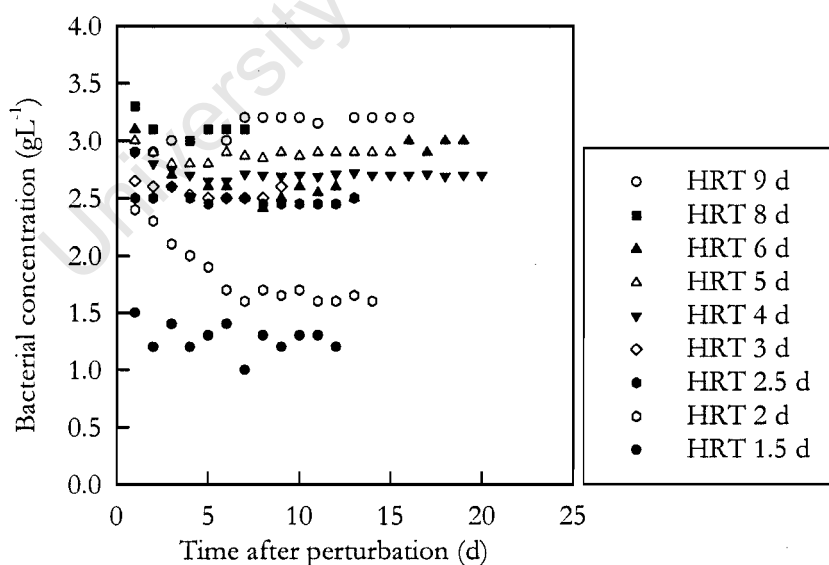


Figure B11. Bacterial concentration profiles as a function of time after perturbation at 35°C at retention times between 10 and 4 d. Data taken from a continuous bioreactor operating at pH 7.8 with feed sulphate and acetate concentrations of 5.0 and 17.5 kgm^{-1} respectively.

APPENDIX B

CALCULATION OF HYDROGEN SULPHIDE SPECIES IN THE LIQUID PHASE

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DISSOCIATION BETWEEN LIQUID AND GASEOUS PHASE

The equilibrium distribution hydrogen sulphide between the gaseous and aqueous phase can be represented by the following reaction:



Using Henry's Law with $K_D = 0.102$ (Pourbaix) the amount of hydrogen sulphide found in the gaseous phase can be calculated by:

$$[\text{H}_2\text{S}]_{\text{gas}} = 9.8 \times [\text{H}_2\text{S}]_{\text{aq}} \quad \text{A1.2}$$

LIQUID PHASE SPECIATION

The aqueous hydrogen dissociates to HS^- and S^{2-} according to the equilibrium reaction:



$$K_1 = \frac{[\text{HS}^-][\text{H}^+]}{[\text{H}_2\text{S}]} \quad \text{A1.6}$$

$$K_2 = \frac{[\text{S}^{2-}][\text{H}^+]}{[\text{HS}^-]} \quad \text{A1.7}$$

To calculate the fraction of each species it can be assumed that:

$$[\text{H}_2\text{S}] + [\text{HS}^-] + [\text{S}^{2-}] = 1 \quad \text{A1.8}$$

Using the above equations the fraction $[\text{H}_2\text{S}]_{\text{aq}}$ can be calculated by:

$$\text{Fraction}[\text{H}_2\text{S}]_{\text{aq}} = \frac{1}{\left(1 + \frac{K_1}{10^{-\text{pH}}} + \frac{K_1 K_2}{(10^{-\text{pH}})^2}\right)} \quad \text{A1.9}$$

The fraction $[\text{HS}^-]$ can be represented by:

$$\text{Fraction}[\text{HS}^-] = \frac{1}{\left(1 + \frac{10^{-\text{pH}}}{K_1} + \frac{K_2}{10^{-\text{pH}}}\right)} \quad \text{A1.10}$$

The fraction $[S^{2-}]$ is calculated by the following equation:

$$\text{Fraction}[S^{2-}] = \frac{1}{\left(1 + \frac{(10^{-\text{pH}})^2 K_2}{K_1} + \frac{10^{-\text{pH}}}{K_2}\right)} \quad \text{A1.11}$$

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