2012

Effect of Linoleic Acid and COD/SO42- Ratio on Anaerobic Sulphate Reduction in Semi-Continuous Reactors

Tapas Biswas
University of Windsor

Effect of Linoleic Acid and COD/SO_4^{2-} Ratio on Anaerobic Sulphate Reduction in Semi-Continuous Reactors

by

Tapas Biswas

A Thesis
Submitted to the Faculty of Graduate Studies
Through Civil and Environmental Engineering
in Partial Fulfillment of the Requirements for
the Degree of Master of Applied Science at the
University of Windsor

Windsor, Ontario, Canada

2012

Effect of Linoleic Acid and COD/SO_4^{2-} Ratio on Anaerobic Sulphate Reduction in Semi-Continuous Reactors

by

Tapas Biswas

APPROVED BY:

_____

Dr. Joel Gagnon, Outside Program Reader
Department of Earth and Environmental Sciences

_____

Dr. Paul Henshaw, Department Reader
Department of Civil and Environmental Engineering

_____

Dr. Rajesh Seth, Advisor
Department of Civil and Environmental Engineering

_____

Dr. Nihar Biswas, Co-Advisor
Department of Civil and Environmental Engineering

_____

Dr. Iris Xu, Chair of Defence
Department of Civil and Environmental Engineering

June 15, 2012

DECLARATION OF ORIGINALITY

I hereby certify that I am the sole author of this thesis and that no part of this thesis has been published or submitted for publication.

I certify that, to the best of my knowledge, my thesis does not infringe upon anyone's copyright nor violate any proprietary rights and that any ideas, techniques, quotations, or any other material from the work of other people included in my thesis, published or otherwise, are fully acknowledged in accordance with the standard referencing practices. Furthermore, to the extent that I have included copyrighted material that surpasses the bounds of fair dealing within the meaning of the Canada Copyright Act, I certify that I have obtained a written permission from the copyright owner(s) to include such material(s) in my thesis and have included copies of such copyright clearances to my appendix.

I declare that this is a true copy of my thesis, including any final revisions, as approved by my thesis committee and the Graduate Studies office, and that this thesis has not been submitted for a higher degree to any other University or Institution.

ABSTRACT

Anaerobic sulphate reduction method has the potential for being effective and economically viable over conventional treatment methods for the treatment of sulphate rich wastewater such as acid mine drainage (AMD). However, a major challenge in anaerobic sulphate reduction is the diversion of a fraction of organic carbon towards methane production. Use of long chain fatty acids (LCFA) as a methanogenic inhibitor to enhance sulphate reduction has the potential for being economically attractive since it is easily available at low cost. The present study investigated the effect of linoleic acid (LA) and COD/SO_4^{2-} ratio on anaerobic dissimilatory sulphate reduction in semi continuous suspended growth system at 37 ^oC. Without LA, sulphate reduction of 50% was observed at a COD/SO_4^{2-} ratio of 0.75. Sulphate reduction increased with increasing LA concentrations and at 1000 mg/L, almost 100% sulphate reduction was achieved.

DEDICATION

I dedicate this research work to my loving and proud parents Mr. B. N. Biswas and Mrs. Shova Biswas who had always been the source of my inspiration.

ACKNOWLEDGEMENTS

I would like to express my sincerest gratitude to my advisors, Dr. Rajesh Seth and Dr. Nihar Biswas for their continuous guidance, motivation and support. This research work would not have been completed without their guidance, ideas and expertise. I would also like to acknowledge Dr. Paul Henshaw and Dr. Joel Gagnon for taking time to review my work and providing invaluable comments.

I would also like to thank my peers, Mr. Biman Paudel and Mrs. Purnima Mallelwar who helped me in analytical works. I would like to extend my appreciation to Mr. Bill Middleton and Mr. Matt St. Louis.

A very special thanks to Mr. Sailesh Singh. All the discussions with coffee cups, chasing deadlines, moral support during long days, nights and weekends are invaluable to me. I think myself fortunate to have a friend, peer and mentor like you along this journey.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

NOMENCLATURE

AMD	acid mine drainage
AMPB	aceticlastic methane prodicing bacteria
ASRB	aceticlastic sulphate reducing bacteria
COD	chemical oxygen demand
CSTR	continuous stirred tank reactor
HMPB	hydrogenotrophic methane producing bacteria
HRT	hydraulic retention time
HSRB	hydrogenotrophic sulphate reducing bacteria
IC	inorganic carbon
LA	linoleic acid
LCFA	long chain fatty acid
MPB	methane producing bacteria
OA	oleic acid
SA	Stearic acid
SCFA	short chain fatty acid
SCSTR	semi-continuous stirred tank reactor
SRB	sulphate reducing bacteria
TC	total carbon
TOC	total organic carbon
TS	total sulphide
UASB	upflow anaerobic sludge blanket
VFA	volatile fatty acid

CHAPTER I

INTRODUCTION

1.1 Background

Sulphate rich wastewaters from a variety of industries, pose a severe threat to the environment. Pulp and paper, food processing, metallurgical, petroleum, edible oil, etc. are some of the industries that produce this type of wastewater (Lens et al., 1998). However, the mining industry is a major producer of acidic sulphate containing wastewater from their tailings ponds, which is commonly known as Acid Mine Drainage or AMD (Johnson & Hallberg, 2005). High level of heavy metals (iron, zinc, nickel, copper, chromium, lead, cadmium etc. and others) are present in the AMD, which run the risk of additional contamination to the environment. For example, approximately 19,300 km of streams and rivers, and 72,000 ha of lakes and reservoirs worldwide were estimated in 1989 to be seriously damaged due to AMD (Johnson & Hallberg, 2005).

Neutralization and chemical precipitation are the most widely used conventional treatment methods to treat wastewater with heavy metals and high sulphate concentrations (Kaksonen & Puhakka, 2007). These methods are not cost effective as they require chemicals for treatment and generate waste that is difficult and expensive to dispose of. This has prompted research to look for alternate technologies to remove heavy metals and sulphates from sulphate rich wastewater. Major advances in anaerobic digestion in the last three decades resulted in widespread adoption of this process due to low sludge production and low energy requirement (Ghosh & Pohland, 1974). Sulphate reducing bacteria (SRB), in an anaerobic environment, can remove sulphate from

sulphate containing wastewaters including AMD by dissimilatory sulphate reduction where sulphate can act as terminal electron acceptor. In addition, microbial species consume a small portion of sulphur from sulphate for their growth and activity. This process is referred as assimilatory sulphate reduction.

Two major challenges in the dissimilatory sulphate reduction are 1) the low organic carbon concentrations in AMD that serve as the electron donor to the SRB and 2) co-existence of methanogenic bacteria (MPB) that consume a fraction of organic carbon and divert the electron flux towards methane production. This competition between SRB and MPB for organic carbon or the by-product such as acetate or hydrogen, make the anaerobic digestion process less efficient (Weijma et al., 2002).

Long chain fatty acids (LCFAs) have the potential to inhibit gram-positive bacteria such as methanogens (Kabara et al., 1977). Kramer (1971) reported that wastewaters from dairies, food manufacturing and vegetable oil industries contain elevated levels of LCFAs. Use of LCFA can be effective as well as more economically viable than conventional inhibition processes such as heat treatment (Sung et al., 2002; Lay, 2000; Okamoto, 2000) or chemical inhibition (Chen et al., 2008). However, knowledge on the effect of LCFA on sulphate reduction is limited. A recent study in batch operation (Sharma & Biswas, 2010) has shown that it may be possible to use LCFA to selectively inhibit methanogens and divert more electron flux towards sulphate reduction. This possibility of the same being true in semi-continuous or continuous process applications has not been tested.

1.2 Objective

The overall objective of this study was to examine the effect of linoleic acid and COD/SO_4^{2-} ratio on anaerobic sulphate reduction in semi-continuous stirred tank reactors (SCSTRs). The experiments were conducted in two phases.

1.2.1 Phase I

To assess the effect of COD/SO_4^{2-} ratio alone on anaerobic sulphate reduction in SCSTRs.

1.2.2 Phase II

To study the effect of LCFA (linoleic acid) and COD/SO_4^{2-} ratio on anaerobic sulphate reduction in SCSTRs.

1.3 Scope

The scope of the present study was as follows:

1.3.1 Phase 1

i. To obtain healthy culture of SRB for subsequent experiments;

ii. To investigate the effect of COD/SO_4^{2-} ratio (4.66, 1.96 and 0.75) alone on anaerobic sulphate reduction in 3 sets (duplicate) of semi-continuous stirred tank reactors (SCSTRs).

1.3.2 Phase 2

i. To investigate the effect of COD/SO_4^{2-} ratio (4.66, 1.96 and 0.75) with and without LCFA (linoleic acid) on anaerobic sulphate reduction in semi-continuous stirred tank reactors (SCSTRs).

CHAPTER II

REVIEW OF LITERATURE

2.1 Overview

The focus of this chapter is to provide sufficient information regarding anaerobic sulphate reduction to treat sulphate rich wastewater especially acid mine drainage. An understanding of anaerobic digestion concepts, involvement of various microorganisms at different stages, competition between sulphate reducing bacteria and methane producing bacteria for organic substrates, and factors affecting this competition such as pH, temperature, HRT, COD/SO_4^{2-} ratio are discussed in this chapter.

In anaerobic sulphate reduction, the major challenge is to inhibit methanogens and divert reducing equivalents towards sulphate reduction. This chapter discusses the conventional technologies of inhibiting methanogens by physical or chemical inhibitors and their limitations. A recent batch study (Sharma & Biswas, 2010) has shown that long chain fatty acids (LCFAs) have the potential to selectively inhibit methanogens to enhance sulphate reduction. This chapter describes the advantage of LCFA, inhibition mechanism, and types of toxicity effects due to different LCFAs.

2.2 Concepts of Anaerobic Digestion

Anaerobic treatment of wastewater has become a practical requirement in many full-scale facilities because of its cost effectiveness and energy saving (Lettinga, 1995). The conversion of complex organic substrates to either methane or hydrogen sulphide or both, is anaerobically mediated by a consortium of different microbial populations, which includes hydrolytic microorganisms, acidogens, acetogens, methanogens and sulphate

reducers (Bagley & Brodkorb, 1999; Veeken et al., 2000). The four step process includes hydrolysis, acidogenesis, acetogenesis, methanogenesis and/or sulphidogenesis (Gujer & Zehnder, 1983). In this four-step process (Figure 2.1), by-products from one reaction serve as substrate, for other reactions and the major end products along with methane or sulphide are biomass, water and carbon dioxide (CO_2). However, methane is not the terminal product in the presence of alternative terminal electron acceptors such as sulphate.

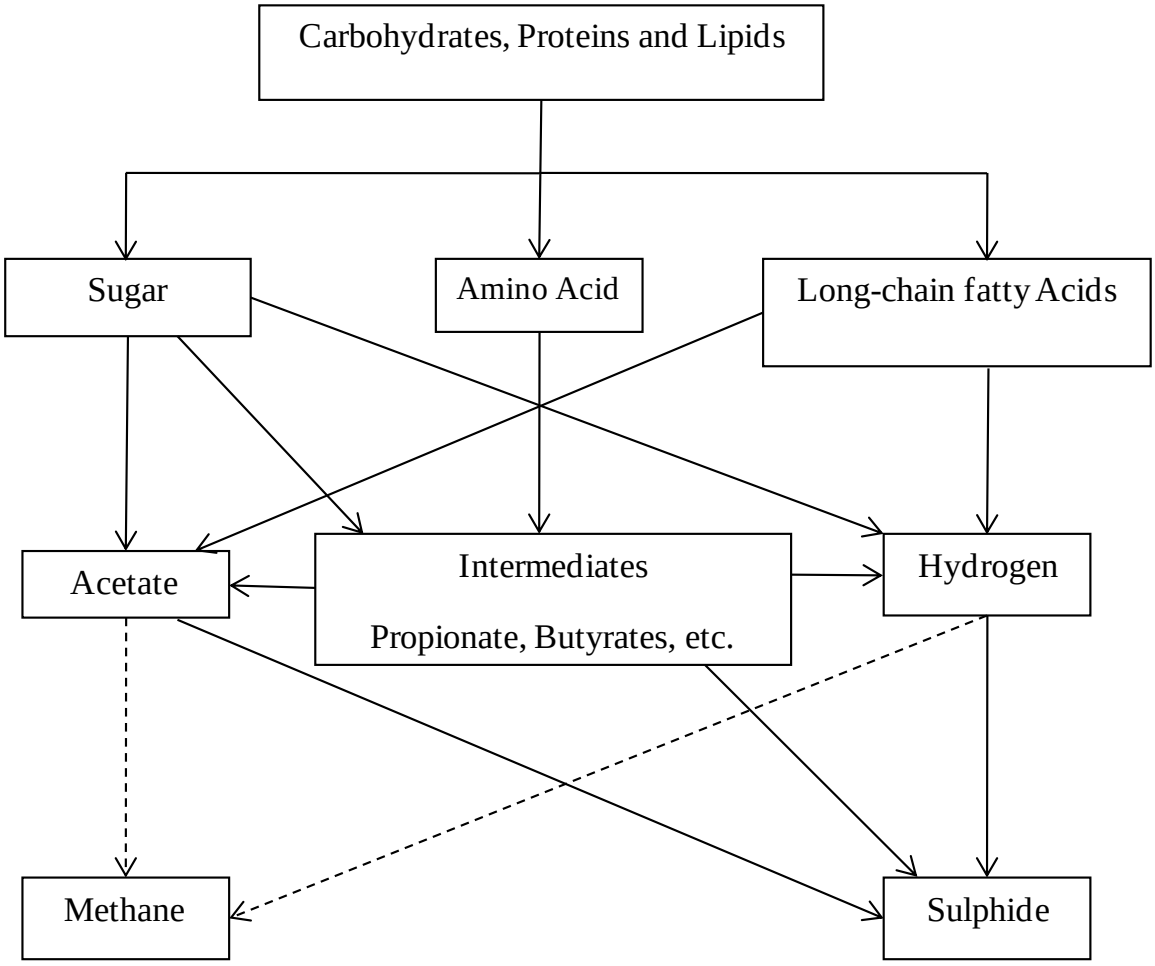


Figure 2.1 Pathway of anaerobic biodegradation (adapted from Gujer & Zehnder, 1983)

2.2.1 Hydrolysis

Hydrolytic microorganisms are responsible for hydrolysis, the first step of anaerobic biodegradation (Gujer and Zehnder, 1983). In this process, hydrolytic microorganisms excrete extra cellular enzymes and break down large complex organic polymers into simple monomers (Annachhatre, 1996; Veeken et al., 2000). Noike et al. (1985) and Eastman & Ferguson (1981) reported this step as the rate limiting step for overall hydrolysis process which is a function of pH, temperature, composition, particle size of the substrates and high concentrations of intermediate products (Gujer & Zehnder, 1983; Veeken & Hamelers, 1999).

2.2.2 Acidogenesis

The second step is acidogenesis where the products of hydrolysis (simple monomers, amino acids, long chain fatty acids) are converted to volatile fatty acids (VFAs), some intermediate by-products (alcohols), hydrogen and carbon dioxide (Boone, 1985; Veeken et al., 2000). Malina & Pohland, (1992) reported that fast growing fermentative bacteria such as, *Enterobacteraerogenes* and *Escherichia coli* mediate these reactions. Table 2.1 presents the most significant organic acids (volatile and non-volatile) produced at this stage. Volatile acids shown in bold are the most prevalent intermediates found in the process.

Table 2.1 Organic acids of significance in Acidogenesis (adapted from Environmental Biotechnology, Rittman and McCarty, 2001)

Volatile acids	Non-volatile acids
Formic acid	
Acetic acid	
Propionic acid	
n-Butyric acid	Lactic acid
Iso-Butyric acid	Pyruvic acid
n-Valeric acid	Succinic acid
Isovaleric acid	
Caproic acid	
Heptanoic acid	
Octanoic acid	

2.2.3 Acetogenesis

Acetogenic bacteria are responsible for the third step of anaerobic digestion. Higher VFAs and intermediate alcohols are converted into acetate, hydrogen and carbon dioxide at relatively low hydrogen partial pressure in this step. The important reactions involved in this step are given in Table 2.2.

Table 2.2 Reactions involved in anaerobic digestion (Thauer, 1977)

Example Hydrolytic Reactions	$\Delta G^{o\prime}$ (kJ·mole^{-1})	Eq.
B - Lactose + $H_2O \rightarrow$ α –D - galactose + α –D -glucose	-106.5	2.1
β - Maltose + $H_2O \rightarrow 2\alpha$ – D -glucose	-45.3	2.2
Sucrose + $H_2O \rightarrow$ D -fructose + α - D - glucose	-43.6	2.3
Example Acidogenic Reactions		
$C_6H_{12}O_6 + 4\ H_2O \rightarrow 2\ CH_3COO^- + 2\ HCO_3^- + 4\ H_2 + 4\ H^+$	-206.0	2.4
$C_6H_{12}O_6 + 5\ H_2O \rightarrow CH_3CH_2COO^- + 3\ HCO_3^- + 5H_2 + 4H^+$	-177.9	2.5
$C_6H_{12}O_6 \rightarrow CH_3CH(OH)COO^- + 2\ H^+$	-198.5	2.6
$C_6H_{12}O_6 + 2\ H_2O \rightarrow CH_3(CH_2)_2COO^- + 2\ HCO_3^- + 2\ H_2 + 3H^+$	-253.8	2.7
Example Acetogenic Reactions		
$CH_3CH_2COO^- + 3\ H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 3\ H_2$	357.6	2.8
$CH_3CH(OH)COO^- + 2\ H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 2\ H_2$	277.2	2.9
$CH_3(CH_2)_2COO^- + 2\ H_2O \rightarrow 2\ CH_3COO^- + H^+ + 2\ H_2$	48.3	2.10
$CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + H^+ + 2\ H_2$	9.6	2.11
Example Methanogenic Reactions		
Aceticlastic Methanogenesis:		
$CH_3COO^- + H^+ \rightarrow CO_2 + CH_4$	-27.5	2.12
Hydrogenotrophic Methanogenesis:		
$CO_2 + 4\ H_2 \rightarrow CH_4 + 2\ H_2O$	-139.1	2.13
Example Sulphidogenic Reactions		
Aceticlastic Sulphidogenesis:		
$CH_3COO^- + SO_4^{2-} \rightarrow HS^- + 2HCO_3^-$	- 47.6	2.14
Hydrogenotrophic Sulphidogenesis:		
$SO_4^{2-} + 4H_2 + H^+ \rightarrow HS^- + 4H_2O$	-151.9	2.15

$\Delta G^{o\prime}$ is the free energy for the reactor under standard conditions (temperature, 237^{o}K; pressure, 1.0 atm; pH, 7.0 and products at 1 M)

2.2.4 Methanogenesis

Methanogenesis is the terminal step in the absence of any other electron acceptor such as sulphates. A group of strictly anaerobic archaea called methane producing bacteria (MPB) carries out this step by converting acetate and hydrogen to methane and carbon dioxide. Studies have shown that two major pathways for methane production exist in the terminal anaerobic reaction. In one pathway, hydrogen consuming MPB or hydrogenotrophic methanogens (HMPB) utilize hydrogen as electron donor and in another pathway, aceticlastic MPB (AMPB) use acetate as the carbon source. Compared to hydrogenotrophic methanogens, the growth of aceticlastic methanogens is approximately 5 to 10 times slower since the free energy of reaction for acetate conversion to methane and carbon dioxide is less than that for reduction of carbon dioxide to methane and water. Hence, biomass yield of aceticlastic methanogens per unit of chemical oxygen demand (COD) substrate is less than that of hydrogenotrophic methanogens. Both aceticlastic and hydrogenotrophic methanogens compete for substrate in presence of sulphate reducers.

2.2.5 Sulphidogenesis

Sulphidogenesis can occur simultaneously with methanogenesis when sulphate is present with the activity of sulphate reducing bacteria (SRB). Co-existence with methanogens causes a competition for various electron donors (McCartney & Oleszkiewicz, 1993). The typical reactions involved in this step are shown in Table 2.2. SRB can utilize a variety of organic matter as a carbon source in comparison to methanogens. The major by-product of sulphidogenesis is sulphide, which is a potent

toxin to both methanogens and sulphate reducers. A proper balance between acid production rate (hydrolysis and acidogenesis) and acid consumption rate (acetogenesis, methanogenesis and sulphidogenesis) is a major operational challenge in anaerobic digestion. Lack of stability in maintaining equilibrium can cause VFA accumulation and eventually has the potential for system failure.

2.3 Competition for organic substrate between SRB and MPB

Sulphate reduction and methane formation can take place simultaneously in anaerobic digestion. Both sulphate reducers (SRB) and methane formers (MPB) can use hydrogen and acetate produced in the process as electron donor. Therefore, a competition for organic substrates such as hydrogen and acetate exists between SRB and MPB (Lens et al., 1998). Methane formation is undesirable for dissimilatory sulphate reduction since a fraction of reducing equivalents from the substrate is utilized for methane formation resulting in low sulphate reduction (Weijma et al., 2002). SRB are much more versatile in terms of substrate utilization than MPB (Kaksonen & Puhakka, 2007). Stams et al. (2005) reported that compounds such as propionate, butyrate etc. are degraded directly by SRB species (*Desulfovibrio* and *Desulfomicrobium*) in sulphate rich environments, whereas MPB can utilize only hydrogen and acetate. Hence, the competition is mainly for these two electron donors - hydrogen and acetate. Several factors determine the outcome of this competition. Thermodynamic and kinetic considerations, substrate affinity and COD/SO_4^{2-} ratio are the main guiding factors to determine the outcome of the competition.

2.3.1 Competition for Hydrogen

From thermodynamic, kinetic and substrate affinity considerations, hydrogen consuming SRB (HSRB) should effectively out-compete hydrogenotrophic methanogens (HMPB) under anaerobic conditions while treating sulphate rich wastewater (Zinder, 1993; Mulder, 1984; Rinzema et al., 1986 Alphenaar et al., 1993; Bhattacharya et al., 1996b; Harada et al., 1994; McCartney & Oleszkiewicz, 1993; Rinzema & Lettinga, 1988; Uberoi & Bhattacharya, 1995; Visser et al., 1993a; Widdel et al., 1988; Colleran et al., 1995; O'Flaherty & Colleran, 1999; Omil et al., 1996). As indicated in Table 2.3, lower values of ΔG, $\Delta G^{o'}$ and K_m favour SRB to win the competition for hydrogen over MPB, due to their comparative higher values for reactions with the same. SRB have higher affinity for hydrogen than MPB. The location of the hydrogenase enzyme in the periplasmic space of SRB rather than in the cytoplasm as in MPB is the reason for this higher affinity (Tursman and Cork, 1989). Moreover, HSRB function at a lower hydrogen threshold concentration than HMPB (Chen et al., 2008; Colleran et al., 1995; Elferink et al., 1994; Lovley, 1985).

Table 2.3 Free energy, apparent K_m and minimum substrate threshold values for hydrogenotrophic and aceticlastic methanogens and sulphate reducers. (adapted from Colleran et al., 1995)

Reactions	ΔG° (kJ/mole)	ΔG°' (kJ/rxn)	Apparent K_m (μM)	Minimum threshold (nM)
$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$	- 32.7	- 135	5-13	23-75
$4H_2 + HSO_4^- \rightarrow HS^- + 4H_2O$	- 38.0	- 152	2	7
$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$	- 28.2	-31	*3-5 x 10^3	0.5-1.2 × 10^6
			**0.5-1 × 10^3	5-70 × 10^3
$CH_3COO^- + SO_4^{2-} \rightarrow HS^- + 2HCO_3^-$	- 39.5	-47	0.2 x 10^3	±1 × 10^3

* Methanosarcinasp, ** Methanothrix sp.

2.3.2 Competition for Acetate

From thermodynamic and kinetic points of view, aceticlastic SRB (ASRB) are expected to out-compete acetate-utilizing MPB (AMPB) because of their low K_m value and free energy values (Table 2.3). ASRB gain more energy from the acetate than AMPB and have higher growth rates (Colleran et al., 1995). Elferink et al. (1994) have observed that ASRB out-compete AMPB especially at low acetate concentration. Lovley and Phillips (1987) confirmed the ability of SRB to out-compete MPB for acetate in freshwater sediments at a concentration of 5 μM. However, the outcome of the competition for acetate in anaerobic digesters is contradictory (Colleran et al., 1995) and

less clear (Stams et al., 2005). Some authors have reported preferential acetate utilization by SRB species whereas the majority of them have indicated successful utilization by MPB species in the presence of sulphate.

The type of reactor involved in the investigation may play a crucial role, to some extent, in determining the outcome of the competition for acetate (Colleran et al., 1995; Lens et al., 1998). Experiments in CSTRs and contact processes showed preferential acetate consumption by SRB species (Olthof et al., 1985). On the other hand, the outcome of the competition is less predictable in modern high rate reactors with sludge immobilization (Lens et al., 1998).

Several studies have reported complete conversion of acetate into methane via methanogenesis indicating preferential consumption of acetate by MPB, even in excess of sulphate (Mulder, 1984; Rinzema et al., 1986; Isa et al., 1986a, b; Polprasert & Haas, 1995; Yoda et al., 1987), while others have reported a predominance of ASRB (Choi & Rim, 1991; Omil et al., 1997; Omil et al., 1996; Stucki et al., 1993; Visser et al., 1993a). Hence, there is no agreement in the scientific community in terms of acetate utilization and factors affecting the competition for acetate.

Researchers have put forward various theories to explain the apparent competitive advantage of AMPB in retained biomass system. Isa et al. (1986 a, b) have reported that relatively superior capability of MPB to colonize on support material may attribute to successful competition. Yoda et al. (1987) found the predominance of MPB at acetate concentrations higher than 8 mg COD/L. Several authors have reported other factors such as COD/SO_4^{2-} ratio (Bhattacharya et al., 1996b; Choi & Rim, 1991; Isa et al., 1986b), pH (Isa et al., 1986b), temperature (Shin et al., 1996), HRT (Isa et al., 1986b; Omil et al.,

1998), and organic and sulphate loading rates (Yoda et al., 1987) can potentially determine the outcome of the competition for acetate.

2.4 Factors affecting the Competition

2.4.1 Effect of pH

The literature shows that there are direct and indirect effects of pH on sulphate reduction and methane formation. Studies have reported the optimal pH range of 7.3 to 7.6 and 6.5 to 7.8 for SRB and MPB, respectively (Widdel, 1988; Vogels et al., 1988). ASRB can tolerate pH higher than 7.6. Visser et al. (1996) investigated the kinetic properties of acetotrophic SRB (ASRB) and acetotrophic MPB (AMPB). Their results indicate that ASRB win the competition at a pH levels higher than 7.7. Omil et al. (1997) have reported that SRB show higher growth rate than that of MPB at higher pH level. Their findings indicate that at pH values greater than ~ 7.7, ASRB will out-compete AMPB because under these conditions ASRB have a higher maximal specific growth rate and are less inhibited by sulphide than AMPB. The pH up to which the sulphidogenic bacteria can survive and grow is 10 whereas it is 8.5 for AMPB (Visser et al., 1996).

AMPB have advantage over ASRB at a pH level less than 6.9. Both have comparable growth rates in the pH range of 6.9 to 7.7. In this pH range, both are equally inhibited by sulphide and the outcome of the competition is governed by the sulphate concentration in the bulk solution. In addition to the direct pH effect, the outcome of the competition is also subjected to an indirect effect due to the pH dependence of sulphide toxicity on SRB and MPB. Section 2.4.4 discusses the sulphide toxicity and its pH dependence.

2.4.2 Effect of Temperature

Temperature can play a crucial role in determining the outcome of the competition. The literature shows that mesophilic ASRB and AMPB have similar temperature ranges and optima. Visser et al. (1992) have reported that both ASRB and AMPB respond similarly to temperature changes in the range of 10 – 50 ^{o}C. In general, increasing temperature is more favourable to SRB growth compared to methanogens. Methanogens were strongly suppressed with a large fraction of electron flow distributed to SRB in mixed culture system operating at elevated temperature (Shin et al., 1996). Visser et al. (1993b) have reported that SRB are less sensitive to high temperature shocks (65 ^{o}C for 8 - 9 hrs.) compared to methanogens in granular sludge. Shin et al., (1996), reported contradictory finding. In their continuous reactors, observed that electron flow towards SRB increased from 43% to 80% when the temperature was decreased from 35 ^{o}C to 25 ^{o}C. The spore forming ability of few SRB species in adverse conditions may attribute to their lesser sensitivity compared to MPB. Hence, temperature shock can be instrumental in determining the outcome of the competition.

2.4.3 Effect of HRT

The competition between SRB and MPB decreases with increasing hydraulic retention time (HRT). Apparently, higher HRT is more advantageous to SRB leading to increase in sulphate reduction. Polo et al. (2006) has reported a significant decrease in effluent sulphide concentration and washout of biomass at retention time lower than 10 hours. Isa et al. (1986b) worked with acetate in high rate anaerobic reactors and observed an increased sulphate reduction by 7.6% when the HRT was increased from 0.5 to 10

days. However, it is noteworthy that the SRB population was dominant at start-up. MPB out-competed SRB after 7 weeks of re-inoculation with anaerobic liquor. Competition between ASRB and AMPB in mesophilic (30 ^{o}C) UASB reactors fed with two different media: VFA mixture (acetate : propionate : butyrate ratio of 5:3:2 on COD basis) and acetate as sole substrate was examined by Omil et al. (1998). They concluded that ASRB became predominant in prolonged reactor operation with excess sulphate in the influent. The amount of acetate used by SRB increased from 50% to 90% in reactors when HRT was increased from 250 to 400 days, respectively. These findings were in agreement with that of Harada et al. (1994) who have also reported that ASRB became predominant after prolonged (more than 100 days) reactor operation.

2.4.4 Sulphide Inhibition

Sulphide in high concentrations in an anaerobic digester can be toxic to sulphate reducers and methane producers. Its accumulation can cause severe inhibition in bacterial activities even result in process failure. Sulphide can be present in both unionized (H_2S) and ionized forms (HS^- and S^{2-}) in the solution. The unionized H_2S dissociates in water according to the following equations (Garrels and Christ, 1965):

$$H_2S \rightleftharpoons H^+ + HS^-$$

$$HS^- \rightleftharpoons H^+ + S^{2-}$$

It is presumed that only unionized H_2S exhibits inhibitory effects as only neutral molecules can permeate the cell membrane (Schlegel, 1981) driven by osmotic gradients (McCartney and Oleszkiewicz, 1993), although Khan and Trottier (1978) rated the inhibition potential for various sulphur compounds as H_2S > total sulphide (TS) >

sulphite > thiosulphite > sulphate. The exact mechanism of inhibition due to H_2S is not yet clear. The H_2S crosses the cell wall and affects the intracellular pH. This change in pH denatures the native proteins and essential metabolic coenzymes through the formation of sulphide and disulphide cross-links between the polypeptide chains (Lens et al, 1998). It can also form insoluble FeS that can result in Fe deficiency for the cell constituents such as ferrodoxin and cytochrome (Reis et al., 1992). This metal sulphide formed can also act as a barrier that prevents the access of essential reactants to the enzymes (Utgikar et al., 2002).

The pH plays a crucial role in sulphide inhibition as the chemical equilibrium of different sulphide species is pH dependant (Okabe et al., 1995; Hao et al., 1996). Figure 2.2 shows the variation of distribution of sulphide species with pH. At high pH level of 8 to 9, all dissolved sulphide is in ionized forms (HS^- and S^{2-}). At low pH level of 6 to 7, most of the sulphide remains in unionized form (H_2S) which potentially causes inhibition.

Researchers have different opinions regarding the effect of sulphide inhibition. Hilton & Oleszkiewicz (1988) have reported that SRB were inhibited in proportion to the total sulphide (TS) concentrations and not the hydrogen sulphide concentrations, while acetotrophic methanogens were inhibited more by free hydrogen sulphide. As per Visser et al. (1996), sulphide inhibition depends on sludge characteristics. They concluded that above pH 7, inhibition in granular sludge is caused by total sulphide concentration (TS = $H_2S + HS^- + S^{2-}$), while in suspended sludge, free H_2S determines the toxicity. Studies have reported 50% inhibition at H_2S concentrations ranging from 50 to 130 mg/L in suspended sludge (Lens et al., 1998).

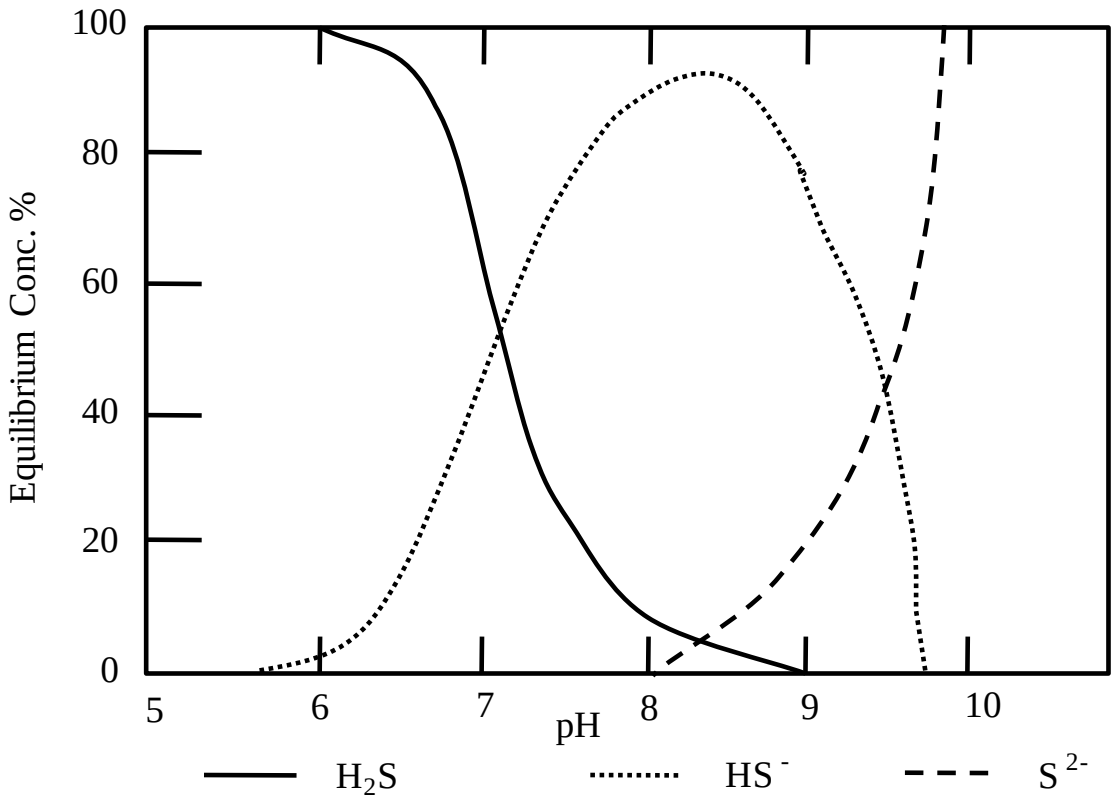


Figure 2.2 Distribution of sulphide species as a function of pH (adapted from Hao et al., 1996)

On the other hand, in sludge granules, 50% inhibition was found at H_2S concentrations of 250 and 90 mg/L at pH values of 6.4 to 7.2 and 7.8 to 8.0, respectively (Table 2.4). The inhibition of MPB is significantly higher at high pH values compared to that at lower pH range (Table 2.4). Internal pH gradients in granules (Koster et al., 1986), and mass transfer limitation from bulk liquid to bio-film (Overmeire et al., 1994; Visser et al., 1996) may explain why higher sulphide concentrations can be tolerated in bio-film reactors operating at neutral pH values (Parkin et al., 1991; Maillacheruvu et al., 1993).

Sensitivity of sulphide also depends on substrate utilized as per Millacheruvu et al. (1993). They concluded that lactate and glucose fed system can tolerate higher

sulphide level (100 - 150 mg/L as H_2S and 200 - 400 mg/L as TS) than acetate or propionate fed system. Although, in long term studies, they observed a cyclic pattern of variation in H_2S and TS levels in acetate, propionate, lactate and glucose system resulting in process failure. A complex interaction between SRB and MPB, inhibition, acclimation seemed to be potential factors for this cyclic pattern.

Sulphide inhibition was demonstrated as reversible by many researchers. Parkin et al. (1983) have reported that methane production was completely inhibited with a shock load of 500 mg/L as TS, but was fully recovered within 10 days when the TS concentration was lowered. Methane production recovered even after longer exposure (4 days) to higher TS concentration (1500 mg/L) in their study. Similar finding was reported by Reis et al. (1992) for SRB growth. They observed a complete inhibition of SRB growth with 547 mg/L as hydrogen sulphide. But the inhibitory effect was reversible as the SRB activity increased with H_2S stripping. Another research team also observed increased COD removal and sulphate reduction using an H_2S-stripped reactor concluding reversible sulphide toxicity (Oleszkiewicz & Hilton, 1986; Hilton & Oleszkiewicz, 1988). Isa et al. (1986b) have indicated a contradictory finding as they didn't observe any significant effect of hydrogen sulphide inhibition to SRB and MPB. Isa et al. (1986b) reported a 50% inhibition of methanogenesis at an extraordinary high hydrogen sulphide concentration of 1200 mg/L using both acetate and acetate/ethanol as substrate. Adaptation of MPB to hydrogen sulphide could be a probable explanation as they reported in their attached film reactor.

Table 2.4 Unionized Sulphide (H_2S) and Total Sulphide (TS) Concentrations (mg/L) causing a 50% Inhibition of Sulphate Reduction and Methanogenesis (adapted from Lens et al., 1998)

Sludge Type	Substrate	T (^oC)	pH	H_2S (mg/L)	TS (mg/L)	Reference
Sulphate reduction						
Sludge suspension	Lactate/acetate	35	7.2-7.6	NR	83	McCartney and Oleszkiewicz (1991)
Sludge suspension	Lactate	35	7.0	> 300	NR	McCartney and Oleszkiewicz (1993)
			8.0	185	2244	
Sludge suspension	Propionate	35	6.5-7.4	100	NR	Oleszkiewicz et al. (1989)
			7.7-7.9	60	NR	
Sludge suspension	Butyrate	35	6.5-7.4	235	NR	Oleszkiewicz et al. (1989)
			7.7-7.9	>200	NR	
Sludge suspension	Lactate	35	6.5-7.4	320	NR	Oleszkiewicz et al. (1989)
			7.7-7.9	390	NR	
Sludge granules	Acetate	30	7.2-7.4	171	615	Visser et.al. (1996)
			8.1-8.3	57	1125	

Table 2.4 continued

Sludge Type	Substrate	T (^{o}C)	pH	H_2S (mg/L)	TS (mg/L)	Reference
Sludge granules	Propionate	30	7.0-7.5	140	NR	Rinzema and Lettinga (1988)
Methanogenesis						
Sludge suspension	Acetate	35	6.5-7.4	125	NR	Oleszkiewicz et al. (1989)
			7.7-7.9	100	NR	
Sludge suspension	Lactate/acetate	35	7.2-7.6	NR	240	McCartney and Oleszkiewicz (1991)
Sludge suspension	Lactate	35	7.0	100	270	McCartney and Oleszkiewicz (1993)
			8.0	100	1258	
Sludge granules	Acetate	30	6.4-6.6	246	357	Koster et al. (1986)
			7.0-7.2	252	810	
			7.8-8.0	90	841	
Sludge granules	Acetate	30	7.2-7.4	184	564	Visser et al. (1996)
			8.1-8.3	38	590	

Note: NR = Not reported.

2.4.5 COD/SO_4^{2-} ratio

Sulphate reduction and methane formation can take place simultaneously during anaerobic digestion. Both sulphate reducers (SRB) and methane formers (MPB) can use hydrogen and acetate produced in the process as electron donors. Therefore, a competition for organic substrate exists between SRB and MPB. This competition is strongly dependant on the COD/SO_4^{2-} ratio (Isa et al., 1986a,b) in organic substrate. The importance of this ratio increases with the decrease of COD/SO_4^{2-} ratio in wastewater. Each mole of sulphate (96 g) needs 8 moles of electrons to be reduced which can be derived from a suitable electron donor such as acetate. Since each mole of electron is equivalent to 8 g of COD, the total theoretical COD requirement is 64 g to reduce one mole (96 g) of sulphate. Sulphate reduction by SRB follows the reaction below (Lens et al., 2002):

$$SO_4^{2-} + 8e^- + 4H_2O \rightarrow S^{2-} + 8OH^-$$

In the waste streams with COD/SO_4^{2-} ratio of 0.67, there is theoretically enough sulphate available for complete removal of organic matter as COD by sulphate reducing bacteria only (Rinzema and Lettinga, 1988). If sufficient organic matter in not present in the wastewater, addition of extra substrate is required for sulphate reduction (Omil et al., 1998).

Choi and Rim (1991) have reported that SRB out-compete MPB at COD/SO_4^{2-} ratios less than 1.7 (sulphate rich condition). They observed an active competition between them at COD/SO_4^{2-} ratios between 1.7 and 2.7. With a COD/SO_4^{2-} ratio of more than 2.7 (sulphate limiting condition), it was observed that MPB out-competed SRB. This

finding was supported by Freese & Stuckey (2004) who have reported a possible shift towards sulphate reduction when the COD/SO_4^{2-} ratio was decreased from 2 to 1. Colleran et al. (1995) have reported the work of Finnegan (1994) stating an increase in sulphidogenic activity from 38% to 52% when the COD/SO_4^{2-} ratio was decreased from 1.9 to 1.2. This clearly indicates that partitioning of reducing equivalents via sulphidogenic or methanogenic activity is governed mainly by influent COD/SO_4^{2-} ratio. It is also noteworthy; though the sulphate removal rate or sulphidogenic activity is higher with lower COD/SO_4^{2-} ratio, the degree of sulphate reduction improves with increasing COD/SO_4^{2-} ratios (Erdirencelebi et al., 2007). Lopes et al. (2007), Wang et al., (2008) also have reported similar findings though the operating conditions were slightly different.

The COD/SO_4^{2-} ratio plays a key role in determining the metabolic pathways for sulphate reduction. Several studies have reported completely different metabolic pathways for sulphate reduction based on different COD/SO_4^{2-} ratios (Uberoi and Bhattacharya, 1995; Colleran et al., 1995; McCartney and Olesziewicz, 1991). SRB are found to be more versatile in terms of their metabolic possibilities than MPB (Elferink et al., 1994). Some species of SRB can perform complete oxidation of organic substrate, while some others govern incomplete oxidation based on the relative sulphate level (COD/SO_4^{2-} ratio) in the influent (Lens et.al., 1998). In addition to the competitive interaction with MPB; SRB grow much faster than other syntrophic consortia at a relatively high concentration of sulphate to organic substrate (Elferink et al., 1994). Table 2.5 and 2.6 illustrates literature data on sulphate reduction in batch and continuous/semi- continuous operation.

Table 2.5 Sulphate reduction efficiency in batch studies

Reactor Type	COD/SO_4^{2-} ratio	Feed	Organic Carbon (mg/L)	Sulphate (mg/L)	Sulphate reduction (%)	Sulphate reduction (mg/L)	Reference
COD/SO_4^{2-}<1.7							
suspended	0.43	Ethylalcohol		3000	18.7	561	Cao et al. (2011)
suspended	0.6	Acetate	7820	13000	4.5	585	Erdirencelebi et al. (2007)
suspended	0.63-.69	Propionate	200	500	43	215	Uberoi & Bhattacharya (1995)
suspended	0.86	Ethylalcohol		1500	38.3	575	(Cao et al., 2011)
suspended	1.23	Acetate	8300	7500	4	300	Erdirencelebi et al. (2007)
suspended	1.25	Glucose	1870	1500	24	360	Sharma and Biswas (2010)
suspended	1.3	Ethylalcohol		1000	69.7	697	Cao et al. (2011)
suspended	1.7	Ethylalcohol		750	86.6	650	Cao et al. (2011)

Table 2.5 continued

Reactor Type	COD/SO_4^{2-} ratio	Feed	Organic Carbon (mg/L)	Sulphate (mg/L)	Sulphate reduction (%)	Sulphate reduction (mg/L)	Reference
$1.7< COD/SO_4^{2-}< 4$							
suspended	2.16	Ethylalcohol		600	94.3	566	Cao et al. (2011)
suspended	2.6	Ethylalcohol		500	93.2	466	Cao et al. (2011)
suspended	2.7	Acetate	8455	3560	9	320	Erdirencelebi et al. (2007)
suspended	3.03	Ethylalcohol		428.6	92.3	396	Cao et al. (2011)
$COD/SO_4^{2-}> 4$							
suspended	5.3	Acetate	9425	1775	12	213	Erdirencelebi et al. (2007)
suspended	18	Acetate	8940	607	36	219	Erdirencelebi et al. (2007)

Table 2.6 Sulphate reduction efficiency in semi-continuous/continuous studies

Reactor Type	COD/SO_4^{2-} ratio	Feed	Organic Carbon (mg/L)	Sulphate (mg/L)	Sulphate reduction %	Sulphate reduction (mg/L)	Reference
$COD/SO_4^{2-}<1.7$							
suspended	0.66	Propionate	1100	2500	68	1700	Uberoi and Bhattacharya(1995)
suspended	1.33	Propionate	1100	1250	92	1150	Uberoi and Bhattacharya(1995)
suspended	0.9	Acetic acid	6500	7278	12	873	Erdirencelebi et al. (2007)
suspended	1.47	Acetic acid	6900	4697	13	611	Erdirencelebi et al. (2007)
UASB	0.83	Sucrose	500	600	29-64	174-384	Harada et al. (1994)
UASB	1.16	Molasses	520	450	70	315	Annachhatre (2001)
UASB	1	Sucrose	2000	2000	25-35	500-700	Lopes et al. (2007)
Attached	1.5	Ethanol	4500	3000	99	2970	Sarti et al. (2010)

Table 2.6 continued

Reactor Type	COD/SO_4^{2-} ratio	Feed	Organic Carbon (mg/L)	Sulphate (mg/L)	Sulphate reduction %	Sulphate reduction (mg/L)	Reference
$1.7< COD/SO_4^{2-}< 4$							
suspended	2	Propionate	1100	833	99	825	Uberoi & Bhattacharya (1995)
suspended	3.32	Propionate	1000	500	99	495	Uberoi & Bhattacharya (1995)
suspended	3.25	Lactate	2200	1000	87	870	Oyekola et al. (2010)
suspended	3.25	Lactate	5500	2500	54	1350	Oyekola et al. (2010)
suspended	3.25	Lactate	11000	5000	58	2900	Oyekola et al. (2010)
suspended	3.25	Lactate	22200	10000	40	4000	Oyekola et al. (2010)
Suspended	3.15	Glucose	6945	2198	29	637	Erdirencelebi et al. (2007)
Suspended	2.7	Glucose	6760	3728	28	1044	Erdirencelebi et al. (2007)
UASB	3.33	Sucrose	500	150	94	141	Harada et al. (1994)
UASB	3.2	Glucose	4280	1337	70	936	Erdirencelebi et al. (2007)
UASB	3.33		3000	900	> 80	> 720	Annachhatre (2001)
Attached	1.8	Ethanol	5400	3000	99	2970	Sarti et al. (2010)

Table 2.6 continued

Reactor Type	COD/SO_4^{2-} ratio	Feed	Organic Carbon (mg/L)	Sulphate (mg/L)	Sulphate reduction %	Sulphate reduction (mg/L)	Reference
$COD/SO_4^{2-} > 4$							
Suspended	6.7	Glucose	6710	1000	15	150	Erdirencelebi et al. (2007)
UASB	20	Glucose	6270	305	86	262	Erdirencelebi et al. (2007)
UASB	13.2	Glucose	4710	357	73	261	Erdirencelebi et al. (2007)
UASB	10	Glucose	5270	600	80	480	Erdirencelebi et al. (2007)
UASB	5	Glucose	6670	1275	74	944	Erdirencelebi et al. (2007)
UASB	4	Glucose	4495	1125	81	911	Erdirencelebi et al. (2007)
UASB	4	Sucrose	2000	500	65	325	Lopes et al. (2007)
UASB	16.67	Sucrose	500	30	86	26	Harada et al. (1994)

2.5 Methanogenic Inhibition

Diversion of a fraction of reducing equivalents from the substrate towards methane production has been a major challenge for dissimilatory sulphate reduction. This has prompted research on finding methanogenic inhibitors to divert the reducing equivalents towards sulphate reducers to achieve a higher sulphide yield. Many researchers have studied the effect of different inhibitors to inhibit methanogenic activities. This section discusses some of the inhibitors, inhibition methods and challenges associated to them.

2.5.1 Heat Treatment

Many researchers have studied the effect of heat treatment on blocking certain species of methanogens selectively (Duangmanee et al., 2007; Lay, 2000; Okamoto, 2000). Heat treatment can inhibit non-spore forming methanogens. However, the high cost involved in this physical inhibition method makes it unpopular in full-scale application.

2.5.2 Chemical Inhibitors

Ammonia, produced by the biological degradation of nitrogenous matter, mostly in the form of proteins and urea (Kayhanian, 1999) can selectively inhibit methanogens among four types of anaerobic microorganisms. Koster and Letttinga (1988) reported a 56.5% inhibition in methanogenic activities when ammonia concentration was increased in the range of 4051 mg/L to 5734 mg/L. Free ammonia (FA) was suggested to be the main cause of inhibition since it is freely membrane-permeable (Kroeker et al., 1979; de

Baere et al., 1984). Several researchers have studied the problems associated with ammonia inhibition like pH (Kroeker et al., 1979; Hashimoto, 1983, 1984; Hansen et al., 1999; Borja et al., 1996; Zeeman et al., 1985), temperature (Braun et al., 1981), antagonistic effects due to the presence of Na^{+}, Ca^{2+} and Mg^{2+} (McCarty & McKinney, 1961; Braun et al., 1981; Hendriksen & Arhing, 1991). In addition, acclimation is another major factor associated with ammonia inhibition. Studies have reported methanogens to perform actively at high concentrations of ammonia, far exceeding the initial inhibitory concentration after adaptation (Kroeker et al., 1979; Parkin & Miller, 1983; Bhattacharya & Parkin, 1989; Angelidaki & Arhing, 1993).

Inhibition to methanogenic activities was shown using 2-bromoethanesulphonate or BES (Oremland & Capone, 1988; Scholten et al., 2000). BES is an analogue of a cofactor (mercaptoethanesulfonic acid, known as HS^{-} coenzyme M) unique to methanogens and is highly effective in blocking methanogens (Gunsalus & Wolfe, 1978). The cost of BES and its toxic discharge to the environment are the major disadvantages in full-scale application. Organic compounds which have been reported to be toxic to the anaerobic processes include alkyl benzenes (Yang & Speece, 1986; Renard et al., 1993), halogenated benzenes (van Beelen and van Vlaardingen, 1994), nitro benzenes (Bhattacharya et al., 1996a), phenol and alkyl phenols (Sierra-Alvarez & Lettinga, 1991; Soto et al., 1991; Fang et al., 1995), halogenated phenols (Shin & Kwon, 1998), nitrophenols (Borja et al., 1997; Uberoi & Bhattacharya, 1997; McCue et al., 2003), alkanes (Mormile & Suflita, 1996), halogenated aliphatics (Stuckey et al., 1980; Boucquey et al., 1995), alcohols (Dimirer & Speece, 1998), halogenated alcohols (Blum & Speece, 1991), aldehydes (Gonzales-Gil et al., 2002), ethers (Playne & Smith, 1983;

Hayward & Lau, 1989), ketones (Playne & Smith, 1983; Hayward & Lau, 1989), acrylates, carboxylic acids, amines, nitriles, amides (Blum & Speece, 1991; Stergar et al., 2003), pyridine and its derivatives (Liu et al., 1998). Most of the organic inhibitors are not specific inhibitors to methanogens and inhibit different microorganisms in anaerobic digestion.

2.5.3 Long Chain Fatty Acids (LCFAs)

Studies have reported LCFAs to be inhibitory for gram-positive microorganisms because their cell wall structure typically lacks the outer membrane, which is present in the gram-negative bacteria (Kabara et al., 1977). Hence, LCFAs can inhibit methanogens that have similar cell wall structure as gram-positive bacteria (Zeikus, 1977). Cherrington et al. (1991) have reported that before entering into the cells, LCFAs may exert antibacterial effects by disrupting several cell membrane components and inactivating many energy-linked reactions. For example, they interfere with K^+, Na^+, regular proteins and other cell proteins involved in maintaining cell homeostasis (Cherrington et al., 1991). Demeyer & Henderickx (1967); Rinzema et al. (1994) and Hwu et al. (1998) have reported that unsaturated LCFAs adhere to the bacterial cell wall by adsorption and alter the permeability of the cell, henceforth, limiting the transport of important nutrients.

In addition, Rinzema et al. (1989) have reported that flotation of sludge and consequent sludge washout may occur due to the sorption of LCFAs to biomass. Later, Hwu et al. (1996) supported this stating the dependence of LCFA toxicity was more based on physical characteristics of sludge (specific surface are, size distribution) than

their biological characteristics. For example, suspended and flocculent sludge having higher specific surface area, suffered much greater inhibition than granular sludge.

The advantages of using LCFA as an inhibitor to methanogens to divert electron fluxes towards sulphate reducers are two-fold: i) LCFAs are cost effective, readily available and can be derived from lipids and fats, which in turn, are produced from edible oil refineries, slaughterhouse and dairy products industries (Kramer, 1971), ii) LCFAs are degradable and electron equivalents from the degradation can be used by terminal electron acceptors.

Several researchers have studied the anaerobic degradation of LCFAs (Lalman & Bagley, 2002; Lalman & Bagley, 2001; Alves et al., 2001; Weng & Jeris, 1976; Novak & Carlson, 1970). Hydrogen producing acetogens can degrade LCFAs to acetate via a β-oxidation mechanism:

$$CH_3(CH_2)_nCOOH + 2H_2O \rightarrow CH_3(CH_2)_{n-2}COOH + CH_3COOH + 2H_2$$

Biodegradation of LCFA proceeds via several steps including adsorption onto the cell wall, movement across the cell membrane and LCFA conversion into a lower molecular weight component like acetate. The degradation products from each step are acetate, hydrogen and a LCFA with a reduction of two carbons in the alkyl group.

Many researchers have studied the effect of different LCFAs like linoleic acid - LA (C18:2), oleic acid - OA (C18:1), stearic acid – SA (C18:0), palmitic acid (C16:0), myristic acid (C14:0) etc. with varying concentrations. Angelidaki & Arhing (1995) have concluded that inhibition effect is concentration dependent. Their finding was supported by Lalman & Bagley (2001); Hwu et al. (1998); and Koster & Cramer (1987).

The extent of inhibition is known to increase with the increase of carbon chain length and with the increase in number of carbon double bonds. Lalman & Begley (2001) and Lalman & Bagley (2002) have reported that LA and OA bearing carbon double bonds are more inhibitory to both aceticlastic and hydrogenotrophic methanogens when compared to SA bearing no carbon double bonds. LA reduces the interfacial tension between the bacterial membrane and the bulk aqueous phase of growth medium and thus, acts as a surfactant. SA bearing no carbon double bond was reported to be a poor surfactant when compared to LA (Greenway & Dyke, 1979). Supporting this finding, Lalman (2000) has reported that the surfactant property due to the difference in chemical structure is responsible for high methanogenic inhibition in LCFAs bearing double bonds such as LA when compared to SA.

Sharma (2008) has studied the effect of LA (C18:2), OA (C18:1) and SA (C18:0) in mixed microbial communities in batch operation and concluded that LA diverts more than 30% electron fluxes towards sulphate reduction whereas OA contributes more than 20%. No significant effect due to SA was reported in this study. Sharma & Biswas (2010) have reported the potential for LA in inhibiting methanogens and diverting electron fluxes toward enhanced sulphate reduction (more than 92%) in batch studies. No study has reported the effect of LA in semi-continuous or continuous operations.

2.6 Two-Step Process

The low pH, high sulphate, and high metal concentrations are inhibitory to SRB and hinder the successful implementation of treatment technologies. To address this challenge, Al-Ani et al. (1995) proposed a two-step process, separating the SRB activities (biological reactor) from metal precipitation (chemical reactor). In the first step,

sulphides and alkalinity are produced by SRB that are channelled through the second step (chemical reactor) for metal sulphide precipitation from AMD. The metal free, sulphate rich effluents from the chemical reactor are added to the biological reactor to provide sulphate to SRB. Later on, Prasad & Henry (2009) proposed a three-step process by adding an alkaline unit to increase the pH for SRB activities. This system avoids the direct exposure of SRB to low pH and high metal concentrations in AMD. Figure 2.3 illustrates the two-step process for AMD treatment.

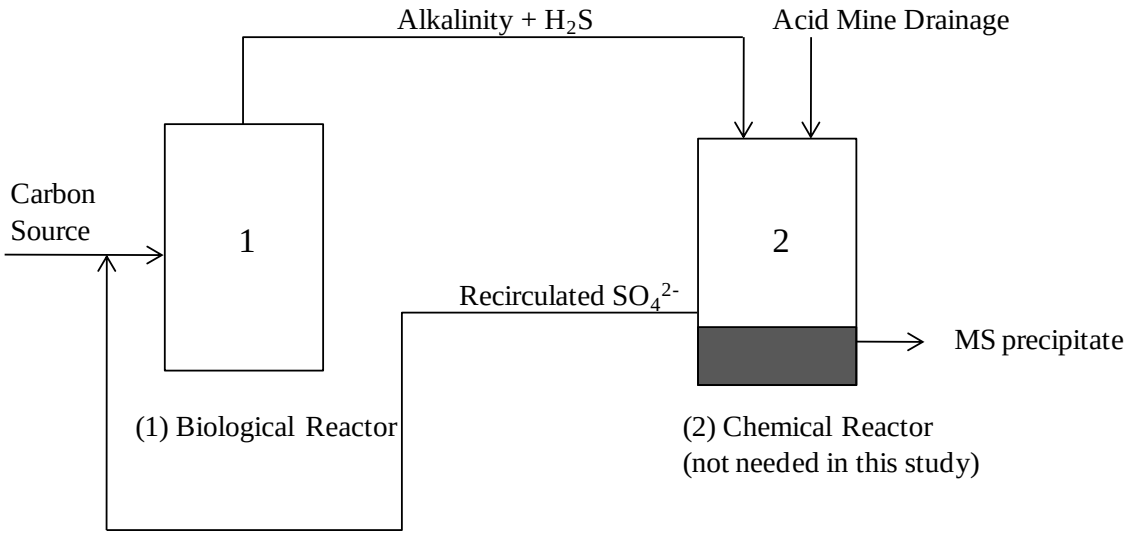


Figure 2.3 Two-step process for treatment of AMD (adapted from Al-Ani et al., 1995)

The current study focuses on the first step (biological reactor) of the two-step process to enhance sulphate reduction by inhibiting methanogens with linoleic acid.

CHAPTER III

EXPERIMENTAL DESIGN AND METHODOLOGY

This chapter focuses on the detailed experimental design and methodology adopted with an intention to accomplish the objectives of the study. Collection of inoculums, enrichment of SRB culture, substrate composition, operational conditions, analytical parameters and methods are discussed in this chapter along with experimental set-ups of six semi-continuous stirred tank reactors (SCSTRs).

3.1 Inoculums Source and Start-up

3.1.1 Seed Source

The seed for bacterial growth (10 L) was collected from anaerobically digested sludge from Municipal Wastewater Treatment Plant, Chatham, Ontario. The sludge was greyish black in colour and had distinctive odour of H_2S. The sludge was transported to University of Windsor and used to develop enriched SRB culture in the mother reactors.

3.1.2 Growth Medium

Modified Postgate Medium C (Postgate, 1984) was used for microbial growth. In the Postgate Medium C, lactate is proposed as a sole organic substrate or electron donor. Glucose was used instead of lactate in the current study for economic advantage. Na_2SO_4 was used for the sulphate source as electron acceptor. Table 3.1 shows the composition of modified Postgate Medium C.

Table 3.1 Composition of growth medium (modified Postgate medium C, adapted from Postgate, 1984)

Name	Chemical formula	Concentration (g/L)	Manufacturer
Potassium Phosphate	KH_2PO_4	0.5	ACP, Quebec, CA
Ammonium Chloride	NH_4Cl	1	ACP, Quebec, CA
Sodium Sulphate	Na_2SO_4	4.5	ACP, Quebec, CA
Calcium Chloride	$CaCl_2.6H_2O$	0.06	Aldrich, USA
Magnesium Sulphate	$MgSO_4.7H_20$	0.06	BDH, Toronto, CA
Glucose	$C_6H_{12}O_6$	6	ACP, Quebec,CA
Yeast Extract	-	1	Bio Basic, CA
Ferrous Sulphate	$FeSO_4.7H_2O$	0.004	BDH, Toronto, CA
Sodium Citrate	$Na_3C_6H_5O_7.2H_2O$	0.3	ACP, Quebec, CA
Ascorbic Acid	$C_6H_8O_6$	0.1	Sigma-Aldrich, CA

3.1.3 Start-up

Two SCSTRs, each of 4 L capacity, were termed as mother reactor M1 and M2. The substrate to sludge ratio was maintained at 2:2 and 1:3 in M1 and M2, respectively. Both reactors were operated semi-continuously at hydraulic retention time (HRT) of 40 days, replacing 400 ml of contents at an interval of every 4 days. The temperature was not controlled and ranged from 20 ^oC to 24 ^oC (ambient temperature). SRB growth was monitored by analyzing the samples at interval of 4 days. Data obtained from M1 and M2 are presented in Appendix A and B.

3.2 Experimental Design

Three sets of SCSTRs with 600 ml enriched microbial communities (from M1) were termed as R1 & R1C, R2 and R2C, R3 and R3C, respectively. Glucose was varied to maintain three different COD/SO_4^{2-} ratios in the influent. However, the concentration of sulphate in influent of all six reactors was maintained constant at 3095 mg/L. During Phase I, the effect of varying COD/SO_4^{2-} ratios on sulphate reduction was investigated.

After the end of Phase I, all six reactors were carried through Phase II to investigate the effect of linoleic acid (LA). Initially, LA was added to R1, R2, and R3 and its concentration in the reactors was maintained at 250 mg/L. The effect of LA on sulphate reduction was observed. LA was not added in the remaining three reactors (R1C, R2C and R3C). These reactors were used as controls. The effect of LA was also investigated with two higher concentrations of LA (500 mg/L, 1000 mg/L) in reactor with lower COD/SO_4^{2-} ratio of 0.75. Table 3.2 shows the design matrix of the experiment.

Table 3.2 Design Matrix of Experiments

Reactor	Phase I	Phase II
	COD/SO_4^{2-} ratio	Linoleic acid
R 1	4.66	√
R 1C	4.66	×
R 2	1.96	√
R 2C	1.96	×
R 3	0.75	√
R 3C	0.75	×

3.3 Experimental Duration

All six reactors were operated semi-continuously for 90 days during phase I to investigate the effect of COD/SO_4^{2-} ratios. After the end of phase I, same reactors were carried through phase II. Table 3.3 shows the experimental durations for both phases.

Table 3.3 Experiments duration for Phase I and Phase II

Reactors	COD/SO_4^{2-}	Operational duration (Days)	
		Phase I	Phase II
R1	4.66	0 – 90	LA: 250 mg/L; 0 – 40
R1C	4.66	0 – 90	0 – 40
R2	1.96	0 – 90	LA: 250 mg/L; 0 – 100
R2C	1.96	0 – 90	0 – 215
R3	0.75	0 – 90	LA: 250 mg/L; LA: 500 mg/L (0 – 70); LA: 1000 mg/L (170 – 215)
R3C	0.75	0 – 90	0 – 140
R3 Prime	0.75		LA: 1000 mg/L; 140 – 170 – 215

3.4 Experimental Set-Up

Figure 3.1 and Figure 3.2 show the individual and combined reactor configurations, respectively. The setups of all six SCSTRs were identical. They were kept in a water bath to maintain the temperature at 37 ± 1 ^{o}C. Each reactor consisted of the following components:

- Sampling tube (sealed)
- Feeding tube (sealed)
- Gas collection tube
- Vent tube (sealed)
- Magnetic stirrer
- Gas collection bottles

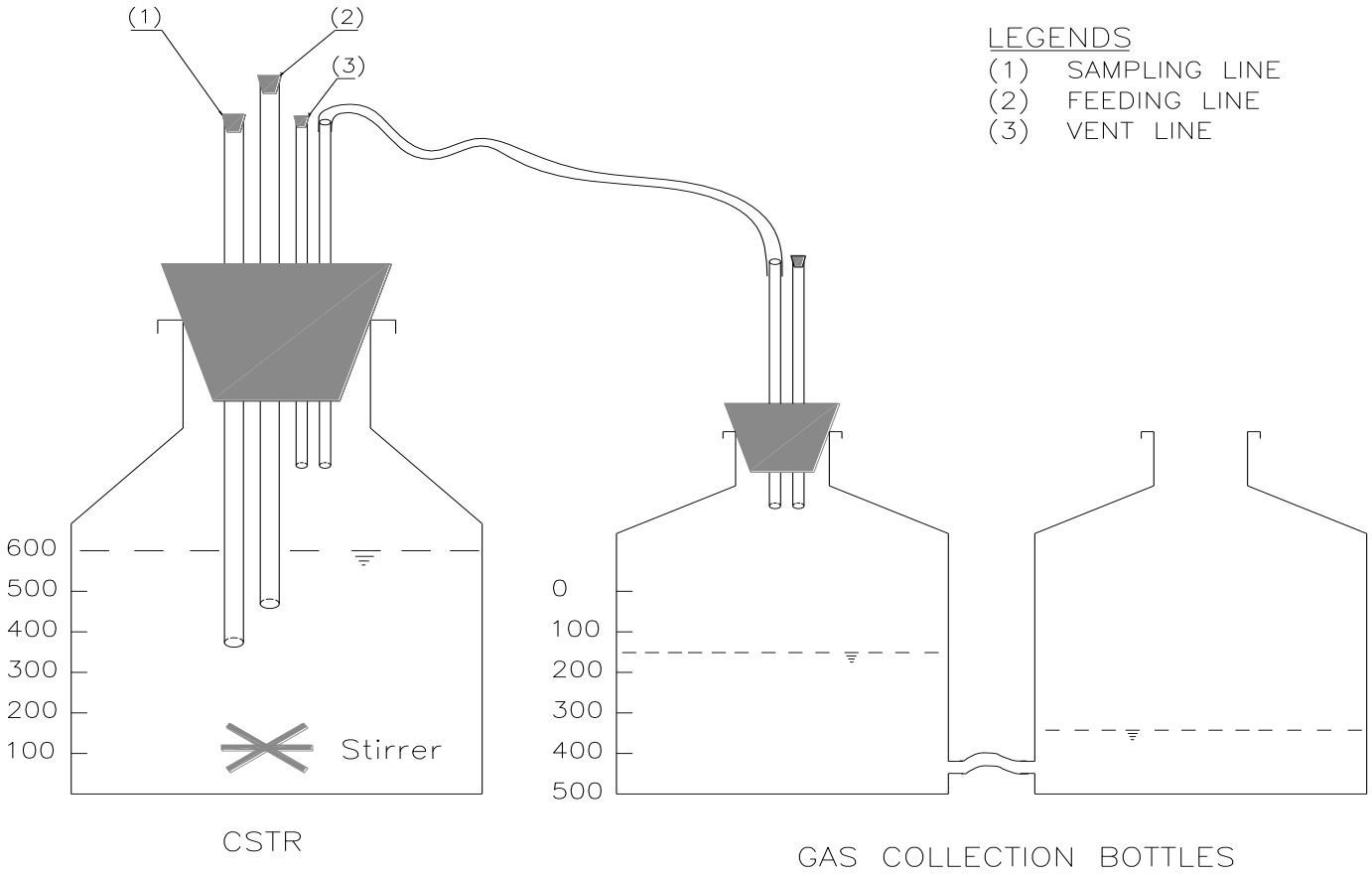


Figure 3.1 Individual reactor configuration

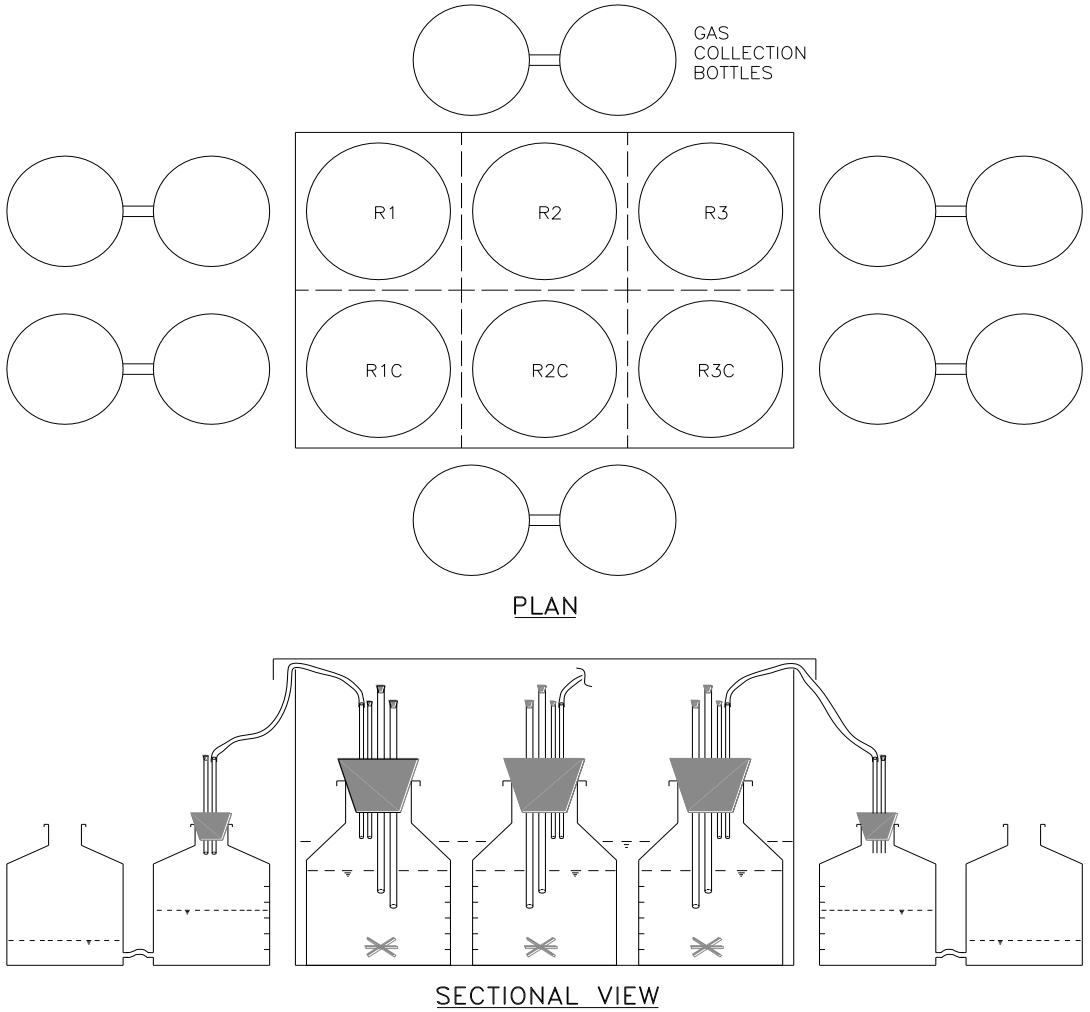


Figure 3. 2 Combined reactor configuration

3.5 Operating Procedure

Reactors were operated semi-continuously at HRT of 40 days. A volume of 75 ml of the content was withdrawn every 5^{th} day and was replaced with synthetic substrate. A gas-bag was connected to the vent tube while withdrawing samples as well as while feeding to maintain pressure and anaerobic conditions inside the reactor. Separate sampling and feeding lines were used to avoid contamination. Temperature was maintained at 37 ± 1 ^{o}C using a water bath for optimal bacterial growth.

3.6 Analytical Parameters

Reactor samples were analyzed in duplicate for pH, oxidation-reduction potential (ORP), sulphate, total organic carbon (TOC), alkalinity and total volatile fatty acids (VFA). All samples were filtered with Glass Micro fibre filters (Whatman 934-AH) prior to sulphate, TOC, alkalinity and total VFA analyses.

3.6.1 pH

Measurement of pH was used as an indicator of the environmental conditions of the reactors as well as their performance. The pH of the samples were measured immediately after sampling. A VWR Symphony pH electrode in combination with Oaklon pH meter as per the Standard Method (APHA, 1998. The pH meter was calibrated with pH buffers, 4 and 7, prior to each day's measurements.

3.6.2 Oxidation Reduction Potential

Oxidation Reduction Potential (ORP) was measured with Orion 9678BNW ORP Probe as per the Standard Methods for the Examination of Water and Wastewater (1998). The reading was taken after 5 minute of contact time of probe with sample so that the probe was at equilibrium.

3.6.3 Alkalinity and Total Volatile Fatty Acids

Alkalinity and total volatile fatty acids (VFA) were determined by the direct titration method (DiLallo & Albertson, 1961). These two parameters are indicative of biochemical environment of the reactors. Total alkalinity and total VFA were measured by titrating to pH 4 with 0.1N H_2SO_4 and titrating from pH 4 to pH 7 with 0.05N NaOH, respectively. NaOH was standardized each time prior to analysis. A sample volume of 10 mL was used for each analysis.

3.6.4 Sulphate

Sulphate was analyzed by the Gravimetric Method with Drying of Residue as per Standard Methods: 4500-SO_4^{2-} D (APHA, 1998). This method was selected because of its flexibility of analyzing samples with high sulphate concentrations (>10 mg/L). The basic principle of this method is the precipitation of $BaSO_4$ in acidic medium with $BaCl_2$. Sulphate values are determined by weighing $BaSO_4$. In the current study, all the samples were analyzed in duplicates. The sulphate values were reported as SO_4^{2-}. A sample volume of 10 mL was used for each analysis.

3.6.5 Total Organic Carbon

Total organic carbon (TOC) is a convenient parameter to measure organic carbon since it does not measure other organically bound elements like nitrogen, hydrogen and other inorganics that can contribute to BOD and COD. TOC was measured with TOC analyzer (Shimadzu TOC-V_{CSH}) as per Standard Methods: 5310 B (APHA, 1998). Samples were acidified with H_2SO_4 and the pH values were reduced to less than 2 prior to analysis. Calibration curves were prepared for both total carbon (TC) and inorganic carbon (IC) with known standards (Appendix D and E). TOC values were obtained by

calculating the difference between TC and IC values and multiplying by respective dilution factors. A coefficient of variance (CV) of < 2% was accepted in duplicate injections.

3.6.6 Chemical Oxygen Demand (COD)

Chemical Oxygen Demand (COD) was measured by Closed reflux, Colorimetric Method as per Standard Methods: 5220 D (APHA, 1998) using 20 x 150 mm culture tubes. A sample volume of 5 mL with digestion solution and acid reagent was digested in a Bioscience COD reactor followed by measuring the absorbance at 600 nm using a Varian-Cary 50 spectrophotometer. Standard potassium hydrogen phthalate was used to prepare a calibration curve in the range of 100 mg/L to 800 mg/L concentrations (Appendix F).

3.6.7 Gas production

Gas production from each reactor was measured by volume displacement technique using calibrated aspirator bottles that were filled with saturated NaCl in water.

CHAPTER IV

ANALYSIS OF RESULTS

Phase I

Three sets of reactors with varying COD/SO_4^{2-} ratios of 4.66, 1.96 and 0.75 were operated semi-continuously with a hydraulic retention time (HRT) of 24 days at a temperature of 37 ^oC. Each set consisted of two identical reactors. The detailed of operating conditions are noted in Chapter 3. Comparison in terms of sulphate reduction of the current study with respect to batch and semi-continuous or continuous operations in previous studies is discussed in this phase.

4.1 Reactor Start-up

All three sets of reactors were operated with a HRT of 24 days maintaining an organic loading rate of 625 mg COD/L/d, 312 mg COD/L/d and 156 mg/L/d for reactors with COD/SO_4^{2-} ratios of 4.66, 1.96 and 0.75, respectively. The pH was observed to gradually drop in all the reactors. After an operational period of 20 days, pH was in the range of 5 – 6. The difference in growth rates of acid formers and SRBs/MPBs may cause this pH drop. Acid formers are relatively fast growing microorganisms compared to SRB or MPB. The slow growing terminal electron acceptors (SRB and MPB) may not have been able to consume the VFAs and short chain fatty acids (SCFAs) produced by the acid formers causing the pH to drop. To allow the SRB/MPB to recover, the feeding was skipped for two consecutive feeding cycles to allow sufficient time for the terminal electron acceptors to consume the built up VFAs. The pH was expected to rise up. Instead, the pH kept on decreasing to 4.5 ± 0.3 giving indications of reactor upset. The results

suggested that the chosen HRT of 24 days might be too short to allow the slow growing SRB/MPB to consume the produced VFAs. Hence, it was decided to shut down the reactors and start afresh with a longer HRT of 40 days.

4.2 Reactors operation with 40 days of HRT:

All six reactors of 600 ml were inoculated from reactor M1 (2:2) and were operated with similar conditions with a HRT of 40 days. The initial sulphate, TOC and pH were 19 mg/L, 70 mg/L and 6.9 respectively. The sulphate loading rate (77 mg SO_4^{2-}/L/d) was kept constant for all reactors. The organic loading rates were maintained as 361, 152 and 58 mg COD/L/d, in reactors with COD/SO_4^{2-} ratios of 4.66, 1.96 and 0.75 respectively. Data obtained in terms of sulphate reduction and other controlling parameters are discussed during Phase I.

Reactor operation was disrupted due to transfer of the reactor set-up from one laboratory to another. The reactors were at ambient temperature (~ 22 C) for Days 74 - 76. $NaHCO_3$ at a concentration of 4500 mg/L began to be added with substrate from Day 80 onwards in both reactors to enhance buffering and maintain identical conditions in all six reactors. Both reactors (with same COD/SO_4^{2-} ratio) were mixed anaerobically at the end of Phase I to allow for the reactors to have a similar starting point during Phase II of the experiments.

4.2.1 Reactors with COD/SO_4^{2-} = 4.66

The results are presented in Figures 4.1 – 4.4. Though both the reactors were started with the same culture in similar conditions, it took almost 3 weeks to reach at a steady sulphate reduction. Another group (Sam-soon et al., 1991) worked with the same substrate (glucose) in their UASB system and reported the same time duration for steady sulphate reduction. Almost complete removal (99%) of influent sulphate (3095 mg/L) was observed. TOC showed a significant variability at the beginning, but from Day 35 onwards, similar TOC levels for both the reactors were observed. Other controlling parameters such as pH, VFA (Day 50 onwards) were within a similar range for both reactors (pH: 6.7 ± 0.1 and 6.9 ± 0.1; VFA: 706 ± 106 and 723 ± 134 mg/L respectively).

The previous study of Erdirencelebi et al. (2007) reported 74 - 81% of sulphate reduction in a continuous UASB system with an influent sulphate level of 1100 – 1200 mg/L. Lopes et al. (2007) and Harada et al. (1994) worked with similar substrate (sucrose) and reported 65% and 86% sulphate reduction respectively. But the sulphate levels in these studies were significantly lower than the current study. Though, limited data is available for glucose-fed suspended growth systems, in general, the current study has observed a highest sulphate removal than most other previous studies.

Reactor operation was upset due to transfer of the reactor set-up from Day 70 onwards. One feeding cycle was skipped to allow the reactors to recover. Despite that, significantly higher VFA level was observed in R1 (1438 mg/L) than R1C (909 mg/L) which lead R1 to have a pH of 6.1 whereas the pH of R1C was observed as 7.06. $NaHCO_3$ at a concentration of 4500 mg/L was added with the substrate from Day 80 onwards in both the reactors to enhance buffering and prevent reactor upset due to lowering of the pH.

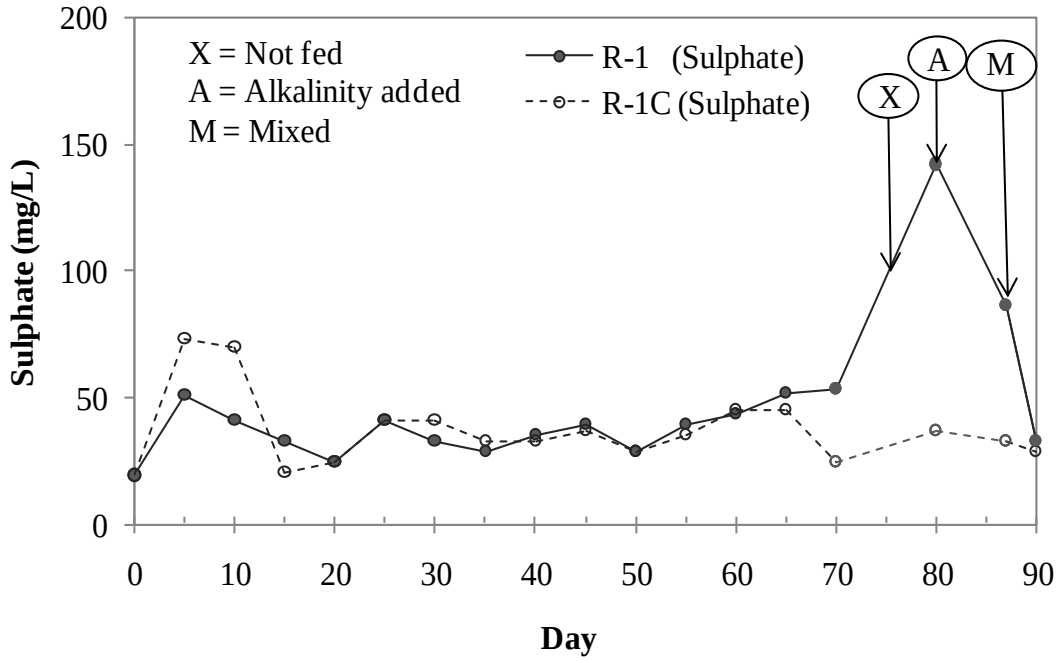


Figure 4.1 Sulphate vs. Time in Reactors with COD/SO_4^{2-} = 4.66

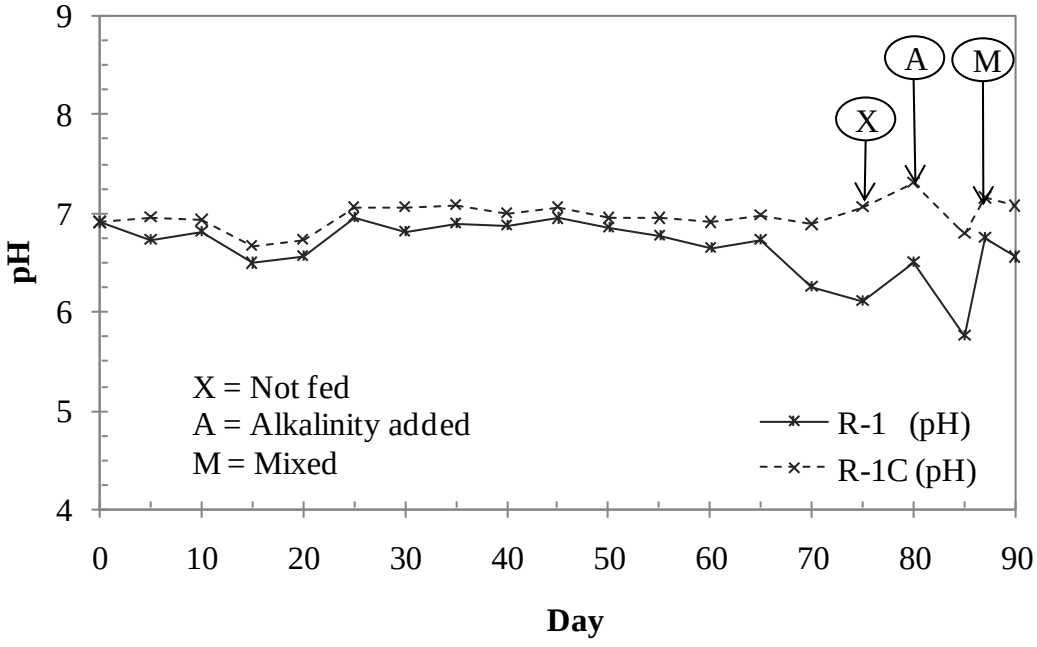


Figure 4.2 pH vs. Time in Reactors with COD/SO_4^{2-} = 4.66

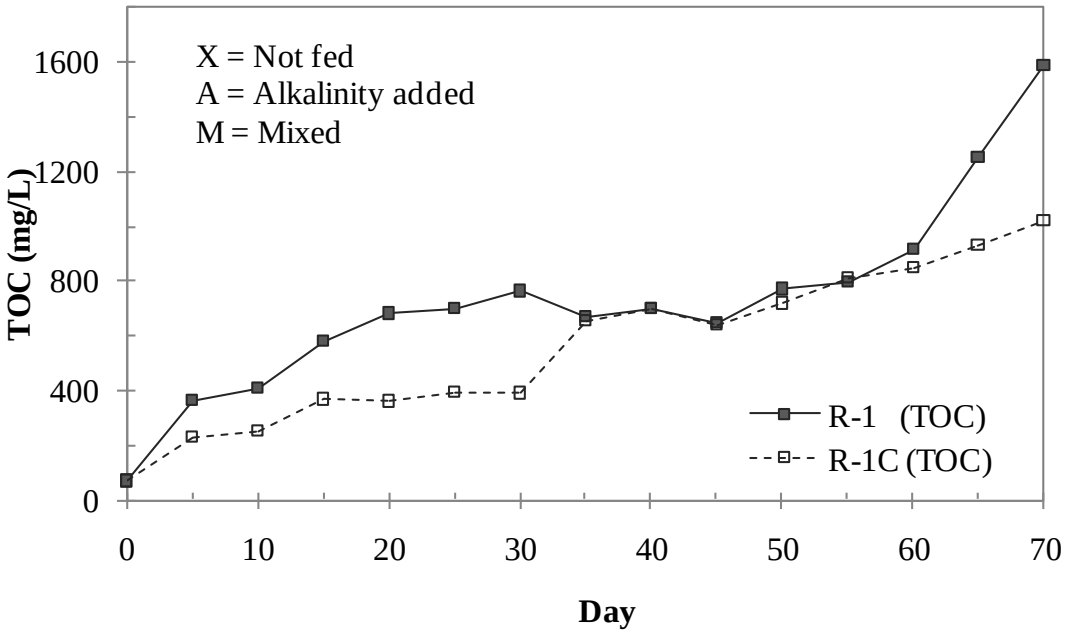


Figure 4.3 TOC vs. Time in Reactors with COD/SO_4^{2-} = 4.66

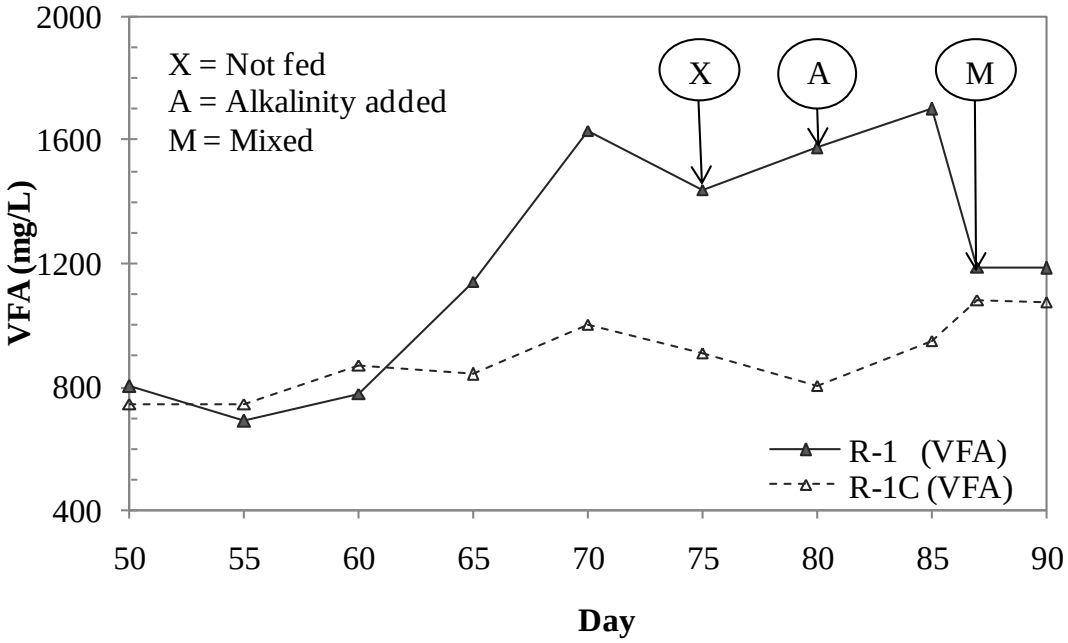


Figure 4.4 VFA vs. Time in Reactors with COD/SO_4^{2-} = 4.66

Despite the identical feeding cycle, the performance of the two reactors was seen to diverge. The exact reason for this diversion is not known. However, maintaining a balance between fast growing acid formers and slow growing terminal electron acceptors (SRB and MPB) has been shown to be major challenge for steady operation of anaerobic sulphidogenesis in suspended cultures, particularly at higher COD/SO_4^{2-} ratios. Stability of such systems has been shown to increase with lowering COD/SO_4^{2-} ratio (Colleran et al., 1995). Another potential reason could be because of sulphide, the end product of anaerobic sulphate reduction. It is known to be a potential toxin to both SRB and MPB. Various studies have reported that toxicity due to the elevated levels of sulphide lead to process failure (Karhadkar et al., 1987; Parkin et al., 1983; Rinzema & Lettinga, 1988; Speece, 1983). But the exact reason for the unexpected behavior of one of the reactors is not quite clear, though both of the reactors were seeded with same culture and operated in similar conditions.

4.2.2 Reactors with COD/SO_4^{2-} = 1.96

Results are presented in Figures 4.5 – 4.8. Sulphate levels were seen to increase during the first three weeks of reactor operation, subsequent to which they started to decline. This suggested acclimation and establishment of SRB in the reactors. Average sulphate reduction of 87 ± 3% and 84 ± 4% of influent sulphate concentration of 3095 mg/L were observed in R2 and R2C respectively during Day 0 - 50. Almost complete TOC removal was observed in both reactors. The pH was observed to be similar in both reactors (R2: 7.1 ± 0.2 and R2C: 7.3 ± 0.2). VFA level in R2 (290 ± 28 mg/L) was higher than in R2C (148 ± 2). This difference in VFA levels in this set of reactors (COD/SO_4^{2-} =

1.96) didn't seem to have much impact on sulphate reduction as the VFA levels were significantly lower (145 – 318 mg/L) than that observed in R1 and R1C (550 – 800 mg/L) during Day 0 – 50.

Greater stability was observed in reactor operation from Day 50 onwards. Sulphate reduction improved slightly, 92 ± 3% and 92 ± 2% in R2 and R2C, respectively. TOC removal was also similar in both reactors and comparable with what observed during Day 0 – 50. Other controlling parameters such as pH, VFA (up to Day 75) were within a similar range for both reactors (pH: 7.0 ± 0.1 and 7.3 ± 0.1; VFA: 210 ± 72 and 173 ± 56 mg/L, respectively). As a result of the disruption of reactor operation, VFA built up of 412 mg/L was observed in R2 on Day 80. It was observed that sulphate reduction was affected when the VFA level increased more than 1600 mg/L in R1 (COD/SO_4^{2-} = 4.66) from Day 70 onwards. More favourable and stable pH and lower VFA levels may be responsible for the more stable performance.

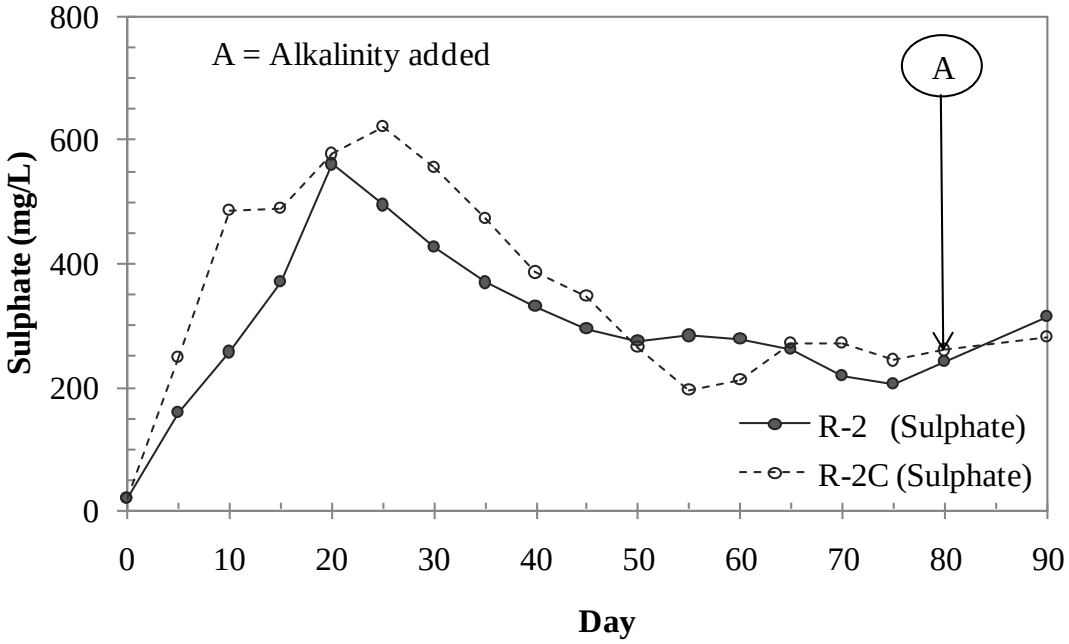


Figure 4.5 Sulphate vs. Time in Reactors with COD/SO_4^{2-} = 1.96

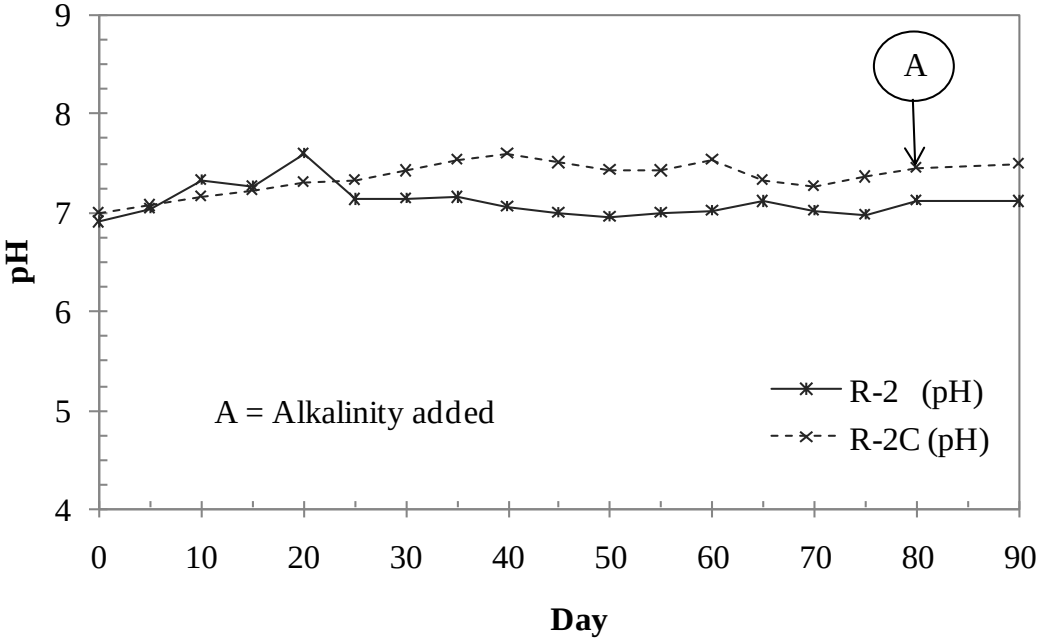


Figure 4.6 pH vs. Time in Reactors with COD/SO_4^{2-} = 1.96

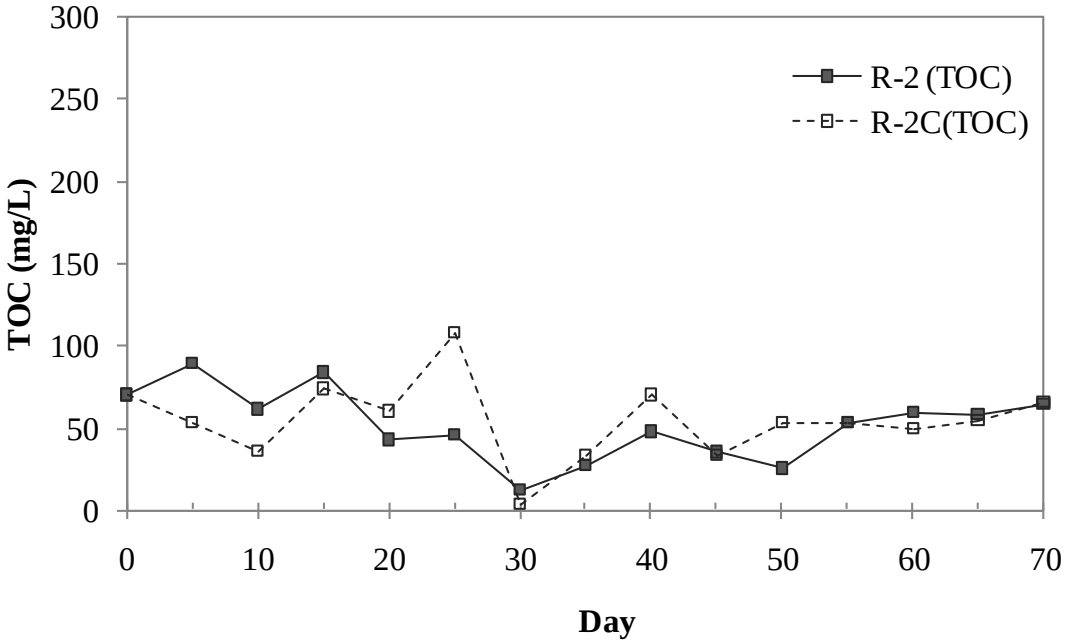


Figure 4.7 TOC vs. Time in Reactors with COD/SO_4^{2-} = 1.96

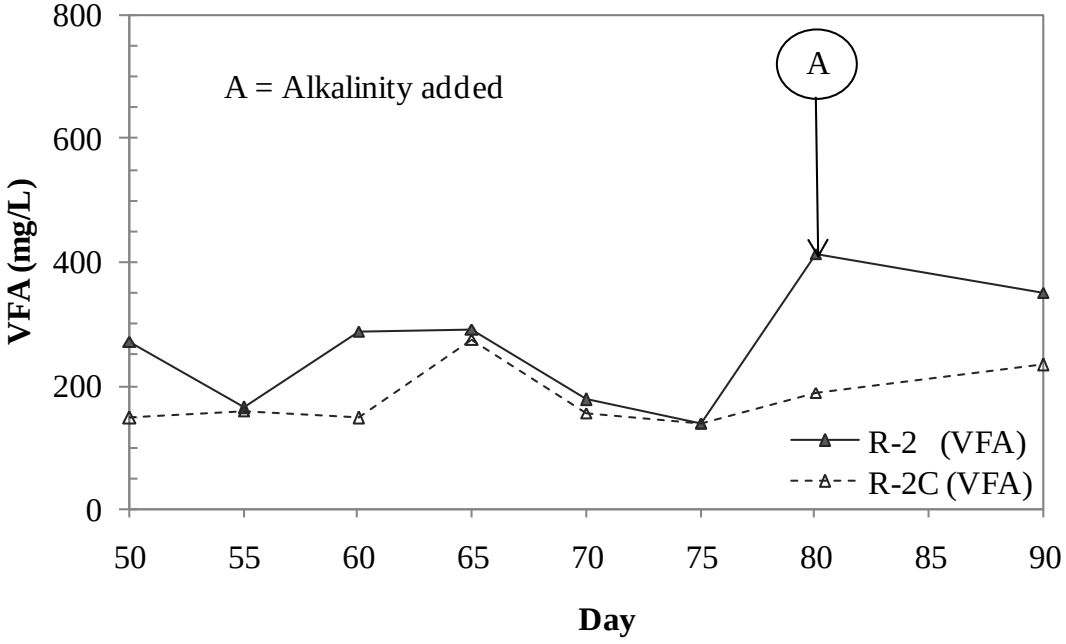


Figure 4.8 VFA vs. Time in Reactors with COD/SO_4^{2-} = 1.96

Limited data is available with regard to studies with suspended growth glucose systems with similar influent sulphate levels. Erdirencelebi et al. (2007), in their glucose-fed suspended system, reported a 28% reduction efficiency with 1044 mg/L of actual sulphate reduction, which was significantly lower than that observed in the current study. Their influent sulphate level (3728 mg/L) was higher than that (3095 mg/L) of the current study. Higher sulphate removal efficiency was observed in continuous or semi-continuous operations in the studies of Oyekola et al. (2010) and Uberoi & Bhattacharya (1995). Uberoi & Bhattacharya (1995) reported 99% sulphate removal efficiency though the influent sulphate level in their study ranged between 200 and 500 mg/L. Oyekola et al. (2010) worked with comparatively higher sulphate levels and reported a removal efficiency of 87%. Though sulphate removal efficiency was observed to be similar with that observed in the current study, the influent sulphate level (1000 mg/L) was significantly lower in their lactate system (Postgate medium B). They also studied the reduction efficiency with increasing influent sulphate levels of 2500, 5000 and 10000 mg/L and reported sulphate reduction efficiencies of 54%, 58% and 40% respectively. Though the sulphate removal efficiency was reported to decrease with increasing sulphate levels in the influent, the actual sulphate reduction (1350, 2900, 4000 mg/L respectively) increased. Sulphate reduction observed in the current study was significantly higher (2610 mg/L) than that of comparable studies.

4.2.3 Reactors with COD/SO_4^{2-} = 0.75

Results are presented in Figures 4.9 – 4.12. Sulphate level started to increase as the starting sulphate level (19 mg/L) was significantly lower in the source culture, which was obtained from M1 (COD/SO_4^{2-} = 2.15). The increasing trend was more rapid up to Day 20 and the rate started to decrease gradually from Day 20 onwards, which is indicative of reactors reaching to a steady state condition. Almost complete TOC removal (94%) was observed in both reactors from an influent TOC concentration of 1000 mg/L. Both reactors were identical during this period in terms of pH and VFA levels as well. Average pH of 7.2 ± 0.1 and 156 ± 5 mg/L of VFA was observed in both reactors during this period. Unlike with other two sets of reactors with COD/SO_4^{2-} ratio of 4.66 and 1.96; this set of reactors (with COD/SO_4^{2-} = 0.75) showed greater stability in their operations. Literature data support the fact that reactors with lower COD/SO_4^{2-} ratios showed greater stability than with high COD/SO_4^{2-} ratios (Colleran et al., 1995).

The increasing trend of sulphate levels in the previous period, stabilized during Day 50 onwards. Slightly higher sulphate reduction (47 ± 3%) was observed in R3C than in R3 (44 ± 2%) from an influent sulphate level of 3095 mg/L. Almost complete removal (95%) of TOC was observed in both reactors. Other controlling parameters such as pH, VFA were within a similar range for both reactors (pH: 7.4 ± 0.1 and 7.3 ± 0.1; VFA: 143 ± 13 and 144 ± 13 mg/L respectively.

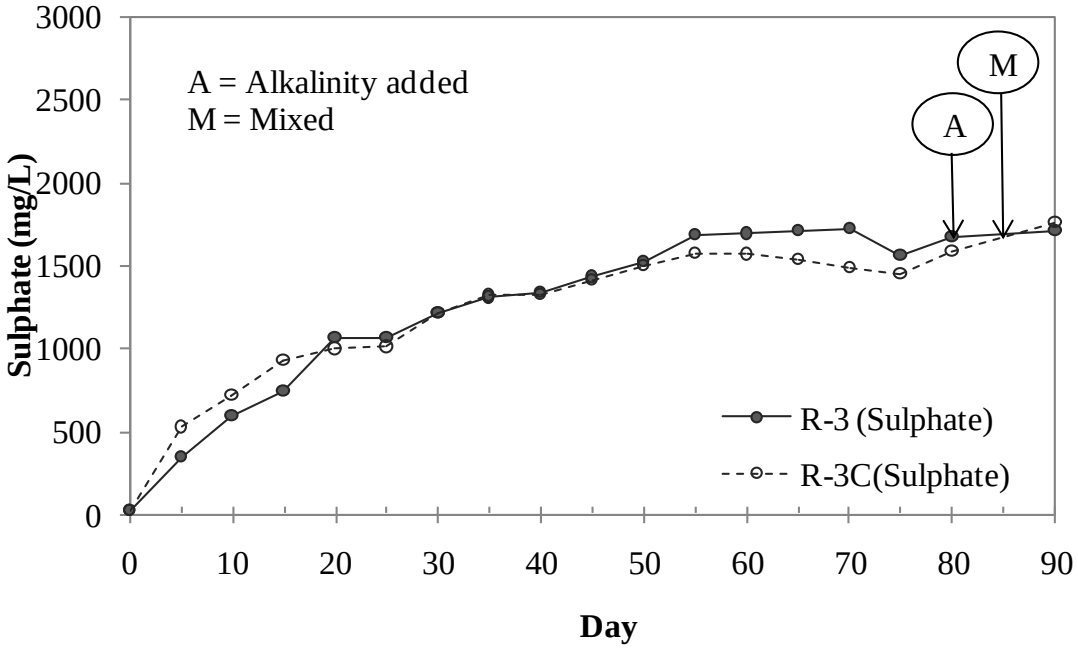


Figure 4.9 Sulphate vs. Time in Reactors with $COD/SO_4^{2-} = 0.75$

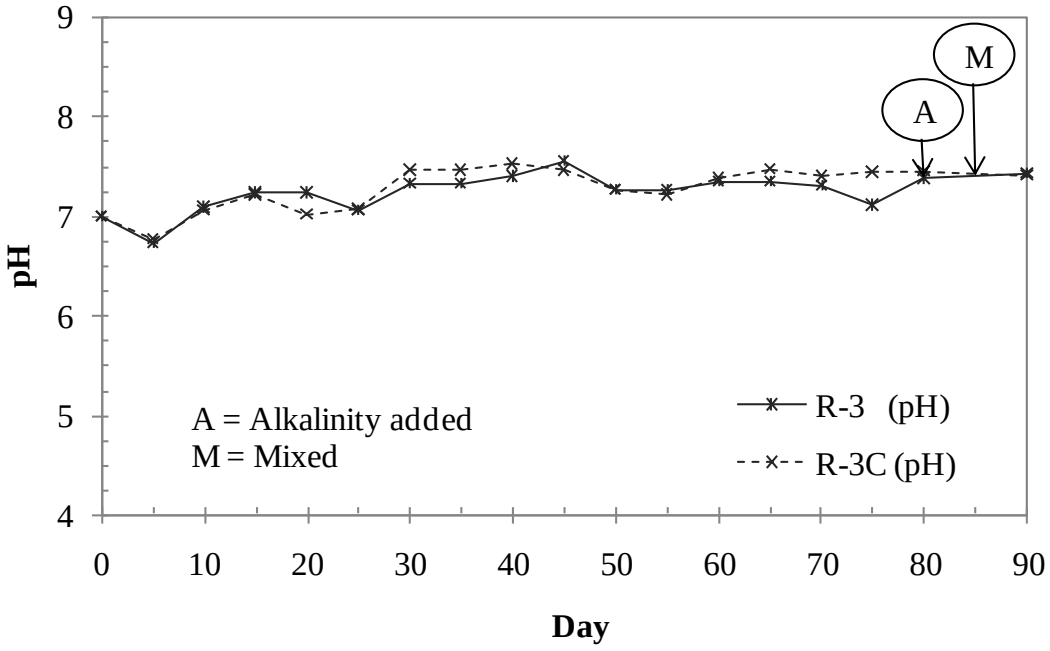


Figure 4.10 pH vs. Time in Reactors with $COD/SO_4^{2-} = 0.75$

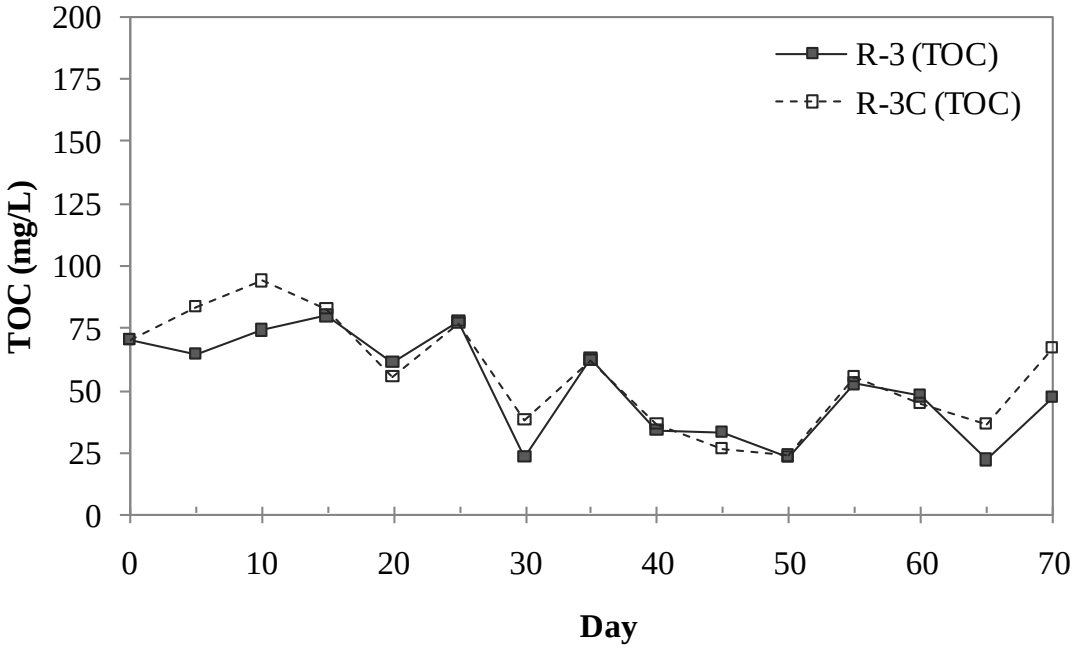


Figure 4.11 TOC vs. Time in Reactors with COD/SO_4^{2-} = 0.75

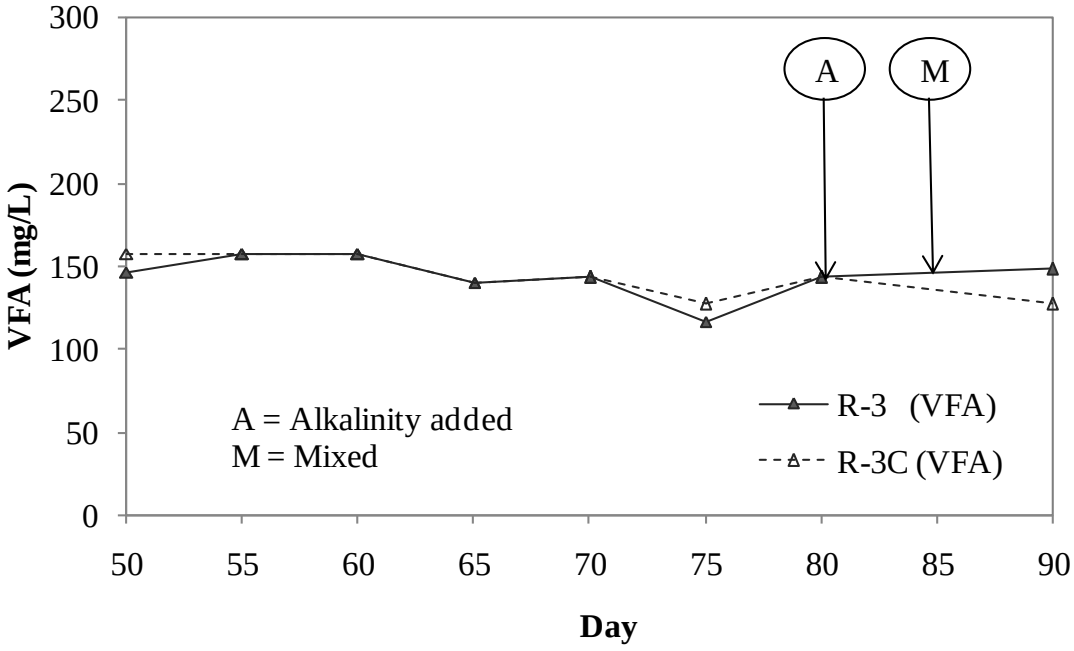


Figure 4.12 VFA vs. Time in Reactors with COD/SO_4^{2-} = 0.75

Limited data is available with regard to studies with suspended growth glucose system in similar influent sulphate levels. Erdirencelebi et al. (2007) reported 13% of sulphate reduction in a continuous UASB system with an influent sulphate level of 4697 mg/L that is higher than that in the current study. Lopes et al. (2007) and Harada et al. (1994) worked with similar substrate (sucrose) and reported 25% to 35% and 29% to 64% sulphate reduction, respectively. The later study worked with low sulphate level of 600 mg/L whereas, Lopes et al., (2007) worked with comparatively higher influent sulphate of 2000 mg/L. Both the sulphate levels in these studies were significantly lower than that (3095 mg/L) in the current study. Sarti et al. (2010) worked with sulphate level of 3000 mg/L, same as the current study, and reported very high sulphate removal efficiency of 99%. But they worked with attached growth system instead of suspended system. However, the current study involved suspended growth system, and therefore, the results should be compared with comparable system, such as the study by Uberoi & Bhattacharya (1995). They worked with two different sulphate levels, 1250 mg/L and 2500 mg/L, of which, the latter is not too different from the current study. In their study, the reduction was 92% and 68%, respectively. The current study observed an average sulphate reduction of 47-52% at steady state operation with glucose.

Phase II

4.3 Effect of Linoleic Acid (LA)

In this phase, linoleic acid (LA) was added to R1 (COD/SO_4^{2-} = 4.66), R2 (COD/SO_4^{2-} = 1.96) and R3 (COD/SO_4^{2-} = 0.75) to maintain a concentration 250 mg/L of LA in the reactors. Modified substrate with LA at a concentration of 250 mg/L of the substrate was added in the subsequent feedings to maintain the same level in the reactors. The other reactors (R1C, R2C and R3C) were not fed with LA and were used as the controls. The effect of LA on sulphate reduction was investigated comparing the reactors with LA to their respective controls. The effect of LA on sulphate reduction was also tested with two higher concentrations of 500 mg/L and 1000 mg/L of LA in R3 (COD/SO_4^{2-}= 0.75). Additional alkalinity continued to be provided to R1/R1C for the entire period of Phase II. For the other two sets of reactors additional alkalinity was discontinued from Day 70 onwards and it was decided to add it whenever needed.

4.3.1 Reactors with COD/SO_4^{2-} = 4.66

Results are presented in Figures 4.13 – 4.16. LA at a concentration of 250 mg/L was added on Day 0 in R1 and R1C was used as control. Sulphate reduction was similar (98%) in R1C until Day 20, as before. The TOC and VFA levels rose slightly. The initial pH on Day 0 was higher (7.5) than previously observed (6.9 ± 0.1). Addition of $NaHCO_3$ as buffer may potentially cause this increase in pH. But the average pH (7.1 ± 0.2) didn't seem to change significantly. After the addition of LA, sulphate reduction did not increase in R1 during Day 0 to Day 25. Similar sulphate levels of 49 ± 35 mg/L and 47 ± 30 mg/L were observed in R1 and R1C (control) respectively, showing 98% sulphate reduction in both reactors from Day 0 to Day25. Although, sulphate reduction did not increase in R1, a

more significant increase in VFA level was observed in R1 than in R1C due to the LA addition. High VFA level (1901 ± 61 mg/L) in R1 than R1C (1157 ± 50 mg/L), is indicative of methanogenic inhibition due to LA. Hence, the fraction of organic carbon, which was being consumed, previously by MPB resulted in elevated VFA level in R1due to the inhibition. Sulphate reduction was expected to increase due to this methanogenic inhibition. It did not increase because of sulphate limiting conditions as 98% sulphate was already reduced without adding LA.

The control (R1C) started to deviate from the steady state after Day 25 onwards. Decrease in sulphate reduction efficiency from 98% to 86% was clearly indicative of this deviation. A similar sulphate level (107 mg/L) was observed in both reactors on Day 25 and was observed to be first affected on Day 30 showing a level of 268 mg/L. This increasing trend continued until Day 40 showing sulphate concentration as high as 415 mg/L. Hence, it was decided to discontinue the operation R1C. The exact reason for this reduction in sulphate reduction efficiency is not quite clear. A possible explanation could be the increasing trend of VFA built-up in R1C, resulting slight decrease in pH. However, similar pH and even higher VFA level in the other reactor (R1) did not seem to have much impact on sulphate reduction, which illustrates that the VFA levels or pH was not the reason for this diversion. Maillacheruvu et al. (1993) observed a cyclic pattern of variation of hydrogen sulphide, a potential toxin and end product of sulphidogenesis and organic carbon consumption in glucose, lactate, acetate and propionate system. Vavilin et al. (1994) worked on developing a model based on the study of Parkin et al. (1990) and reported a self oscillating coexistence of methanogens and sulphate reducers.

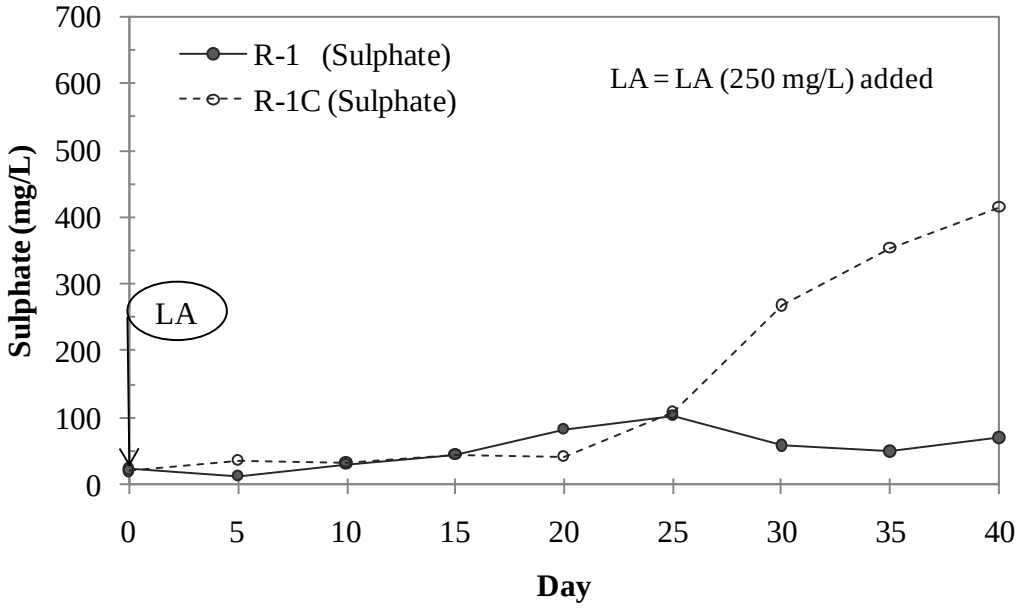


Figure 4. 13 Sulphate vs. Time in Reactors with COD/SO_4^{2-} = 4.66 and LA at a concentration of 250 mg/L

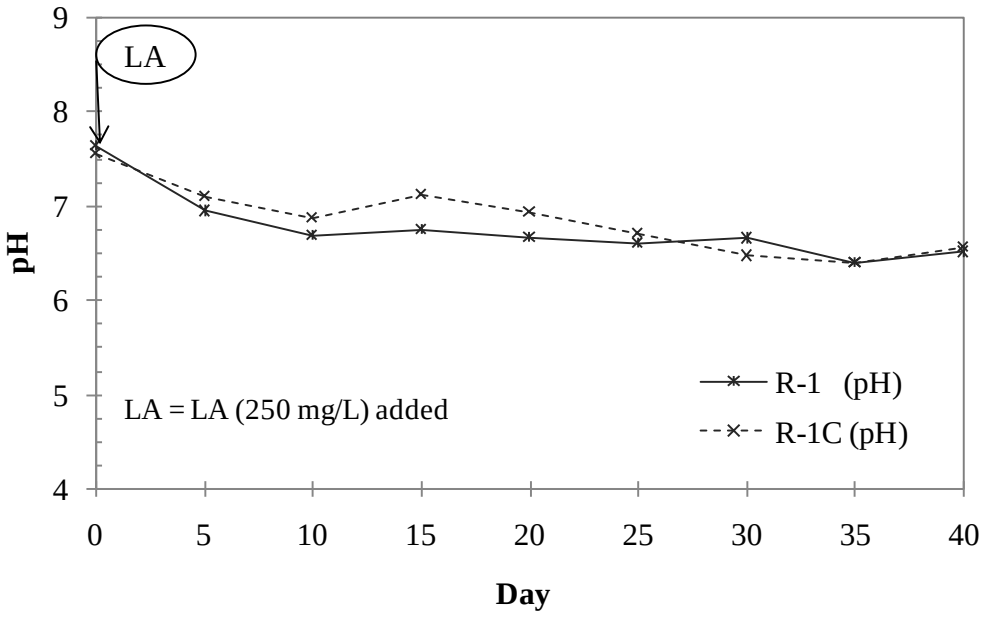


Figure 4.14 pH vs. Time in Reactors with COD/SO_4^{2-} = 4.66 and LA at a concentration of 250 mg/L

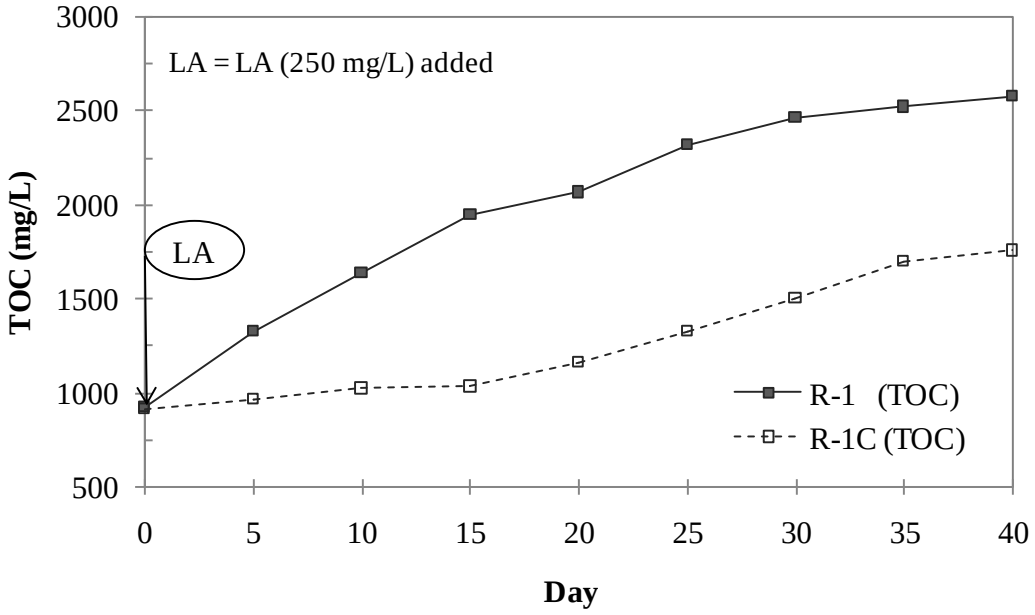


Figure 4.15 TOC vs. Time in Reactors with COD/SO_4^{2-} = 4.66 and LA at a concentration of 250 mg/L

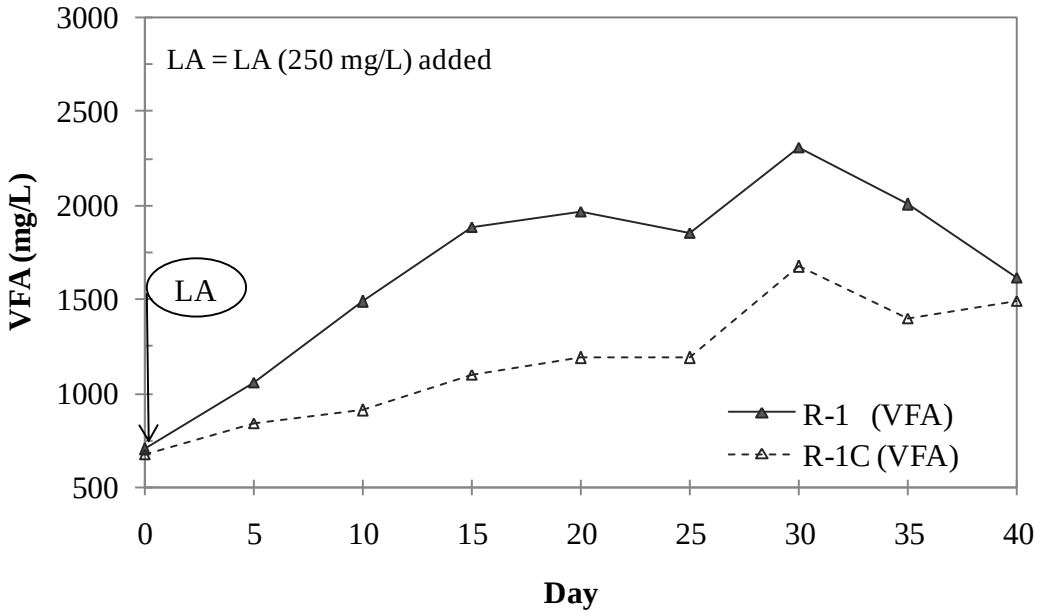


Figure 4.16 VFA vs. Time in Reactors with COD/SO_4^{2-} = 4.66 and LA at a concentration of 250 mg/L

Based on these two findings, it can be interpreted that methanogens became more active than sulphate reducers from Day 25 onwards. Hence, poor sulphate reduction efficiency was observed in R1C (control), whereas sulphate reduction was not affected in R1 with LA which can be due to the methanogenic inhibition. Advanced knowledge of the complex interactions of different species of microorganisms, slow growth response of SRB, inhibition, acclimation etc. are considered to be key factors which may have been responsible for this phenomenon.

4.3.2 Reactors with $COD/SO_4^{2-} = 1.96$

Results are presented in Figures 4.17 – 4.20. LA was added to R2 to maintain a level of 250 mg/L of LA in the reactor on Day 0 and R2C was used as control without LA. Sulphate reduction was affected in the control Day 0 onwards. Sulphate reduction reduced gradually from 94% (173 mg/L) to 86% (279 mg/L) during Day 0 to Day30. Other operational parameters such as TOC consumption, VFA and pH were at steady levels in R2C without LA. TOC consumption was not changed. TOC consumption was observed as high as 98%, similar to that observed previously during Phase I. This phenomenon of reduced sulphate reduction, while other parameters were almost unchanged; indicates enhanced methanogenic activities in the control different than observed previously. Increase in methanogenic and sulphidogenic activities and their cyclic pattern was observed by Parkin et al. (1990); Vavilin et al. (1994) and is discussed in section 4.6 in the extended reactor operations.

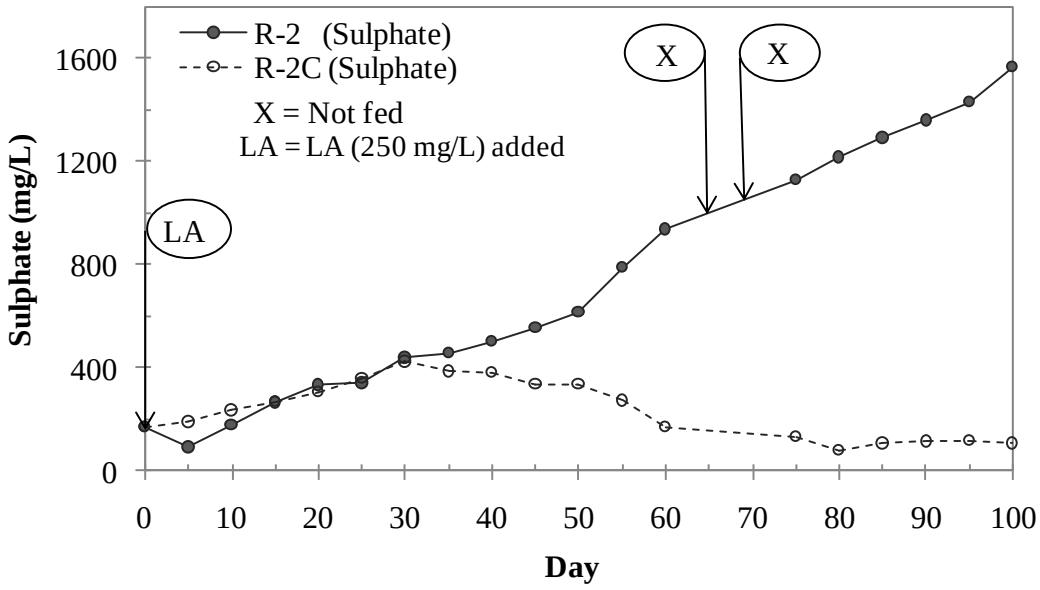


Figure 4.17 Sulphate vs. Time in Reactors with COD/SO_4^{2-} = 1.96 and LA at a concentration of 250 mg/L

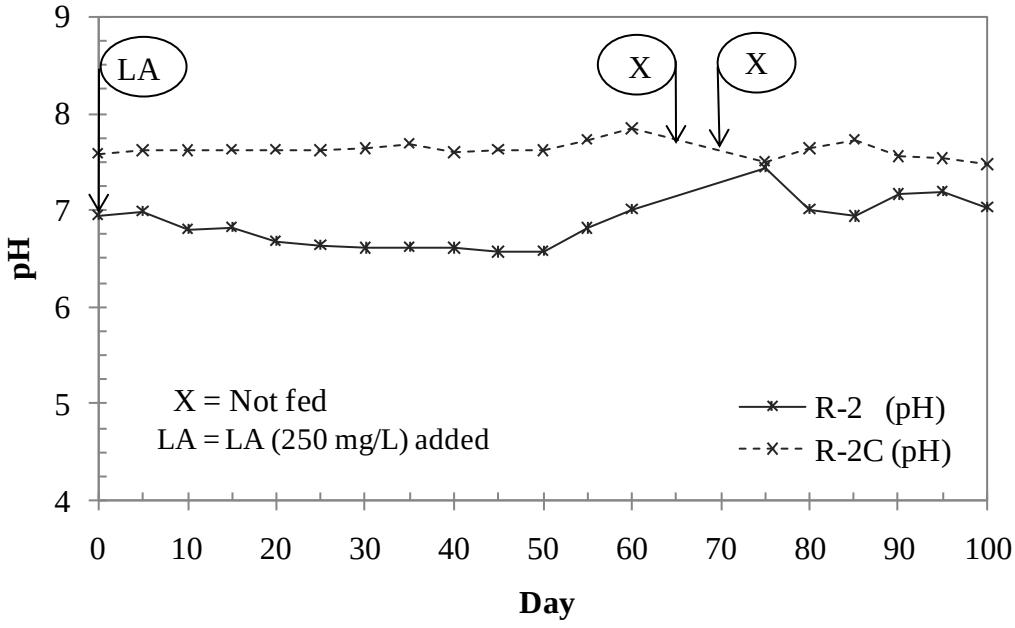


Figure 4.18 pH vs. Time in Reactors with COD/SO_4^{2-} = 1.96 and LA at a concentration of 250 mg/L

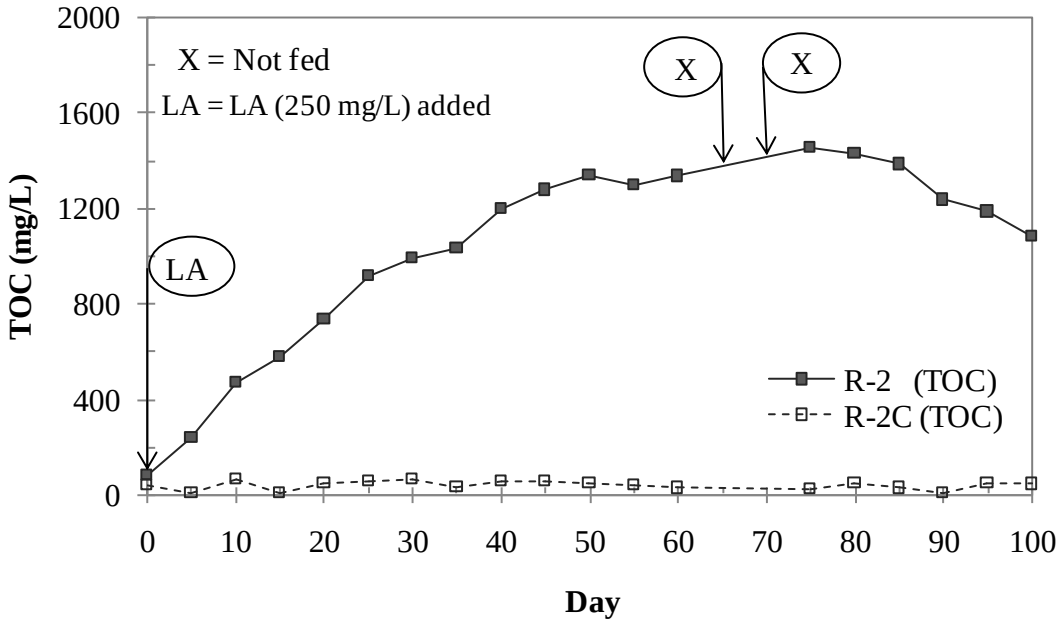


Figure 4.19 TOC vs. Time in Reactors with COD/SO_4^{2-} = 1.96 and LA at a concentration of 250 mg/L

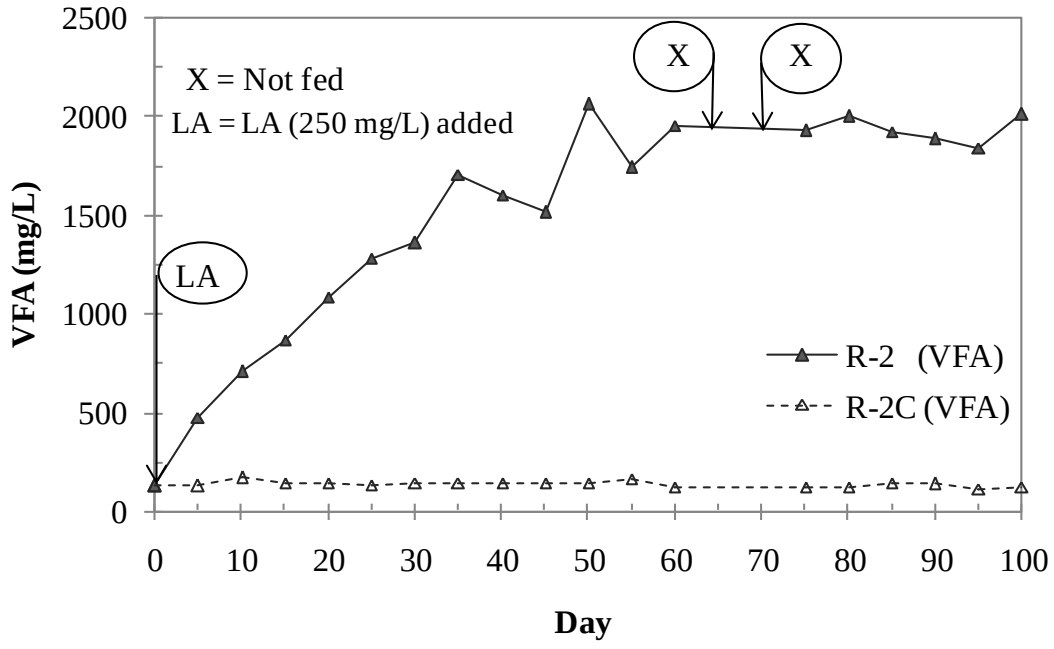


Figure 4.20 VFA vs. Time in Reactors with COD/SO_4^{2-} = 1.96 and LA at a concentration of 250 mg/L

After the addition of LA, the sulphate removal efficiency increased slightly in R2 compared to the control from 94% to 96%. But the sulphate level started to increase similar to R2C (control) from Day 15 onwards. The average pH was similar in both reactors (6.7 ± 0.1 and 7.6 ± 0.1 respectively). Though the effect of LA was not observed in sulphate reduction, a significant amount of organic carbon was diverted from going towards methane production. VFA level in the control was in similar at a level of 136 ± 14 mg/L, but VFA levels kept on increasing in R2 till Day 60. This high organic carbon could have been utilized by the sulphate reducers to reduce more sulphate if enough sulphate was available.

Sulphate reduction in R2C without LA was similar as previously observed during Day 0 to Day 30. It gradually started to improve from Day 60 onwards and reached at a different steady state showing average sulphate reduction of 96%. The TOC consumption and VFA levels remained unchanged during the entire period in control. But R2 with LA was showing the unexpected increasing trend of sulphate levels. The pH was observed higher in R2C than in R2. Considering the pH difference, alkalinity addition was discontinued from Day 75 in the R2C having higher pH, but was continued in R2 with LA.

The sulphate reduction in R2 kept on decreasing as it was observed previously during Day 0 to Day 30, which was unexpected. It seems LA had a reverse effect on sulphate reducers, as the sulphate reduction efficiency decreased from 85% on Day 30 to 68% on Day 60. This, reduced sulphate reduction efficiency affected the other parameters such as TOC, VFA and pH. TOC removal was reduced up to 47% compared to the control. The increasing VFA trend in the treatment observed during Day 0 to Day 30,

started to maintain a higher level of 1846 ± 172 mg/L after Day 60 onwards, whereas TOC removal and VFA level in R2C (control) was almost constant during the entire period of Day 30 to Day 100. Considering the adverse effect of VFA built-up, the R2 was not fed for two consecutive feeding cycles to allow the existing organic carbon and sulphate to be consumed. As a result of that, the organic carbon level started decreasing from Day 75 onwards, but didn't improve the sulphate reduction till Day 100 and even further. So, it was decided to discontinue the reactor (R2) operation. The control (R2C) was continued to operate further to observe the long term effect that was discussed in section 4.6.

4.3.3 Reactors with $COD/SO_4^{2-} = 0.75$

Results are presented in Figures 4.21 – 4.24. LA was added to R3 on Day 0 to maintain a LA concentration of 250 mg/L in the reactor and R 3C was used as control without LA. Sulphate reduction and other parameters such as TOC consumption, pH, VFA etc., in the R3C (control) didn't change significantly during Day 0 to Day 70. Slightly improved sulphate reduction of 53% in R3C (control) was observed during this period whereas, 47% was observed previously during Phase I. VFA levels were almost unchanged. The effect of LA at a concentration of 250 mg/L was observed immediately on next feeding cycle (Day 5) in R3. Sulphate reduction was observed to be 69% in R3 while, that in R3C (control) was observed 52%. Eventually, sulphate reduction was observed to be 77 ± 3% from Day 10 to Day 55. TOC, VFA levels started to increase indicating inhibition to methanogens. During the first 10 days, the increase was not significant. But TOC and VFA levels started to increase from Day 10 onwards.VFA level was observed to be more than double (362 mg/L) on Day 20 from 169 mg/L on Day 10. And this continued till Day 40 and dropped down slightly from Day 40 onwards. On Day 35 $NaHCO_3$ was not

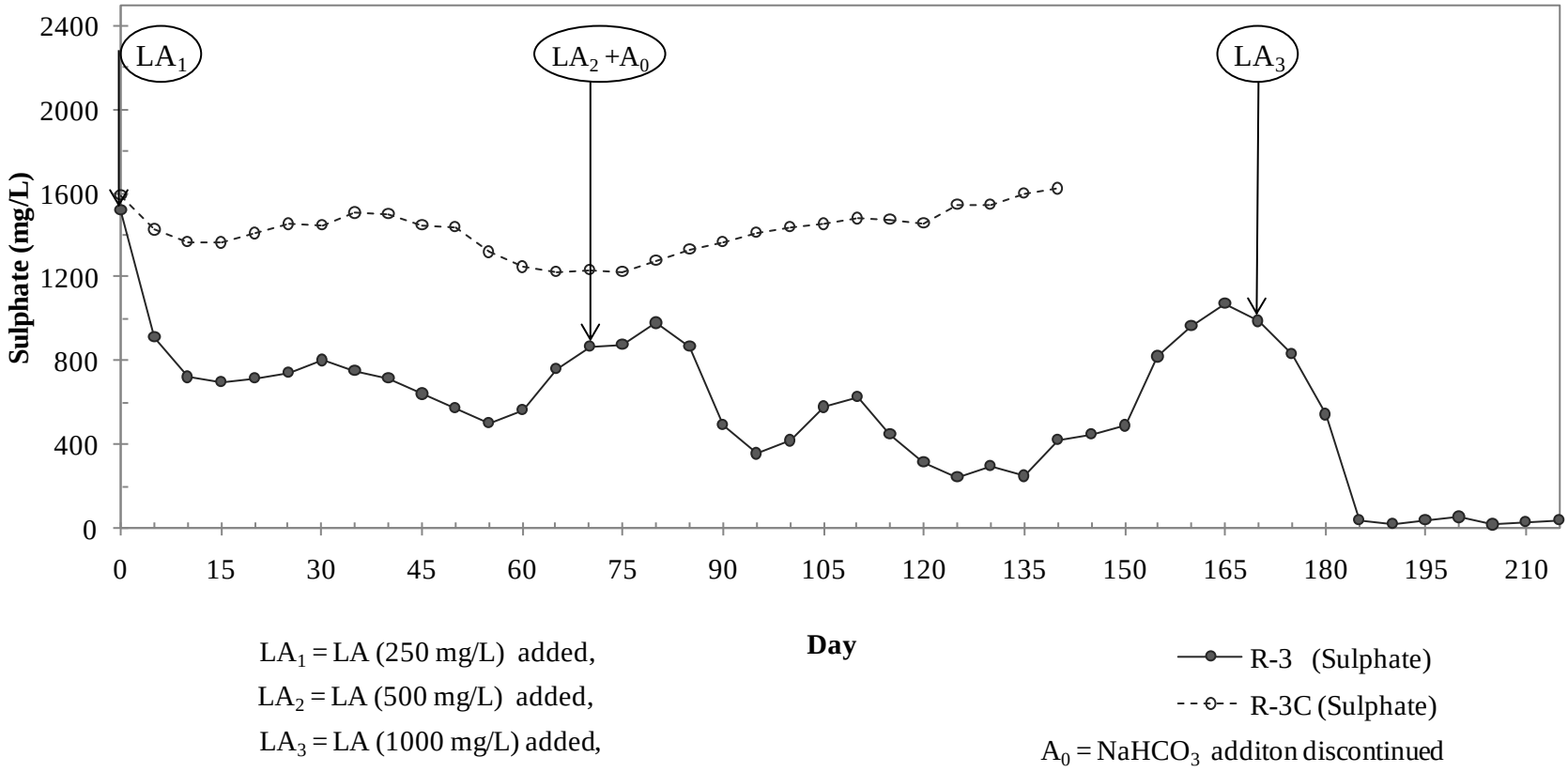


Figure 4. 21 Sulphate vs. Time in Reactors with COD/SO_4^{2-} = 0.75 and LA at a concentration of 250, 500 and 1000 mg/L

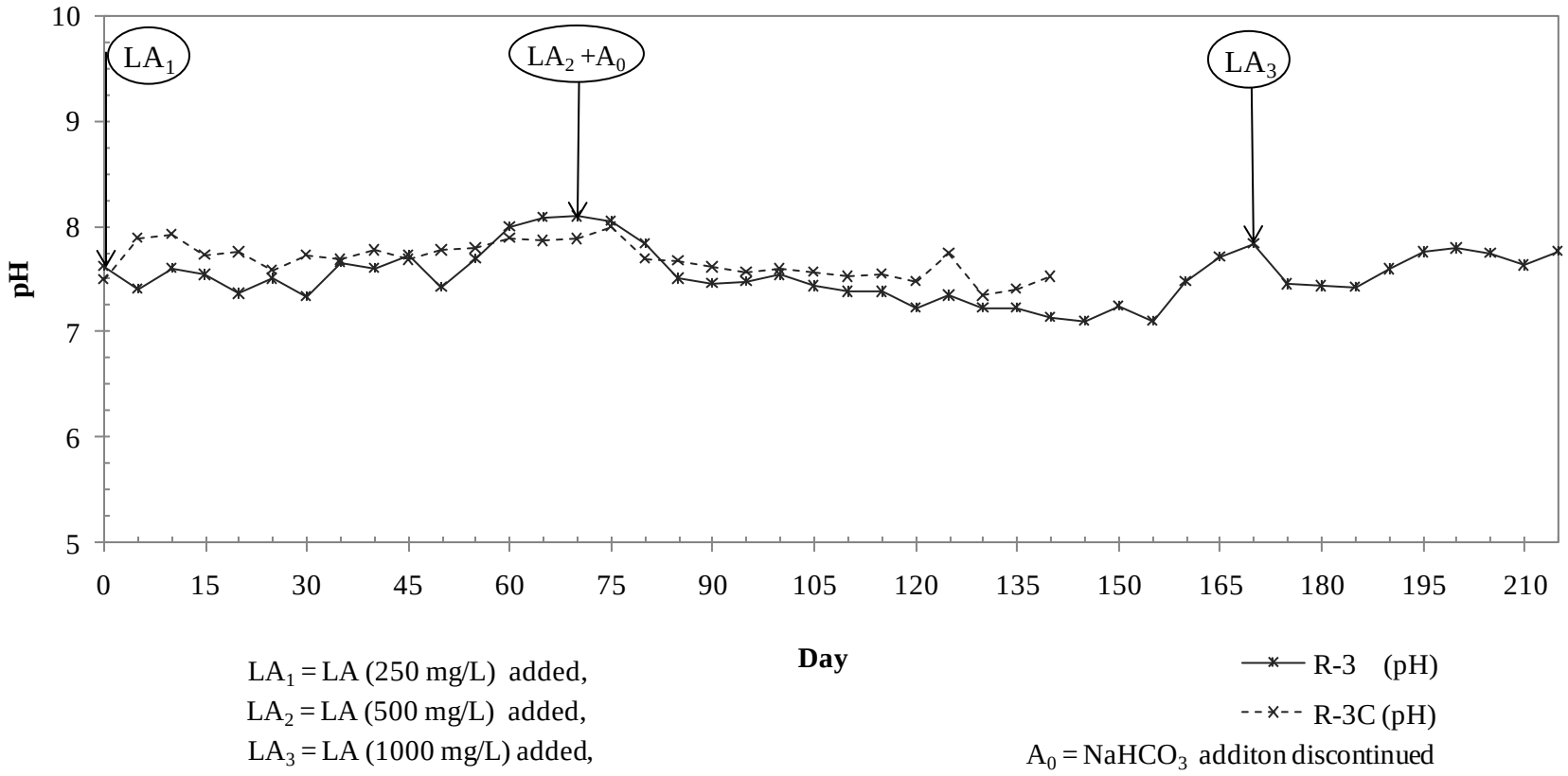


Figure 4.22 pH vs. Time in Reactors with COD/SO_4^{2-} = 0.75 and LA at a concentration of 250, 500 and 1000 mg/L

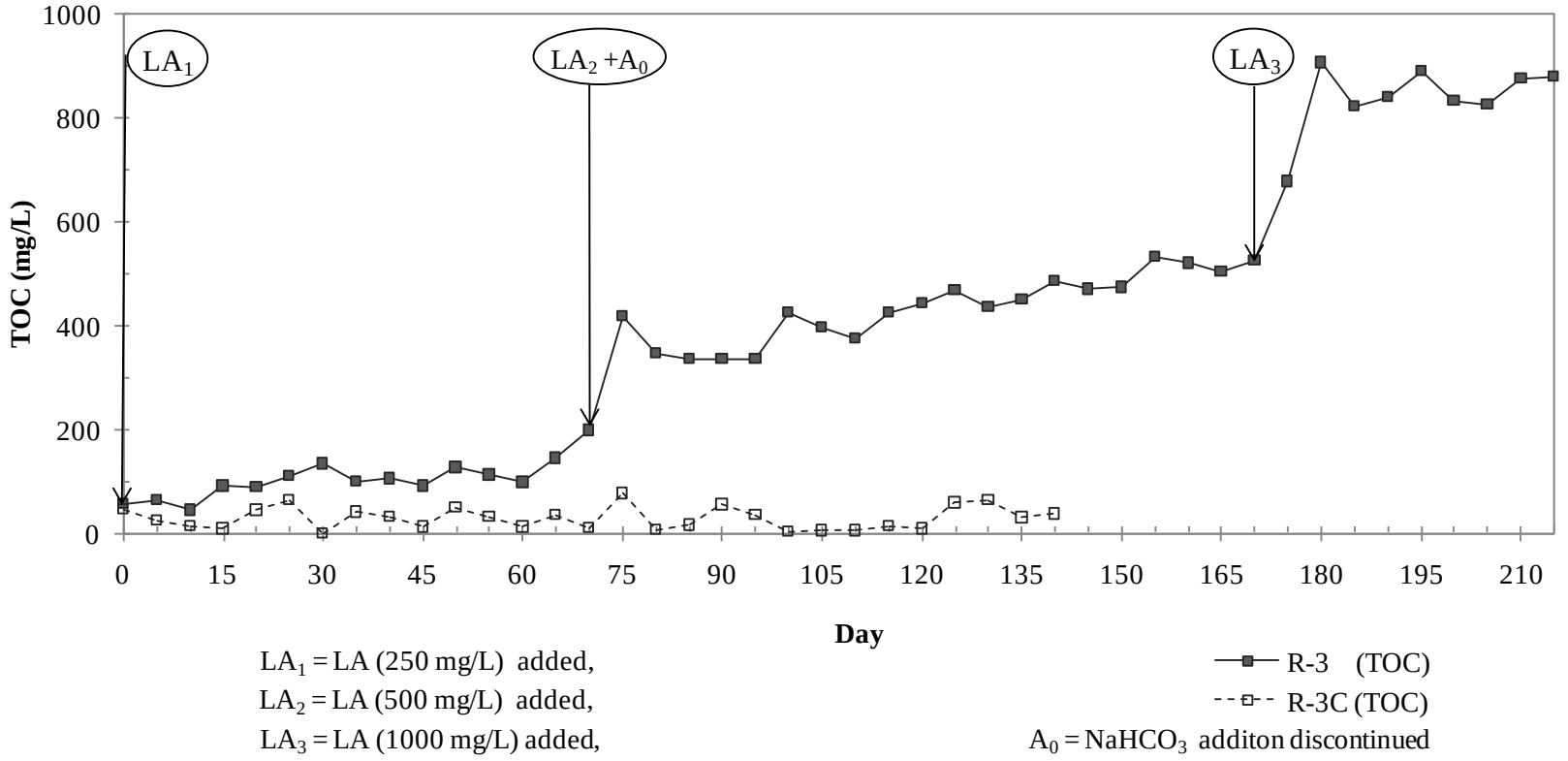


Figure 4.23 TOC vs. Time in Reactors with COD/SO_4^{2-} = 0.75 and LA at a concentration of 250, 500 and 1000 mg/L

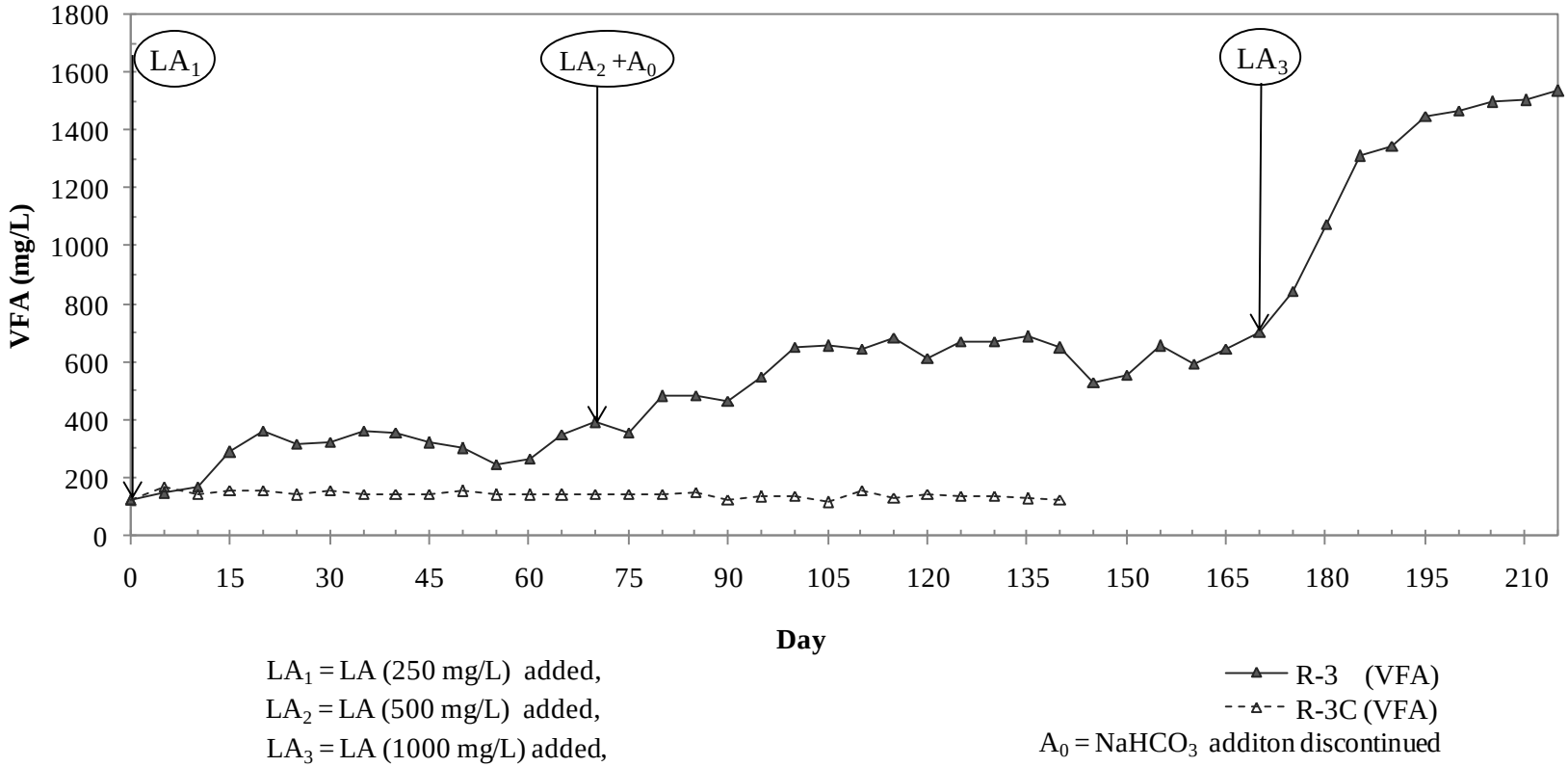


Figure 4.24 VFA vs. Time in Reactors with COD/SO_4^{2-} = 0.75 and LA at a concentration of 250, 500 and 1000 mg/L

added, but dosed double on Day 40. VFA levels clearly affected sulphate reduction. Sulphate reduction was slightly improved with slight decrease in VFA level during Day 45 to Day 55. Similarly, sulphate reduction efficiency reduced with the increase in VFA levels from Day 60 onwards. Due to addition of LA, a significant amount of organic carbon was diverted from going towards methane production. The TOC level in R3C (control) was similar, at a level of 29 ± 18 mg/L for this period, but increased in R3 showing an average TOC level of 385 ± 71 mg/L.

The concentration of LA was elevated to 500 mg/L in the reactor (R3) on Day 70 and was maintained further. R 3C was used as control without LA. $NaHCO_3$ addition was discontinued in both reactors from Day 70 onwards because of the increasing pH trend during the previous period. On Day 70, pH was observed as 8.0 and 7.88 in R3 and R3C respectively. In the aftermath, pH started to drop gradually. On Day 140, reactor pH of 7.1 and 7.5 were observed in R3 and R3C respectively. TOC and VFA levels did not change significantly in R3C (control) during Day 70 to Day 140. The lowering of pH affected sulphate reduction in the control, as higher pH is favourable for SRB activity (Visser et al., 1996). Sulphate reduction dropped down from 59% to 47% at the end of this period and average sulphate reduction was observed as 53 ± 4%.The effect of LA at a concentration of 500 mg/L was observed in sulphate reduction as well as other parameters such as TOC consumption and VFA levels. These parameters varied quite significantly during this period though improved sulphate reduction was observed due to the addition of LA. On Day 70 before adding LA at a concentration of 500 mg/L, sulphate reduction was observed as 71%. That improved during this period showing 92% of removal efficiency on Day 135. Sulphate reduction did fluctuate during Day 100 to Day 115. Average steady

sulphate reduction was observed 89 ± 2% during Day 120 to Day 135. The increasing trend of VFA levels kept on continuing until Day 100 and remained similar afterwards. Average VFA level of 659 ± 23 mg/L was observed during Day 100 to Day 140. The sulphate reduction and other controlling parameters such as TOC, VFA and pH were observed to maintain a similar trend up to Day 150, as before. However, sulphate level started to increase from Day 150. On Day 165, 64% sulphate reduction was observed, which was 20% less than that (84%) observed on Day 150. A similar trend of increase in sulphate level was also observed previously after 70 - 80 days of LA addition. A possible reason could be the acclimation of the SRB, indicating reversible or temporary inhibition due to LA. This trend continued to until a higher concentration of LA (1000 mg/L) was added on Day 170. Another explanation could be the effect of pH. The pH was observed as 7.4 on day 160 whereas, a steady pH of 7.1 was observed previously. The increasing trend of pH continued up to 7.8 on Day 170, which resulted in reduced sulphate reduction efficiency of 67% on Day 170.

After the addition of LA at a concentration of 1000 mg/L, pH dropped slightly and then remained steady at a level of 7.6. Sulphate reduction started to improve significantly followed by a sharp increase in TOC and VFA levels. TOC level increased up to 907 mg/L on Day 180. An average TOC was observed 501 ± 25 mg/L prior to LA addition. Similar increasing trend was observed in VFA levels. It started to stabilize from Day 185 onwards. A comparatively steady VFA level was observed as 1445 ± 85 mg/L during Day 185 to Day 215. From Day 185 onwards, sulphate reduction was observed as high as 99% and this high level of sulphate reduction was achieved till the end of the experiments (Day 215).

4.4 Effect of LA in Slug dose of 1000 mg/L in Semi Continuous Operation

Results are presented in Figures 4.25 - 4.26. A slug dose of LA at a concentration of 1000 mg/L of the reactor volume was added to the control reactor (R3C) on Day 140 and labelled as R3 Prime. The level of LA in this reactor was not maintained by subsequent addition of LA with substrate in every feeding cycle as it was maintained in the case of R3. The sulphate reduction immediately improved from 46% to 65% in 5 days but started to decrease with decreasing LA concentrations. Another single dose of 1000 mg/L (same concentration as before) was added to on Day 170 and sulphate reduction continued to improve up to 98% until Day 185. From Day 190 onwards, sulphate level started to increase showing similar effect as observed after Day 145 onwards; but reached in a lower level of 462 ± 8 mg/L with an average sulphate reduction of 85%. Sulphate reduction again improved to 97% from Day 205 onwards. Higher pH (Visser et al., 1996) of 7.8 ± 0.1and prolonged reactor operation (Omil et al., 1997) may be the possible reasons for this improved sulphate reduction. The highest level of sulphate reduction (97%) due to slug dose was comparable to that (99%) observed in R3 where LA was subsequently added with the substrate in each feeding cycle to maintain a constant level. Hence, it can be concluded that high concentration of LA (1000 mg/L) attributed to almost complete removal of sulphate in both cases.

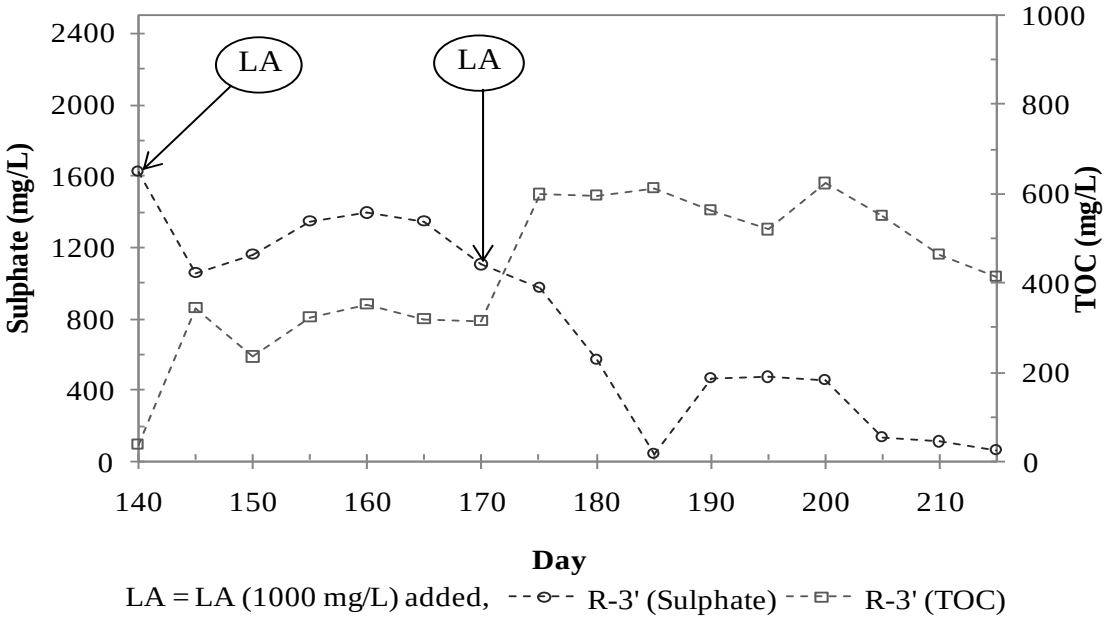


Figure 4.25 sulphate and TOC vs. Time in Reactor with COD/SO_4^{2-} = 0.75 and slug dose of LA at a concentration of 1000 mg/L

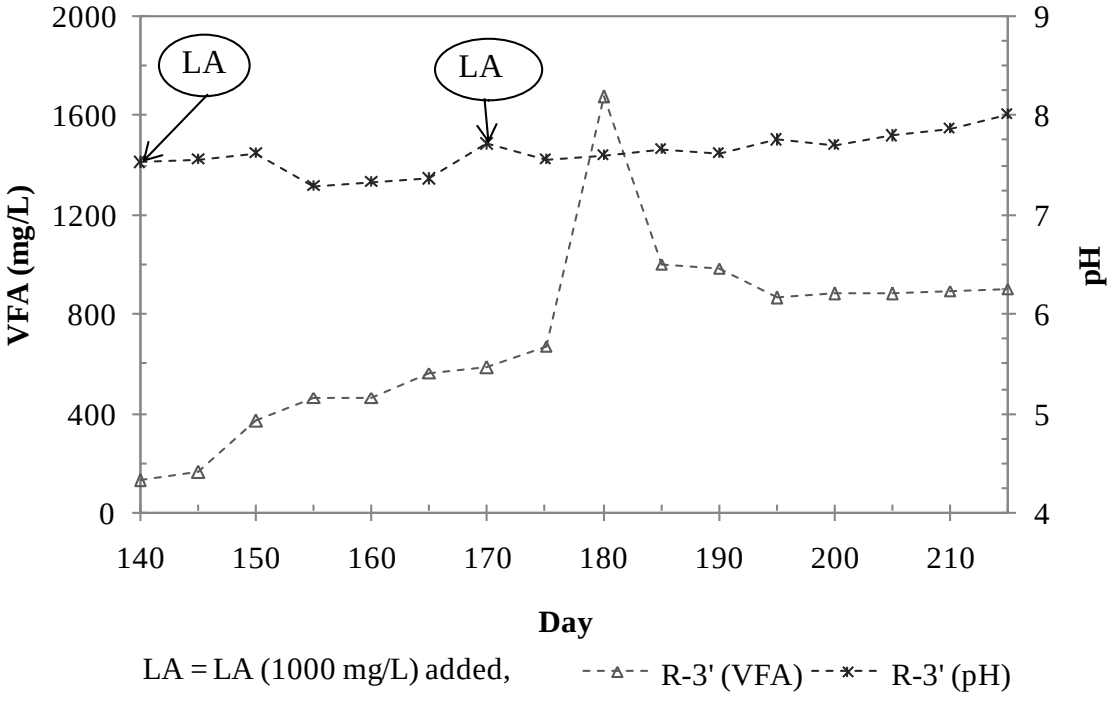


Figure 4.26 VFA and pH vs. Time in Reactor with COD/SO_4^{2-} = 0.75 and slug dose of LA at a concentration of 1000 mg/L

4.5 Effect of LA in Batch study and Semi-continuous study:

A recent study by Sharma & Biswas (2010) reported the efficacy of LA in selectively inhibiting methanogens to enhance sulphate reduction. Glucose (1870 mg/L as COD) was used as the organic carbon source to remove sulphate (1500 mg/L) in batch operation with a COD/SO_4^{2-} = 1.32. Five different concentrations of LA were added and an improved sulphate reduction was observed with increasing LA dosage. Sulphate reduction of 62%, 66%, 77%, 84% and 92% (1375 mg/L) was achieved with LA concentration of 100, 300, 500, 700 and 1000 mg/L, respectively; while a sulphate reduction of 24% was observed in the control without LA.

The present study was carried out in semi-continuous operation with COD/SO_4^{2-} ratio of 0.75 in the influent; but with higher COD (2333 mg/L) and sulphate (3095 mg/L) levels. Sulphate reduction of ~ 77% (2380 mg/L), 89% (2750 mg/L) and 99% (3060 mg/L) were observed with LA concentration of 250, 500 and 1000 mg/L respectively; while a sulphate reduction of ~ 50% was observed in the control without LA. Table 4.1 illustrates the comparative study of the effect of LA between batch and semi-continuous study. A higher level of sulphate reduction was achieved in the current semi-continuous operation than in batch operation by Sharma & Biswas (2010).

Table 4.1: Comparison between Batch and Semi-continuous operation with LA

Batch Study (Sharma & Biswas, 2010)							Semi-continuous Study (Present work)						
COD/ SO_4^{2-}	pH	Organic Carbon (as COD)	Sulphate (mg/L)	LA (mg/L)	Sulphate removal (mg/L)	Sulphate removal (%)	COD/ SO_4^{2-}	pH	Organic Carbon (as COD)	Sulphate (mg/L)	LA (mg/L)	Sulphate removal (mg/L)	Sulphate removal (%)
1.32	7.0-7.2	1870	1500	500 1000	1155 1375	77 92	0.75	7.4-7.6	2333	3095	500 1000	2750 3060	89 99

4.6 Extended Reactor Operation with $COD/SO_4^{2-} = 1.96$

Results of this phase of the study are presented in Figure 4.27. Two significant phenomena were observed during the prolonged operation of the control: 1) oscillating pattern of sulphidogenic activity and 2) improved sulphate reduction over time. In the former case, five different cycles of sulphidogenic activity of varying amplitude were observed. This finding is in line of the results reported by Vavilin et al. (1994), who worked on developing a model using data obtained from the study of Parkin et al. (1990), who observed a fading oscillation pattern within a period of 40 days. In the current study an oscillation pattern was observed within the range of 50 to 70 days. In their study, it was concluded that self-oscillation takes place due to the similarities in the competitive abilities of SRB and MPB and their complex interactions under hydrogen sulphide toxicity. Self buffering capacity of the system, variation in pH, different VFA profiles and adoption of different metabolic pathways in the presence of different microbial species may also play major roles in explaining this phenomenon.

In addition, sulphate reduction efficiency was observed to be improved over an extended reactor operation. During the period of Day 10 to Day 150, the efficiency was observed as 90% which, eventually, improved to 95% and 99% during the period of Day 155 to Day 220 and Day 225 to Day 300, respectively. More than 150 days of reactor operation was needed to achieve a 5% increase in sulphate reduction efficiency while other operational parameters, such as pH, VFA and TOC, remained unchanged. This observation is in agreement with the study of Omil et al. (1997), Harada et al. (1994) but conflicts with Stucki et al. (1993), Mulder (1984) and O'Flaherty et al (1998). Omil et al. (1997) concluded that ASRB out-competed AMPB after extended reactor operation of

more than 100 days; whereas, Harada et al. (1994) reported longer period of 250 to 400 days for the same. A different observation time may be the reason of the conflicting results of Stucki et al. (1993) and Mulder (1984). O'Flaherty et al. (1998) worked with a full-scale fixed-film anaerobic digester and observed that AMPB were predominant even after operating for more than 4 years. Better attachment capability of MPB to the media than SRB and limitation of transport of nutrients to SRB may potentially helped MPB to win the competition. This leads to the conclusion that time required for a population shift may also depend on the reactor technology.

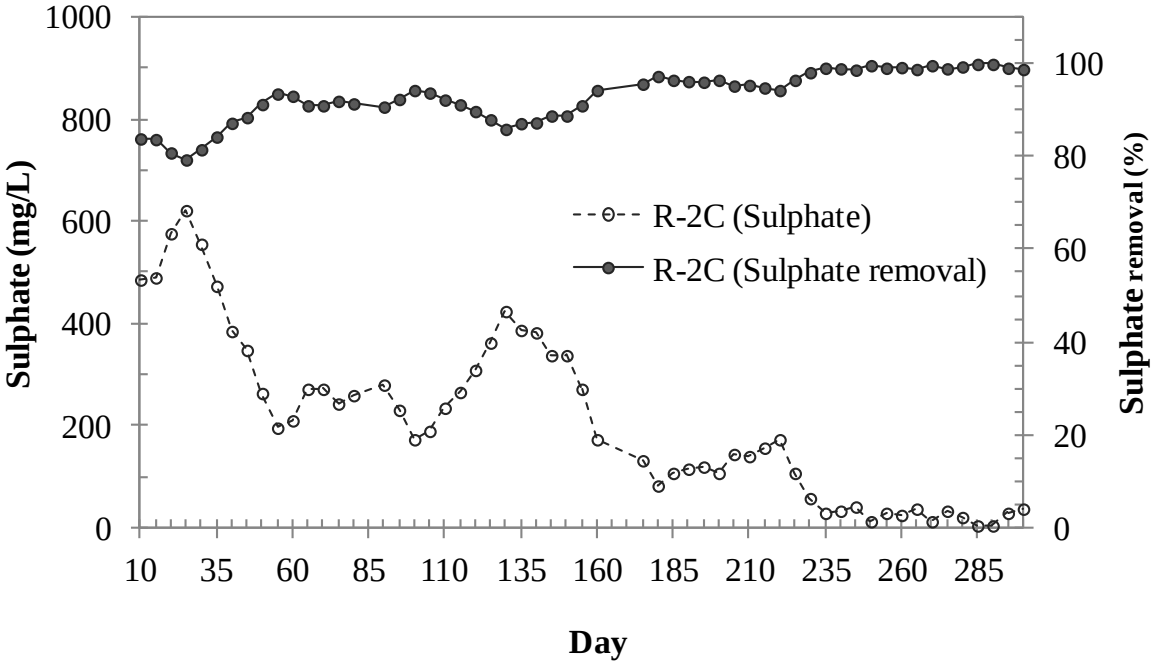


Figure 4. 27 Sulphate reduction in extended operation of reactor with COD/SO_4^{2-} = 1.96

4.7 Summary of Results

Sulphate reduction was observed to increase with increasing COD/SO_4^{2-} ratios. Table 4.2 illustrates the sulphate reduction during phase I with varying COD/SO_4^{2-} ratios:

Table 4.2 Sulphate reduction with varying COD/SO_4^{2-} ratios (Phase I)

Reactors	COD/SO_4^{2-}	Influent sulphate (mg/L)	Sulphate reduction (%)
R1 & R1C	4.66	3095	99 ± 1
R2 & R2C	1.96	3095	89 ± 4
R3 & R3C	0.75	3095	50 ± 3

The effect of LA at a concentration of 250 mg/L did not improve the sulphate reduction in reactors with COD/SO_4^{2-} ratios of 4.66 and 1.96 (Figure 4.28), though increase in VFA levels indicate the methanogenic inhibition (Figure 4.29). During phase I (without LA), VFA levels were observed 1157 ± 50 mg/L, 127 ± 11 mg/L in reactors with COD/SO_4^{2-} ratios of 4.66 and 1.96 respectively. Due to the inhibition of methanogenic activities by LA, the VFA levels increased to 1901 ± 61 mg/L and 1934 ± 61 mg/L in R1 (COD/SO_4^{2-} = 4.66) and R2 (COD/SO_4^{2-} = 1.96) respectively.

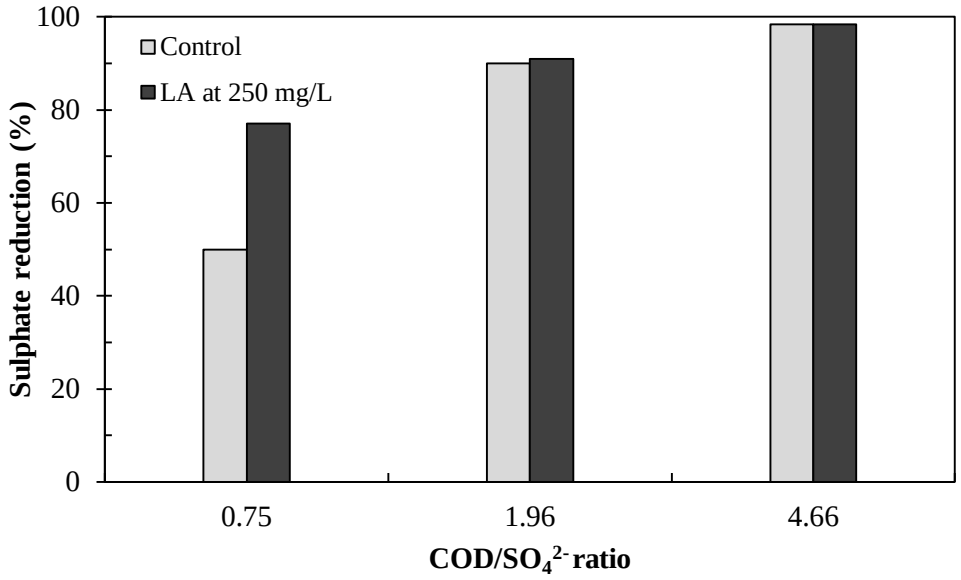


Figure 4.28 Sulphate reduction with varying COD/SO_4^{2-} ratios with LA at 250 mg/L

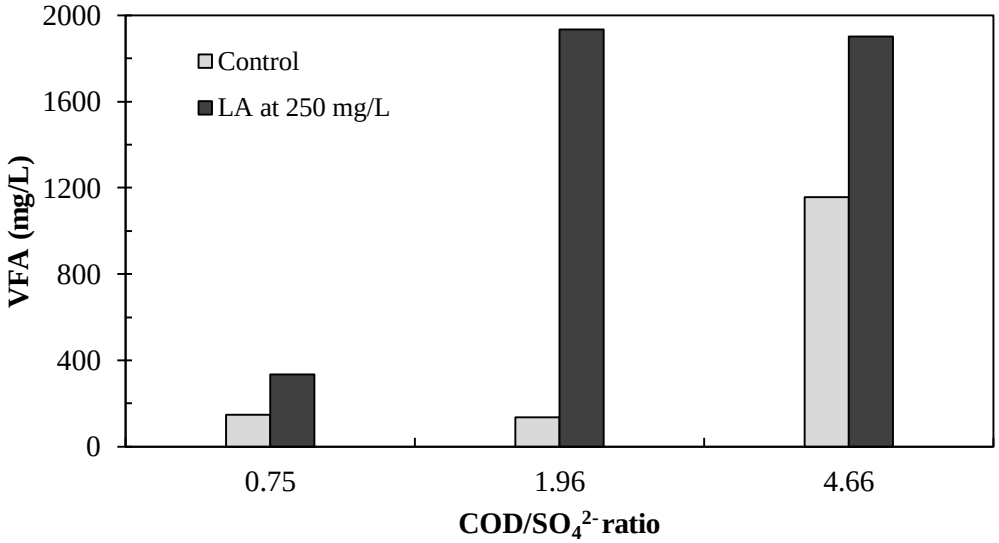


Figure 4.29 VFA levels with varying COD/SO_4^{2-} ratios with LA at 250 mg/L

The effect of LA on sulphate reduction was observed in reactor with lowest COD/SO_4^{2-} ratio of 0.75. Sulphate reduction increased with increasing concentrations of LA. Table 4.3 shows the effect of LA on sulphate reduction in reactor with COD/SO_4^{2-} ratio of 0.75 with varying LA concentrations of 250, 500 and 1000 mg/L.

Table 4.3 Effect of LA with varying concentrations in reactor with $COD/SO_4^{2-} = 0.75$

LA concentration (mg/L)	Influent sulphate (mg/L)	Effluent sulphate (mg/L)	Sulphate reduction (%)
0	3095	1550	50 ± 3
250	3095	710	77 ± 3
500	3095	340	89 ± 2
1000	3095	30	99 ± 1

The effect of LA was also tested with a slug dose of 1000 mg/L. Sulphate reduction increased from 46% to 65% due to first slug dose of LA (1000 mg/L). Almost complete removal of sulphate (98%) was observed after the second dose of LA with same concentration (1000 mg/L). This level of sulphate reduction is comparable with that (99%) observed due to continual LA addition in each feeding cycle in R3.

In case of long-term operation, sulphate reduction improved over time and an oscillation pattern was observed within the range of 50 to 70 days. During the period of Day 10 to Day 150, the efficiency was observed as 90% which, eventually, improved to 95% and 99% during the period of Day 155-220 and Day 225-300, respectively. More

than 150 days of reactor operation was needed to achieve a 5% increase in sulphate reduction efficiency while other operational parameters, such as pH, VFA and TOC, remained unchanged

.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study examined the effect of linoleic acid and COD/SO_4^{2-} ratio on anaerobic sulphate reduction in semi-continuous stirred tank reactors (SCSTRs) with glucose as the carbon source in a synthetic medium. The reactors were operated at a hydraulic retention time (HRT) of 40 days at a temperature of 37 ± 1 ^{o}C. The experiments were conducted in two phases.

5.1.1 Phase I

Phase I examined the effect of COD/SO_4^{2-} ratio at sulphate concentration of 3095 mg/L. The results are as follows:

1. Sulphate reduction increased from ~ 50% to ~ 99% with increasing COD/SO_4^{2-} ratio from 0.75 to 4.66.
2. At COD/SO_4^{2-} ratio of 4.66, ~ 99% (3060 mg/L) of sulphate was reduced. Volatile fatty acids (VFA) accumulation of 800 mg/L and residual TOC concentrations in the range of 800 mg/L suggest organic overloading.
3. At COD/SO_4^{2-} ratio of 1.96, ~ 89% (2750 mg/L) of sulphate was reduced. VFA accumulation was less than 350 mg/L and residual TOC concentrations were below 100 mg/L.
4. At COD/SO_4^{2-} ratio of 0.75, sulphate reduction of ~ 50% (1550 mg/L) was observed. VFA accumulation was less than 150 mg/L and residual TOC concentrations were below 100 mg/L.

5.1.2 Phase II

Phase II examined the effect of LCFA (linoleic acid) and COD/SO_4^{2-} ratio on anaerobic sulphate reduction in SCSTRs. The results are as follows:

1. At COD/SO_4^{2-} ratio of 4.66, sulphate reduction was maintained at ~ 98% in both the control SCSTR (no LA) and SCSTR receiving 250 mg/L of LA concentration in the feed during a period of 40 days (1HRT). VFA accumulation of ~ 1700 mg/L in the reactor receiving LA as compared to ~ 800 mg/L the control reactor (no LA) indicated inhibition of methanogenic bacteria by the added LA.
2. At COD/ SO_4^{2-} ratio of 1.96, sulphate reduction in the range of 84% to 96% was maintained in both the control SCSTR (no LA) and SCSTR receiving 250 mg/L LA concentration in the feed during the period of 40 days (1 HRT). VFA accumulation of ~ 1900 mg/L in the reactor receiving LA as compared to < 150 mg/L in the control reactor (no LA) indicated inhibition of methanogenic bacteria by the added LA.
3. At COD/ SO_4^{2-} ratio of 0.75, sulphate reduction of ~ 50% was observed in the control SCSTR (no LA) during 140 days of operation. In the SCSTR receiving LA, sulphate reduction was observed to increase with increasing LA concentration. Increasing LA concentrations in the feed from 250 to 500 and 1000 mg/L increased the sulphate reduction to ~ 77%, 89%, and 99%, respectively.

5.2 Recommendations

1. Future experiments should be conducted to optimize a two-stage treatment process for industrial wastewater with high concentration of sulphate. In the first reactor, mixed microbial consortia enriched with SRB and inhibitors to MPB should be

assessed to produce sulphide-rich effluent. This sulphide-rich effluent could then be used in the second reactor to optimize the removal of heavy metals from industrial wastewater.

2. Future work is required to investigate whether LCFA can serve as electron donors to sulphate reducers.
3. The effect of elevated LCFA levels on sulphate reduction could be examined as effluents from many industries contain LCFA levels as high as 20000 mg/L (Borja and Banks, 1994).
4. Studies are required to investigate the effect of both COD/SO_4^{2-} ratio and LCFA with elevated levels in high rate reactors.
5. Contradictory findings have been reported regarding the reversibility of LCFA inhibition. Some studies have reported the inhibition to be permanent and irreversible (Sharma & Biswas, 2010; Rinzema et al., 1994) while others have reported this to be temporary and reversible (Pereira et al., 2005; Pereira et al., 2004).This could be interesting to study the nature of inhibition which may reduce the overall treatment cost.

APPENDICES

APPENDIX A

Enrichment of SRB culture: M1 (2:2)

HRT = 40 days

Volume replaced = 400 ml

2 L of substrate + 2L of anaerobically digested sludge were mixed. Semi continuous operation was started after 10 days of mixing.

Day	pH	ORP (mV)	Temperature (^{o}C)	Sulphate (mg/L)	TOC (mg/L)	Gas Production (mL)
0	7.1	-340	19.9	1083.3	20.91	40
4	7.06	-343	20.3	928	30.795	125
8	6.68	-313	20.2	922	74.54	225
12	6.96	-290	19.2	1137.43	205.564	160
16	6.91	-291	20.4	1099.92	354.6	200
20	6.85	-315	20.7	911.64	451.258	80
24	6.9	-330	20.7	857.58	480.416	100
28	6.83	-330	20.1	708.65	634.11	100
32	6.91	-365	22.7	651.28	740.667	150
36	6.75	-358	21.1	738.12	510.427	40
40	6.86	-367	21.4	682.2	995.47	50
44	6.84	-353	24.4	584.6	1025.63	85

Day	pH	ORP (mV)	Temperature (^{o}C)	Sulphate (mg/L)	TOC (mg/L)	Gas Production (mL)
48	6.69	-345	21.6	640	1201	50
52	6.62	-339	19.6	769	1282	40
56	6.51	-331	20	757	1272	20
60	6.62	-336	22.3	624	1496	100
64	6.65	-341	24.8	510	1471	120
68	6.48	-334	21.6	707	1657	0
72	6.35	-327	20.3	911	1682	20
76	6.42	-330	23	755	1686	0
80	6.47	-335	19.7	973	1604	0
84	6.38	-314	24.3	1066	1650	50
88	6.3	-316	24	540	1724	100
92	6.4	-325	21.5	570	1685	50
96	6.31	-309	20.3	611	1760	150
100	6.28	-328	24.1	580	1678	150
104	NR	-312	23.8			
108	6.05	-310	23.6	Data not collected		
112	6.1	-312	24			

APPENDIX B

Enrichment of SRB culture: M2 (1:3)

HRT = 40 days

Volume replaced = 400 ml

1 L of substrate + 3L of anaerobically digested sludge were mixed. Semi continuous operation was started after 10 days of mixing.

Day	pH	ORP (mV)	Temperature (^{o}C)	Sulfate (mg/L)	TOC (mg/L)	Gas Production (mL)
0	6.8	-315	19.9	653	12	440
4	6.9	-311	20.3	694	119	450
8	7.01	-291	20.2	679	103	450
12	6.97	-299	19.2	796	131	400
16	7.02	-303	20.4	788	198	340
20	7.03	-314	20.7	611	298	120
24	7.01	-332	20.7	598	389	190
28	7.06	-331	20.1	418	597	140
32	7.05	-378	22.7	264	546	150
36	7.16	-371	21.1	74	674	30
40	7.18	-374	21.4	140	731	40
44	7.1	-344	24.4	87	810	50
48	7.05	-356	21.6	97	1011	80
52	7.07	-358	19.6	151	1351	60
56	7.06	-348	20	90	1051	50

Day	pH	ORP (mV)	Temperature (^{o}C)	Sulfate (mg/L)	TOC (mg/L)	Gas Production (mL)
60	7.04	-340	22.3	95	1213	100
64	6.92	-346	24.8	121	1152	80
68	6.95	-351	21.6	260	1193	100
72	6.91	-349	20.3	258	1208	100
76	6.94	-350	23	197	1328	50
80	6.85	-355	19.7	112	1485	300
84	6.85	-334	24.3	140	1783	240
88	6.9	-261	24	24	1305	400
92	6.91	-290	21.5	30	1424	500
96	7.18	-347	20.3	47	940	600
100	7.24	-328	24.1	26	1030	700
104	NR	-360	23.8			
108	7.1	-339	23.6	Data not collected		
112	6.98	-353	24			

APPENDIX C

Calculation of Sulphate

Na_2SO_4:

M.W. = 142.06; SO_4^{2-} = 96.06

4.54 g of Na_2SO_4contributes = (96.06X4.54)/142.06 = 3.0699 g of SO_4^{2-}

$MgSO_4.7H_2O$:

M.W. = 246.06; SO_4^{2-} = 96.06

0.061 g of $MgSO_4.7H_2O$ contributes = (96.06X0.061)/246.06 = 0.0238 g of SO_4^{2-}

$FeSO_4.7H_2O$:

M.W. = 277.86; SO_4^{2-} = 96.06

0.004 g of $FeSO_4.7H_2O$ contributes = (96.06X0.004)/277.86 = 0.0013 g of SO_4^{2-}

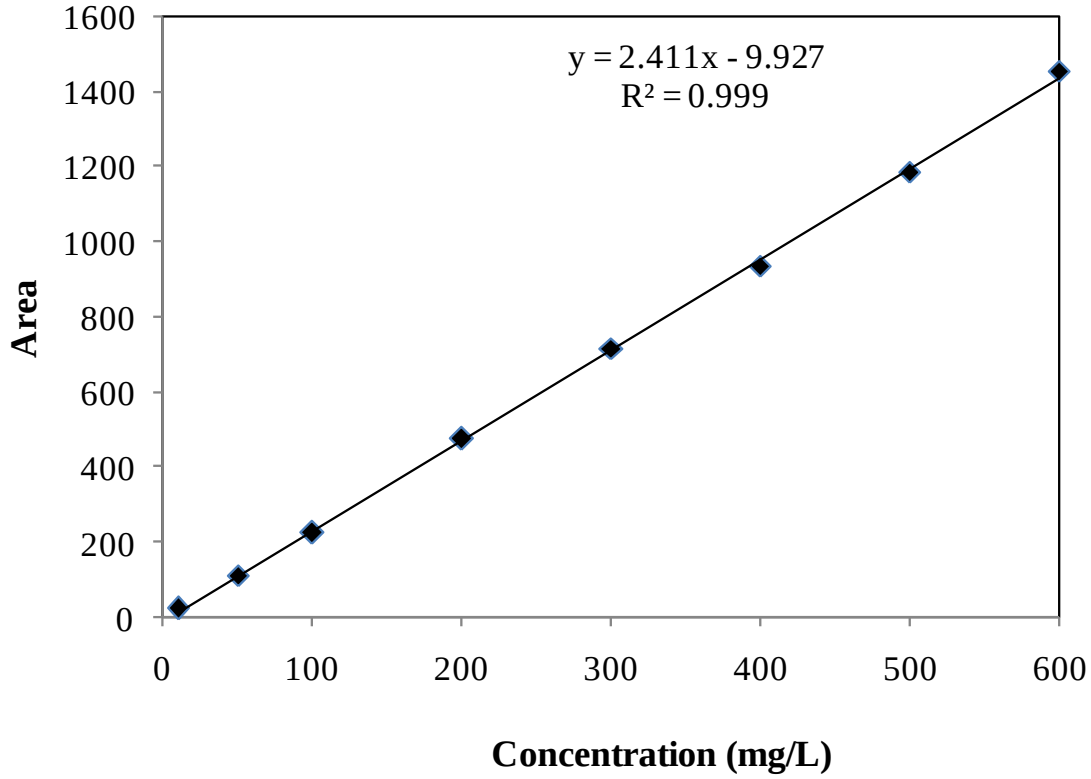
Total SO_4^{2-} concentration = (3.0699 + 0.0238 + 0.0013) g/L

= 3.095 g/L

= 3095 mg/L

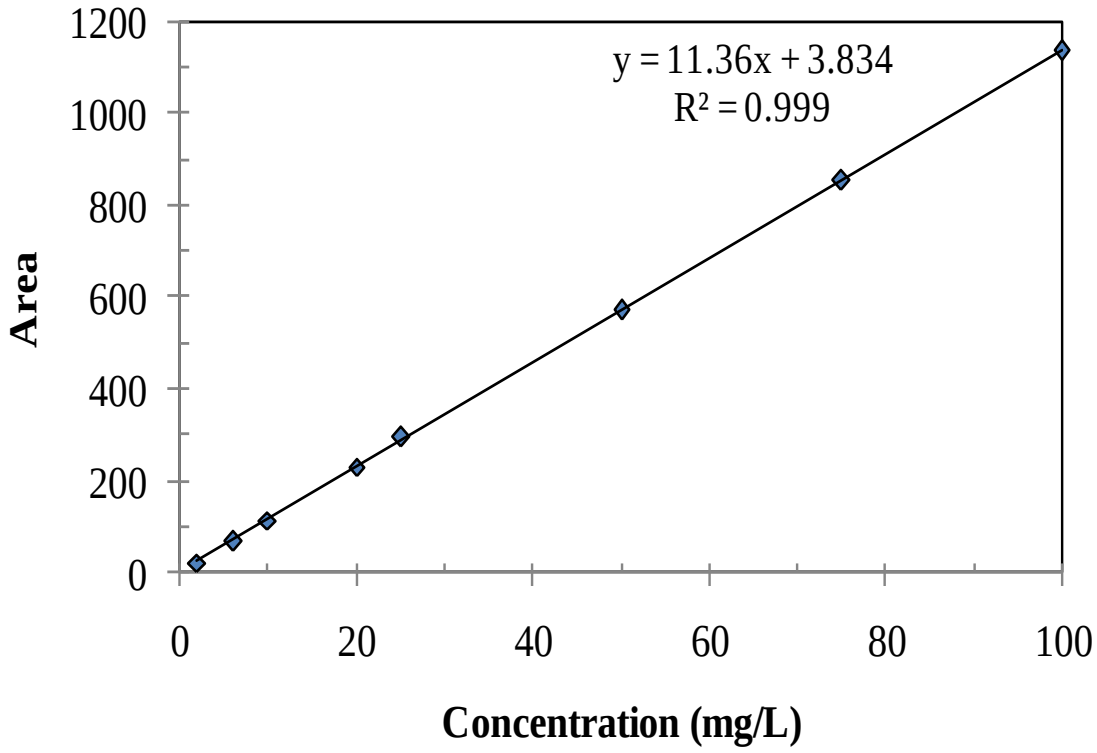
APPENDIX D

TC calibration curve



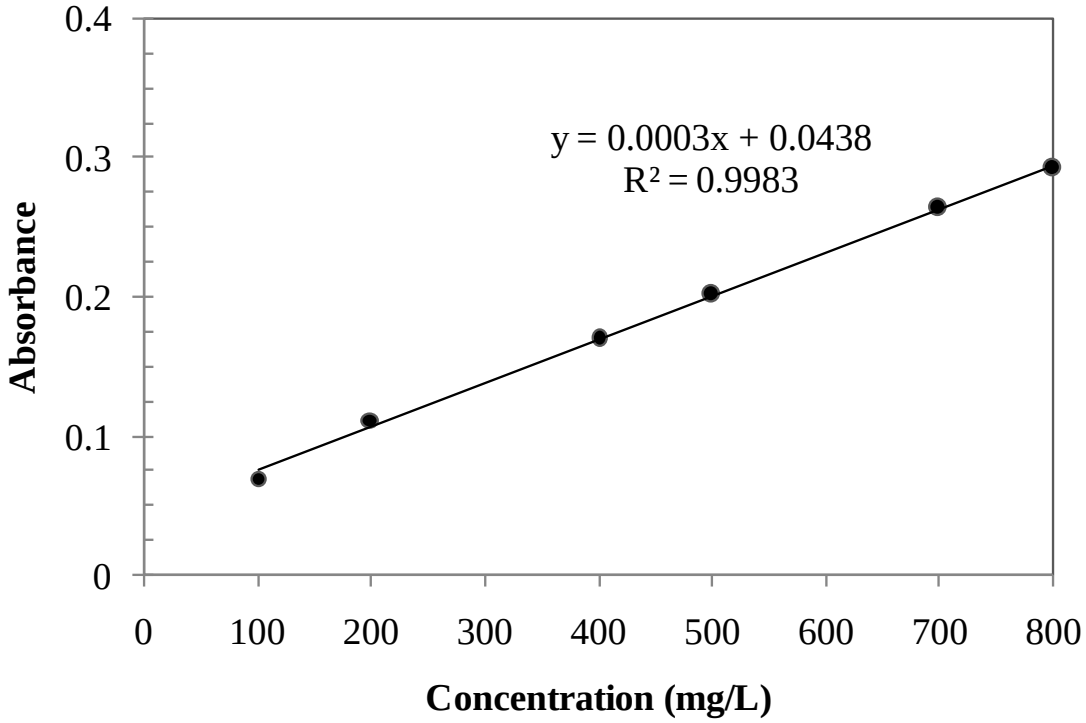
APPENDIX E

IC calibration curve



APPENDIX F

COD calibration curve



REFERENCES

Al-Ani, W.A.G., Henry, J.G., Prasad, D. 1995. Activated sludge as substrate for sulphate-reducing bacteria in Acid mine drainage. in: *50th Purdue Industrial Waste conference*. Chelsea, MI, pp. 461 - 467.

Alphenaar, P.A., Visser, A., Lettinga, G. 1993. The Effect of Liquid Upward Velocity and Hydraulic Retention Time on Granulation in Uasb Reactors Treating Waste-Water with a High Sulfate Content. *Bioresource Technology*, **43**(3), 249-258.

Alves, M.M., Vieira, J.A.M., Pereira, R.M.A., Pereira, M.A., Mota, M. 2001. Effects of lipids and oleic acid on biomass development in anaerobic fixed-bed reactors. Part II: Oleic acid toxicity and biodegradability. *Water Research*, **35**(1), 264-270.

Angelidaki, I., Ahring, B.K. 1995. Establishment and Characterization of an Anaerobic Thermophilic (55-Degrees-C) Enrichment Culture Degrading Long-Chain Fatty-Acids. *Applied and Environmental Microbiology*, **61**(6), 2442-2445.

Angelidaki, I., Ahring, B.K. 1993. Thermophilic Anaerobic-Digestion of Livestock Waste - the Effect of Ammonia. *Applied Microbiology and Biotechnology*, **38**(4), 560-564.

Annachhatre, A.P. 1996. Anaerobic treatment of industrial wastewaters. *Resources Conservation and Recycling*, **16**(1-4), 161-166.

Annachhatre, A.P., Suktrakoolvait, S. 2001. Biological sulfate reduction using molasses as a carbon source. *Water Environment Research*, **73**(1), 118-126.

Bagley, D.M., Brodkorb, T.S. 1999. Modeling microbial kinetics in an anaerobic sequencing batch reactor - Model development and experimental validation. *Water Environment Research*, **71**(7), 1320-1332.

Bhattacharya, S.K., Parkin, G.F. 1989. The Effect of Ammonia on Methane Fermentation Processes. *Journal Water Pollution Control Federation*, **61**(1), 55-59.

Bhattacharya, S.K., Qu, M.B., Madura, R.L. 1996. Effects of nitrobenzene and zinc on acetate utilizing methanogens. *Water Research*, **30**(12), 3099-3105.

Blum, D.J.W., Speece, R.E. 1991. A Database of Chemical Toxicity to Environmental Bacteria and Its Use in Interspecies Comparisons and Correlations. *Research Journal of the Water Pollution Control Federation*, **63**(3), 198-207.

Borja, R., Alba, J., Banks, C.J. 1997. Impact of the main phenolic compounds of olive mill wastewater (OMW) on the kinetics of acetoclastic methanogenesis. *Process Biochemistry*, **32**(2), 121-133.

Borja, R., Banks, C.J. 1994. Treatment of Palm Oil Mill Effluent by Upflow Anaerobic Filtration. *Journal of Chemical Technology and Biotechnology*, **61**(2), 103-109.

Borja, R., Sanchez, E., Weiland, P. 1996. Influence of ammonia concentration on thermophilic anaerobic digestion of cattle manure in upflow anaerobic sludge blanket (UASB) reactors. *Process Biochemistry*, **31**(5), 477-483.

Boucquey, J.B., Renard, P., Amerlynck, P., Modesto, P., Agathos, S.N., Naveau, H., Nyns, E.J. 1995. High-Rate Continuous Biodegradation of Concentrated Chlorinated Aliphatics by a Durable Enrichment of Methanogenic Origin under Carrier-Dependent Conditions. *Biotechnology and Bioengineering*, **47**(3), 298-307.

Braun, R., Huber, P., Meyrath, J. 1981. Ammonia Toxicity in Liquid Piggery Manure Digestion. *Biotechnology Letters*, **3**(4), 159-164.

Cherrington, C.A., Hinton, M., Mead, G.C., Chopra, I. 1991. Organic acids: chemistry, antibacterial activity and practical applications. *Advances in microbial physiology*, **32**, 87-108.

Colleran, E., Finnegan, S., Lens, P. 1995. Anaerobic treatment of sulphate-containing waste streams. *Antonie van Leeuwenhoek*, **67**(1), 29-46.

De Baere, L.A., Devocht, M., Vanassche, P., Verstraete, W. 1984. Influence of High Nacl and NH4Cl Salt Levels on Methanogenic Associations. *Water Research*, **18**(5), 543-548.

Demeyer, D.I., Henderickx, H.K. 1967. The effect of C18 unsaturated fatty acids of methane production in vitro by mixed rumen bacteria. *Biochimica Et Biophysica Acta*, **137**(3), 484-97.

Demirel, B., Yenigun, O., Onay, T.T. 2005. Anaerobic treatment of dairy wastewaters: a review. *Process Biochemistry*, **40**(8), 2583-2595.

Demirer, G.N., Speece, R.E. 1998. Anaerobic biotransformation of four 3-carbon compounds (acrolein, acrylic acid, allyl alcohol and n-propanol) in UASB reactors. *Water Research*, **32**(3), 747-759.

DiLallo, R., Albertson, O.E. 1961. Volatile Acids by Direct Titration. *Journal (Water Pollution Control Federation)*, **33**(4), 356-365.

Duangmanee, T., Padmasiri, S.I., Simmons, J.J., Raskin, L., Sung, S. 2007. Hydrogen production by anaerobic microbial communities exposed to repeated heat treatments. *Water Environment Research*, **79**(9), 975-983.

Elferink, S.J.W.H., Visser, A., Pol, L.W.H., Stams, A.J.M. 1994. Sulfate Reduction in Methanogenic Bioreactors. *Fems Microbiology Reviews*, **15**(2-3), 119-136.

Erdirencelebi, D., Ozturk, I., Cokgor, E.U., Tonuk, G.U. 2007. Degree of sulfate-reducing activities on COD removal in various reactor configurations in anaerobic glucose and acetate-fed reactors. *Clean-Soil Air Water*, **35**(2), 178-182.

Fang, H.H.P., Chen, T., Chan, O.C. 1995. Toxic Effects of Phenolic Pollutants on Anaerobic Benzoate-Degrading Granules. *Biotechnology Letters*, **17**(1), 117-120.

Finnegan, S. 1994. Anaerobic digestion of sulphate-containing wastewater from citric acid production, Vol. Ph.D, University College Galway. Ireland.

Freese, L.H., Stuckey, D.C. 2004. Anaerobic treatment of sulphate-enriched wastewaters. *Proceedings of the Institution of Civil Engineers-Water Management*, **157**(4), 187-195.

Garrels, R.M., Christ, C.L. 1965. *Solutions, minerals, and equilibria*. Harper & Row, New York.

Ghosh, S., Pohland, F.G. 1974. Kinetics of Substrate Assimilation and Product Formation in Anaerobic Digestion. *Journal Water Pollution Control Federation*, **46**(4), 748-759.

Gonzalez-Gil, G., Kleerebezem, R., Lettinga, G. 2002. Conversion and toxicity characteristics of formaldehyde in acetoclastic methanogenic sludge. *Biotechnology and Bioengineering*, **79**(3), 314-322.

Greenway, D.L.A., Dyke, K.G.H. 1979. Mechanism of the Inhibitory Action of Linoleic-Acid on the Growth of Staphylococcus-Aureus. *Journal of General Microbiology*, **115**(Nov), 233-245.

Gujer, W., Zehnder, A.J.B. 1983. Conversion Processes in Anaerobic-Digestion. *Water Science and Technology*, **15**(8-9), 127-167.

Gunsalus, R.P., Wolfe, R.S. 1978. Atp Activation and Properties of Methyl Coenzyme-M Reductase System in Methanobacterium-Thermoautotrophicum. *Journal of Bacteriology*, **135**(3), 851-857.

Hansen, K.H., Angelidaki, I., Ahring, B.K. 1999. Improving thermophilic anaerobic digestion of swine manure. *Water Research*, **33**(8), 1805-1810.

Hao, O.J., Chen, J.M., Huang, L., Buglass, R.L. 1996. Sulfate-reducing bacteria. *Critical Reviews in Environmental Science and Technology*, **26**(2), 155-187.

Harada, H., Uemura, S., Momonoi, K. 1994. Interaction between Sulfate-Reducing Bacteria and Methane-Producing Bacteria in Uasb Reactors Fed with Low Strength Wastes Containing Different Levels of Sulfate. *Water Research*, **28**(2), 355-367.

Hashimoto, A.G. 1984. Methane from Swine Manure - Effect of Temperature and Influent Substrate Concentration on Kinetic Parameter (K). *Agricultural Wastes*, **9**(4), 299-308.

Hashimoto, A.G. 1983. Thermophilic and Mesophilic Anaerobic Fermentation of Swine Manure. *Agricultural Wastes*, **6**(3), 175-191.

Hayward, G., Lau, I. 1989. Toxicity of Organic-Solvents to Fatty-Acid Forming Bacteria. *Canadian Journal of Chemical Engineering*, **67**(1), 157-161.

Hendriksen, H.V., Ahring, B.K. 1991. Effects of Ammonia on Growth and Morphology of Thermophilic Hydrogen-Oxidizing Methanogenic Bacteria. *Fems Microbiology Ecology*, **85**(3), 241-246.

Hilton, B.L., Oleszkiewicz, J.A. 1988. Sulfide-Induced Inhibition of Anaerobic-Digestion. *Journal of Environmental Engineering-Asce*, **114**(6), 1377-1391.

Hoa, T.T.H., Liamleam, W., Annachhatre, A.P. 2007. Lead removal through biological sulfate reduction process. *Bioresource Technology*, **98**(13), 2538-2548.

Hwu, C.S., Donlon, B., Lettinga, G. 1996. Comparative toxicity of long-chain fatty acid to anaerobic sludges from various origins. *Water Science and Technology*, **34**(5-6), 351-358.

Hwu, C.S., Tseng, S.K., Yuan, C.Y., Kulik, Z., Lettinga, G. 1998. Biosorption of long-chain fatty acids in uasb treatment process. *Water Research*, **32**(5), 1571-1579.

Isa, Z., Grusenmeyer, S., Verstraete, W. 1986a. Sulfate Reduction Relative to Methane Production in High-Rate Anaerobic-Digestion - Microbiological Aspects. *Applied and Environmental Microbiology*, **51**(3), 580-587.

Isa, Z., Grusenmeyer, S., Verstraete, W. 1986b. Sulfate Reduction Relative to Methane Production in High-Rate Anaerobic-Digestion - Technical Aspects. *Applied and Environmental Microbiology*, **51**(3), 572-579.

Johnson, D.B., Hallberg, K.B. 2005. Acid mine drainage remediation options: a review. *Science of the Total Environment*, **338**(1-2), 3-14.

Kabara, J.J., Vrable, R., Liekenjie, M.S.F. 1977. Antimicrobial Lipids - Natural and Synthetic Fatty-Acids and Monoglycerides. *Lipids*, **12**(9), 753-759.

Kaksonen, A.H., Puhakka, J.A. 2007. Sulfate reduction based bioprocesses for the treatment of acid mine drainage and the recovery of metals. *Engineering in Life Sciences*, **7**(6), 541-564.

Karhadkar, P.P., Audic, J.M., Faup, G.M., Khanna, P. 1987. Sulfide and Sulfate Inhibition of Methanogenesis. *Water Research*, **21**(9), 1061-1066.

Kayhanian, M. 1999. Ammonia inhibition in high-solids biogasification: An overview and practical solutions. *Environmental Technology*, **20**(4), 355-365.

Khan, A.W., T.M., T. 1978. Effect of sulfur containing compounds on degradation of cellulose to methane by a mixed culture obtained from sewage sludge. *Applied and Environmental Microbiology*(35), 1027-1034.

Koster, I.W., Cramer, A. 1987. Inhibition of Methanogenesis from Acetate in Antigranulocytes Sludge by Long-Chain Fatty-Acids. *Applied and Environmental Microbiology*, **53**(2), 403-409.

Koster, I.W., Lettinga, G. 1988. Anaerobic-Digestion at Extreme Ammonia Concentrations. *Biological Wastes*, **25**(1), 51-59.

Koster, I.W., Rinzema, A., Devegt, A.L., Lettinga, G. 1986. Sulfide Inhibition of the Methanogenic Activity of Antigranulocytes Sludge at Various Ph-Levels. *Water Research*, **20**(12), 1561-1567.

Kramer, G.R. 1971. Hydrolysis of Lipids in Wastewater. *Journal of the Sanitary Engineering Division-Asce*, **97**(Nsa5), 731-&.

Kroeker, E.J., Schulte, D.D., Sparling, A.B., Lapp, H.M. 1979. Anaerobic Treatment Process Stability. *Journal Water Pollution Control Federation*, **51**(4), 718-727.

Lalman, J., Bagley, D.M. 2002. Effects of C18 long chain fatty acids on glucose, butyrate and hydrogen degradation. *Water Research*, **36**(13), 3307-3313.

Lalman, J.A., Bagley, D.M. 2001. Anaerobic degradation and methanogenic inhibitory effects of oleic and stearic acids. *Water Research*, **35**(12), 2975-2983.

Lalman, J.D.A. 2000. Anaerobic Degradation of Linoleic ($C_{18:2}$), Oleic ($C_{18:1}$) and Stearic ($C_{18:0}$) Acids and their Inhibitory Effects on Acidogens, Acetogens and Methanogens. in: *Department of Civil Engineering*, Vol. Ph.D, Univeristy of Toronto. Toronto.

Lay, J.J. 2000. Modeling and optimization of anaerobic digested sludge converting starch to hydrogen. *Biotechnology and Bioengineering*, **68**(3), 269-278.

Lens, P., Vallerol, M., Esposito, G., Zandvoort, M. 2002. Perspectives of sulfate reducing bioreactors in environmental biotechnology. *Reviews in Environmental Science and Biotechnology*, **1**(4), 311-325.

Lens, P.N.L., Visser, A., Janssen, A.J.H., Pol, L.W.H., Lettinga, G. 1998. Biotechnological treatment of sulfate-rich wastewaters. *Critical Reviews in Environmental Science and Technology*, **28**(1), 41-88.

Lettinga, G. 1995. Anaerobic-Digestion and Waste-Water Treatment Systems. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology*, **67**(1), 3-28.

Liu, S.M., Wu, C.H., Huang, H.J. 1998. Toxicity and anaerobic biodegradability of pyridine and its derivatives under sulfidogenic conditions. *Chemosphere*, **36**(10), 2345-2357.

Lopes, S.I.C., Wang, X., Capela, M.I., Lens, P.N.L. 2007. Effect of COD/SO42-ratio and sulfide on thermophilic (55 degrees C) sulfate reduction during the acidification of sucrose at pH 6. *Water Research*, **41**(11), 2379-2392.

Lovley, D.R. 1985. Minimum Threshold for Hydrogen Metabolism in Methanogenic Bacteria. *Applied and Environmental Microbiology*, **49**(6), 1530-1531.

Lovley, D.R., Phillips, E.J.P. 1987. Competitive Mechanisms for Inhibition of Sulfate Reduction and Methane Production in the Zone of Ferric Iron Reduction in Sediments. *Applied and Environmental Microbiology*, **53**(11), 2636-2641.

Maillacheruvu, K.Y., Parkin, G.F., Peng, C.Y., Kuo, W.C., Oonge, Z.I., Lebduschka, V. 1993. Sulfide Toxicity in Anaerobic Systems Fed Sulfate and Various Organics. *Water Environment Research*, **65**(2), 100-109.

Malina, J.F., Pohland, F.G. 1992. *Design of anaerobic processes for the treatment of industrial and municipal wastes*. Technomic Pub. Co., Lancaster.

McCartney, D.M., Oleszkiewicz, J.A. 1993. Competition between Methanogens and Sulfate Reducers - Effect of Cod Sulfate Ratio and Acclimation. *Water Environment Research*, **65**(5), 655-664.

Mccartney, D.M., Oleszkiewicz, J.A. 1991. Sulfide Inhibition of Anaerobic Degradation of Lactate and Acetate. *Water Research*, **25**(2), 203-209.

McCarty, P.L., McKinney, R.E. 1961. Salt Toxicity in Anaerobic Digestion. *Journal (Water Pollution Control Federation)*, **33**(4), 399-415.

McCue, T., Hoxworth, S., Randall, A.A. 2003. Degradation of halogenated aliphatic compounds utilizing sequential anaerobic/aerobic treatments. *Water Science and Technology*, **47**(10), 79-84.

Middleton, A.C., Lawrence, A.W. 1977. Kinetics of Microbial Sulfate Reduction. *Journal Water Pollution Control Federation*, **49**(7), 1659-1670.

Mittal, S.K., Mitra, A.K., Berry, A.M., Ghosh, S. 1974. Study of Heat Hyperpyrexia in Infants and Children. *Indian Pediatrics*, **11**(9), 623-627.

Mizuno, O., Li, Y.Y., Noike, T. 1998. The behavior of sulfate-reducing bacteria in acidogenic phase of anaerobic digestion. *Water Research*, **32**(5), 1626-1634.

Mormile, M.R., Suflita, J.M. 1996. The toxicity of selected gasoline components to glucose methanogenesis by aquifer microorganisms. *Anaerobe*, **2**(5), 299-303.

Mulder, A. 1984. The effects of high sulfate concentrations on the methane fermentation of waste water. *Progr. Ind. Microbiol.*, **20**, 133-143.

Noike, T., Endo, G., Chang, J.E., Yaguchi, J.I., Matsumoto, J.I. 1985. Characteristics of Carbohydrate Degradation and the Rate-Limiting Step in Anaerobic-Digestion. *Biotechnology and Bioengineering*, **27**(10), 1482-1489.

Novak, J.T., Carlson, D.A. 1970. The Kinetics of Anaerobic Long Chain Fatty Acid Degradation. *Journal (Water Pollution Control Federation)*, **42**(11), 1932-1943.

O'Flaherty, V., Colleran, E. 1999. Effect of sulphate addition on volatile fatty acid and ethanol degradation in an anaerobic hybrid reactor. I: process disturbance and remediation. *Bioresource Technology*, **68**(2), 101-107.

O'Flaherty, V., Lens, P., Leahy, B., Colleran, E. 1998. Long-term competition between sulphate-reducing and methane-producing bacteria during full-scale anaerobic treatment of citric acid production wastewater. *Water Research*, **32**(3), 815-825.

Okabe, S., Nielsen, P.H., Jones, W.L., Characklis, W.G. 1995. Sulfide Product Inhibition of Desulfovibrio-Desulfuricans in Batch and Continuous Cultures. *Water Research*, **29**(2), 571-578.

Okamoto, M., Miyahara, T., Mizuno, O., Noike, T. 2000. Biological hydrogen potential of materials characteristic of the organic fraction of municipal solid wastes. *Water Science and Technology*, **41**(3), 25-32.

Oleszkiewicz, J.A., Hilton, B.L. 1986. Anaerobic Treatment of High-Sulfate Wastes. *Canadian Journal of Civil Engineering*, **13**(4), 423-428.

Oleszkiewicz, J.A., Marstaller, T., McCartney, D.M. 1989. Effects of pH on sulfide toxicity to anaerobic processes. *Environmental Technology Letters Environmental Technology Letters*, **10**(9), 815-822.

Olthof, M., Kelly, W. R., O., Weinreb, H.G. 1985. Development of anaerobic treatment process for wastewaters containing high sulfates. *40th Ind. Waste Conf.*, Chelsea, Michigan. Lewis Publishers Inc.,. pp. 871 - 877.

Omil, F., Bakker, C.D., Pol, L.W.H., Lettinga, G. 1997. Effect of pH and low temperature shocks on the competition between sulphate reducing bacteria and methane producing bacteria in uasb reactors. *Environmental Technology*, **18**(3), 255-264.

Omil, F., Lens, P., Pol, L.H., Lettinga, G. 1996. Effect of upward velocity and sulphide concentration on volatile fatty acid degradation in a sulphidogenic granular sludge reactor. *Process Biochemistry*, **31**(7), 699-710.

Omil, F., Lens, P., Visser, A., Pol, L.W.H., Lettinga, G. 1998. Long-term competition between sulfate reducing and methanogenic bacteria in UASB reactors treating volatile fatty acids. *Biotechnology and Bioengineering*, **57**(6), 676-685.

Oremland, R.S., Capone, D.G. 1988. Use of Specific Inhibitors in Biogeochemistry and Microbial Ecology. *Advances in Microbial Ecology*, **10**, 285-383.

Overmeire, A., Lens, P., Verstraete, W. 1994. Mass-Transfer Limitation of Sulfate in Methanogenic Aggregates. *Biotechnology and Bioengineering*, **44**(3), 387-391.

Oyekola, O.O., van Hille, R.P., Harrison, S.T.L. 2010. Kinetic analysis of biological sulphate reduction using lactate as carbon source and electron donor: Effect of sulphate concentration. *Chemical Engineering Science*, **65**(16), 4771-4781.

Pallud, C., Van Cappellen, P. 2006. Kinetics of microbial sulfate reduction in estuarine sediments. *Geochimica Et Cosmochimica Acta*, **70**(5), 1148-1162.

Parkin, G.F., Lynch, N.A., Kuo, W.C., Vankeuren, E.L., Bhattacharya, S.K. 1990. Interaction between Sulfate Reducers and Methanogens Fed Acetate and Propionate. *Research Journal of the Water Pollution Control Federation*, **62**(6), 780-788.

Parkin, G.F., Sneve, M.A., Loos, H. 1991. Anaerobic Filter Treatment of Sulfate-Containing Wastewaters. *Water Science and Technology*, **23**(7-9), 1283-1291.

Parkin, G.F., Speece, R.E., Yang, C.H.J., Kocher, W.M. 1983. Response of Methane Fermentation Systems to Industrial Toxicants. *Journal Water Pollution Control Federation*, **55**(1), 44-53.

Pereira, M.A., Pires, O.C., Mota, M., Alves, M.M. 2005. Anaerobic biodegradation of oleic and palmitic acids: Evidence of mass transfer limitations caused by long chain fatty acid accumulation onto the anaerobic sludge. *Biotechnology and Bioengineering*, **92**(1), 15-23.

Pereira, M.A., Sousa, D.Z., Mota, M., Alves, M.M. 2004. Mineralization of LCFA associated with anaerobic sludge: Kinetics, enhancement of methanogenic activity, and effect of VFA. *Biotechnology and Bioengineering*, **88**(4), 502-511.

Playne, M.J., Smith, B.R. 1983. Toxicity of Organic Extraction Reagents to Anaerobic-Bacteria. *Biotechnology and Bioengineering*, **25**(5), 1251-1265.

Polo, B.C., Bewtra, J.K., Biswas, N. 2006. Effect of hydraulic retention time and attachment media on sulfide production by sulfate reducing bacteria. *Journal of Environmental Engineering and Science*, **5**(1), 47-57.

Polprasert, C., Haas, C.N. 1995. Effect of Sulfate on Anaerobic Processes Fed with Dual Substrates. *Water Science and Technology*, **31**(9), 101-107.

Postgate, J.R. 1984. *The sulphate-reducing bacteria*. Cambridge University Press, Cambridge [Eng.]; New York.

Prasad, D., Henry, J.G. 2009. Removal of sulphates acidity and iron from acid mine drainage in a bench scale biochemical treatment system. *Environmental Technology*, **30**(2), 151-160.

Prasad, D., Wai, M., Berube, P., Henry, J.G. 1999. Evaluating substrates in the biological treatment of acid mine drainage. *Environmental Technology*, **20**(5), 449-458.

Reis, M.A.M., Almeida, J.S., Lemos, P.C., Carrondo, M.J.T. 1992. Effect of Hydrogen-Sulfide on Growth of Sulfate Reducing Bacteria. *Biotechnology and Bioengineering*, **40**(5), 593-600.

Renard, P., Bouillon, C., Naveau, H., Nyns, E.J. 1993. Toxicity of a Mixture of Polychlorinated Organic-Compounds Towards an Unacclimated Methanogenic Consortium. *Biotechnology Letters*, **15**(2), 195-200.

Rinzema, A., Alphenaar, A., Lettinga, G. 1989. The Effect of Lauric Acid Shock Loads on the Biological and Physical Performance of Granular Sludge in Uasb Reactors Digesting Acetate. *Journal of Chemical Technology and Biotechnology*, **46**(4), 257-266.

Rinzema, A., Boone, M., Vanknippenberg, K., Lettinga, G. 1994. Bactericidal Effect of Long-Chain Fatty-Acids in Anaerobic-Digestion. *Water Environment Research*, **66**(1), 40-49.

Rinzema , A., Lettinga , G. 1986. Anaerobic treatment of sulphate containing wastewater. in: *Biotreatment Systems* (Ed.) D.L. Wise CRC Press Boca Raton, pp. 65–109.

Rinzema, A., Lettinga, G. 1988. The Effect of Sulfide on the Anaerobic Degradation of Propionate. *Environmental Technology Letters*, **9**(2), 83-88.

Rinzema , A., Paardekooper, A.H., De Vegt, A.L., Lettinga , G. 1986. Anaerobic treatment of edible oil refinery wastewater in granular sludge UASB reactors. *EWPCA Conf. Anaerobic Treatment, A Grown Up Technology*, Amsterdam, The Netherlands. pp. 205-217.

Rittmann, B.E., McCarty, P.L. 2001. *Environmental biotechnology : principles and applications*. McGraw-Hill, Boston.

Samsoon, P.A.L.N.S., Loewenthal, R.E., Wentzel, M.C., Marais, G.R. 1991. Effect of Sulfate on Pelletization in the Uasb System with Glucose as Substrate. *Water Sa*, **17**(1), 47-56.

Sarti, A., Pozzi, E., Chinalia, F.A., Ono, A., Foresti, E. 2010. Microbial processes and bacterial populations associated to anaerobic treatment of sulfate-rich wastewater. *Process biochemistry.*, **48**(2), 164.

Scholten, J.C., Conrad, R., Stams, A.J. 2000. Effect of 2-bromo-ethane sulfonate, molybdate and chloroform on acetate consumption by methanogenic and sulfate-reducing populations in freshwater sediment. *Fems Microbiology Ecology*, **32**(1), 35-42.

Sharma, M. 2008. Diverting electron fluxes in anaerobic microbial communities using long chain fatty acids (LCFAs). in: *Civil & Environmental Engineering*, University of Windsor. Windsor.

Sharma, M., Biswas, N. 2010. Diverting electron fluxes towards sulfate reduction using linoleic acid in a mixed anaerobic culture. *Canadian Journal of Civil Engineering*, **37**(11), 1492-1504.

Shin, H.S., Kwon, J.C. 1998. Degradation and interaction between organic concentrations and toxicity of 2,4,6-trichlorophenol in anaerobic system. *Biotechnology Techniques*, **12**(1), 39-43.

Shin, H.S., Oh, S.E., Bae, B.U. 1996. Competition between SRB and MPB according to temperature change in the anaerobic treatment of tannery wastes containing high sulfate. *Environmental Technology*, **17**(4), 361-370.

Sierra-Alvarez, R., Lettinga, G. 1991. The Effect of Aromatic Structure on the Inhibition of Acetoclastic Methanogenesis in Granular Sludge. *Applied Microbiology and Biotechnology*, **34**(4), 544-550.

Soto, M., Mendez, R., Lema, J.M. 1991. Biodegradability and Toxicity in the Anaerobic Treatment of Fish Canning Wastewaters. *Environmental Technology*, **12**(8), 669-677.

Speece, R.E. 1983. Anaerobic Biotechnology for Industrial Wastewater-Treatment. *Environmental Science & Technology*, **17**(9), A416-A427.

Stams, A.J.M., Plugge, C.M., de Bok, F.A.M., van Houten, B.H.G.W., Lens, P., Dijkman, H., Weijma, J. 2005. Metabolic interactions in methanogenic and sulfate-reducing bioreactors. *Water Science and Technology*, **52**(1-2), 13-20.

Stergar, V., Zagorc-Koncan, J., Zgajnar-Gotvanj, A. 2003. Laboratory scale and pilot plant study on treatment of toxic wastewater from the petrochemical industry by UASB reactors. *Water Science and Technology*, **48**(8), 97-102.

Stuckey, D.C., Owen, W.F., Mccarty, P.L., Parkin, G.F. 1980. Anaerobic Toxicity Evaluation by Batch and Semi-Continuous Assays. *Journal Water Pollution Control Federation*, **52**(4), 720-729.

Stucki, G., Hanselmann, K.W., Hurzeler, R.A. 1993. Biological Sulfuric-Acid Transformation - Reactor Design and Process Optimization. *Biotechnology and Bioengineering*, **41**(3), 303-315.

Sung, S., Raskin, L., Duangmanee, T., Padmasiri, S., Simmons, J.J. 2002. Hydrogen production by anaerobic microbial communities exposed to repeated heat treatments. *DOE Hydrogen Program Review*, U.S. pp. 1-17.

Thauer, R.K., Jungermann, K., Decker, K. 1977. Energy Conservation in Chemotrophic Anaerobic Bacteria. *Bacteriology Reviews*, **41**(1), 100–180.

Tursman, J.F., Cork, D.J. 1989. Influence of sulphate and sulphate reducing bacteria on anaerobic digestion technology. in: *Biological Waste Treatment*, (Eds.) A. Mizrahi, A. Van Wezel, Alan R Liss Inc. New York, pp. 273-281.

Uberoi, V., Bhattacharya, S.K. 1995. Interactions among Sulfate Reducers, Acetogens, and Methanogens in Anaerobic Propionate Systems. *Water Environment Research*, **67**(3), 330-339.

Uberoi, V., Bhattacharya, S.K. 1997. Toxicity and degradability of nitrophenols in anaerobic systems. *Water Environment Research*, **69**(2), 146-156.

Utgikar, V.P., Harmon, S.M., Chaudhary, N., Tabak, H.H., Govind, R., Haines, J.R. 2002. Inhibition of sulfate-reducing bacteria by metal sulfide formation in bioremediation of acid mine drainage. *Environmental Toxicology*, **17**(1), 40-48.

Van Beelen, P., Van Vlaardingen, P. 1994. Toxic Effects of Pollutants on the Mineralization of 4-Chlorophenol and Benzoate in Methanogenic River Sediment. *Environmental Toxicology and Chemistry*, **13**(7), 1051-1060.

Vavilin, V.A., Vasiliev, V.B., Rytov, S.V., Ponomarev, A.V. 1994. Self-Oscillating Coexistence of Methanogens and Sulfate-Reducers under Hydrogen-Sulfide Inhibition and the Ph-Regulating Effect. *Bioresource Technology*, **49**(2), 105-119.

Veeken, A., Hamelers, B. 1999. Effect of temperature on hydrolysis rates of selected biowaste components. *Bioresource Technology*, **69**(3), 249-254.

Veeken, A., Kalyuzhnyi, S., Scharff, H., Hamelers, B. 2000. Effect of pH and VFA on hydrolysis of organic solid waste. *Journal of Environmental Engineering-Asce*, **126**(12), 1076-1081.

Visser, A., Beeksma, I., Vanderzee, F., Stams, A.J.M., Lettinga, G. 1993. Anaerobic Degradation of Volatile Fatty-Acids at Different Sulfate Concentrations. *Applied Microbiology and Biotechnology*, **40**(4), 549-556.

Visser, A., Gao, Y., Lettinga, G. 1992. Anaerobic Treatment of Synthetic Sulfate-Containing Waste-Water under Thermophilic Conditions. *Water Science and Technology*, **25**(7), 193-202.

Visser, A., Pol, L.W.H., Lettinga, G. 1996. Competition of methanogenic and sulfidogenic bacteria. *Water Science and Technology*, **33**(3), 99-110.

Vogels, G.D., Keltjens, J., van der Drift, C. 1988. Biochemistry of methane production. in: *Biology of Anaerobic Microorganisms*, (Ed.) A.J.B. Zehnder, John Wiley & Sons. New York, pp. 707 - 770.

Wang, A.J., Ren, N.Q., Wang, X., Lee, D. 2008. Enhanced sulfate reduction with acidogenic sulfate-reducing bacteria. *Journal of Hazardous Materials*, **154**(1-3), 1060-1065.

Weijma, J., Bots, E.A.A., Tandlinger, G., Stams, A.J.M., Pol, L.W.H., Lettinga, G. 2002. Optimisation of sulphate reduction in a methanol-fed thermophilic bioreactor. *Water Research*, **36**(7), 1825-1833.

Weng, C., Jeris, J.S. 1976. Biochemical Mechanisms in Methane Fermentation of Glutamic and Oleic Acids. *Water Research*, **10**(1), 9-18.

Yang, J., Speece, R.E. 1986. The Effects of Chloroform Toxicity on Methane Fermentation. *Water Research*, **20**(10), 1273-1279.

Yoda, M., Kitagawa, M., Miyaji, Y. 1987. Long-Term Competition between Sulfate-Reducing and Methane-Producing Bacteria for Acetate in Anaerobic Biofilm. *Water Research*, **21**(12), 1547-1556.

Zeeman, G., Wiegant, W.M., Kostertreffers, M.E., Lettinga, G. 1985. The Influence of the Total Ammonia Concentration on the Thermophilic Digestion of Cow Manure. *Agricultural Wastes*, **14**(1), 19-35.

Zeikus, J.G. 1977. The biology of methanogenic bacteria. *Bacteriological reviews*, **41**(2), 514-41.

Zinder, S.H. 1993. Physiological ecology of methanogens. in: *Methanogenesis: Ecology, Physiology, Biochemistry & Genetics*, (Ed.) J.G. Ferry, Chapman & Hall. New York, NY.

VITA AUCTORIS

Name:	Tapas Biswas
Year of Birth:	1985
Place of Birth:	Kurigram, Bangladesh
Education:	Bachelor of Civil Engineering, 2007
	Bengal Engineering and Science University, Shibpur
	India