EFFECT OF ORP ON ANAEROBIC TREATMENT OF SULFATE-LADEN WASTEWATER

BY

CHI WAI LEUNG, B.Sc.

A Thesis Presented to
The Hong Kong University of Science and Technology
in Partial Fulfillment
of the Requirements for
the Degree of Master of Philosophy
in Civil and Structural Engineering

Hong Kong, January 1998

Copyright© by Chi Wai Leung 1998
Authorization

I hereby declare that I am the sole author of the thesis.

I authorize the Hong Kong University of Science and Technology to lend this thesis to other institutions or individuals for the purpose of scholarly research.

I further authorize the Hong Kong University of Science and Technology to reproduce the thesis by photocopying or by other means, in total or in par, at the request of other institutions or individuals for the purposes of scholarly research.
EFFECT OF ORP ON ANAEROBIC TREATMENT OF SULFATE-LADEN WASTEWATER

BY

CHI WAI LEUNG, B.Sc.

APPROVED:

[Signature]
PROF. HOWARD J.C. HUANG, SUPERVISOR

[Signature]
PROF. WILSON TANG, HEAD OF DEPARTMENT

Department of Civil and Structural Engineering
23 January 1998
Acknowledgements

First I would like to thank God for His guidance and blessing in my study. I would also like to express my gratitude towards my supervisor, Prof. Howard J.C. Huang for his trust, support and advice, especially for the painstaking work of advising my thesis writing. Thanks are also due to my supportive parents, brother and sister who bore with my “missing in action” for such a long time.

I would like to acknowledge Dr. Guanghao Chen and Dr. Lianfa Song for reading my thesis. I have learned a lot from their teaching and advice in the past two years.

My friends in the Environmental Engineering Laboratory, Mr. S.T. Lui, Mr. Johnson Yau and Mr. W.M. Chaw have helped me a lot in the preparation of experimental set up and chemical analysis. I wish to express my sincere thanks for their friendship and encouragement.

Lastly, I would like to dedicate this thesis to my loving Jessica. She shared my difficulties by listening patiently, gave me strength by timely support and encouragement.
TABLE OF CONTENTS

Title Page i
Authorization Page ii
Signature Page iii
Acknowledgements iv
Table of Contents v
List of Figures viii
List of Tables x
Abstract xii

1. INTRODUCTION 1
1.1 Background 1
1.2 Objective 2
1.3 Scope 2

2. LITERATURE REVIEW 4
2.1 Anaerobic Process 4
2.2 Microorganisms 4
2.3 Sulfate-Laden Wastewater 6
2.4 Acid-Forming Bacteria and Acetogens 7
   2.4.1 Environmental Conditions 7
      2.4.1.1 pH 7
      2.4.1.2 Oxygen 7
2.5 Methanogens 8
   2.5.1 Metabolism 8
   2.5.2 Taxonomy 8
   2.5.3 Environmental Conditions 9
      2.5.3.1 Salinity 9
      2.5.3.2 Temperature 9
      2.5.3.3 pH 9
      2.5.3.4 Oxygen 10
      2.5.3.5 Toxicity 10
2.6 Sulfate-Reducing Bacteria 10
   2.6.1 Metabolism 10
   2.6.2 Taxonomy 12
   2.6.3 Environmental Conditions 13
      2.6.3.1 Salinity 13
      2.6.3.2 Temperature 13
      2.6.3.3 pH 14
2.6.3.4 Oxygen 14
2.6.3.5 Inhibition 14

2.7 Oxidation-Reduction Potential 14
2.7.1 Theoretical Background 14
2.7.2 ORP of Bacterial Culture 15
2.7.3 ORP and Anaerobic Systems 15
2.7.4 Interplay of Oxygen and ORP in Anaerobic Systems 16

2.8 Interaction between Methanogens and Sulfate-Reducing Bacteria 16
2.8.1 General 16
2.8.2 Competition 17
2.8.3 Coexistence 18
2.8.4 Synergism 18
2.8.5 Interaction in Anaerobic Treatment Process 19

3. MATERIALS AND METHODS 21

3.1 Apparatus 21
3.1.1 Reactor 21
3.1.2 Reactor Mixing 22
3.1.3 Oxidation-Reduction Potential Measurement 22
3.1.4 Oxidation-Reduction Potential Control 22

3.2 Substrate 23

3.3 Chemical Analysis 23
3.3.1 Sample Preparation 23
3.3.2 pH 24
3.3.3 Gas Compositions 24
3.3.4 Total Organic Carbon 24
3.3.5 Sulfate 25
3.3.6 Sulfides 25
3.3.7 Suspended Solids and Volatile Suspended Solids 25

4. RESULTS AND DISCUSSION 27

4.1 Effects of COD/SO$_4^{2-}$ Ratios and ORP on Anaerobic System Properties 27

4.2 Effects of ORP on System Performance at a COD/SO$_4^{2-}$ Ratio of 28
10000 mg/l: 1000 mg/l
4.2.1 Total Organic Carbon 28
4.2.2 Sulfate 29
4.2.3 Dissolved Sulfides 31
4.2.4 Gases Productions 32
4.2.5 Material Balance 33

4.3 Effects of ORP on System Performance at a COD/SO$_4^{2-}$ Ratio of 34
10000 mg/l: 5000 mg/l
4.3.1 Total Organic Carbon 34
4.3.2 Sulfate 35
4.3.3 Dissolved Sulfides 37
4.3.4 Gases Production 39
4.3.5 Material Balance 41
4.4 Effects of ORP on System Performance at a COD/\text{SO}_4^{2-}\) Ratio of 10000 mg/l: 3000 mg/l

4.4.1 Total Organic Carbon 43
4.4.2 Sulfate 44
4.4.3 Dissolved Sulfides 45
4.4.4 Gases Production 45
4.4.5 Material Balance 47

4.5 Effects of COD/\text{SO}_4^{2-}\) Ratio on System Performance at Same ORP Level 47

4.5.1 At Natural ORP 47
4.5.2 ORP Increment of 50 mV 49
4.5.3 ORP Increment of 100 mV 51

4.6 Effects of pH Change on Properties of Systems 51

4.7 Conceptual Model 53

5. CONCLUSIONS 56

APPENDIX 1 59

APPENDIX 2 63

APPENDIX 3 67

APPENDIX 4 70

REFERENCE 71
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fig. 2-1:</td>
<td>Stages of anaerobic degradation for three-stage and four-stage model</td>
<td>5</td>
</tr>
<tr>
<td>Fig. 2-2:</td>
<td>Competition between methanogenic bacteria and sulfate-reducing bacteria</td>
<td>17</td>
</tr>
<tr>
<td>Fig. 2-3:</td>
<td>Coexistence of methanogenic bacteria and sulfate-reducing bacteria</td>
<td>18</td>
</tr>
<tr>
<td>Fig. 2-4:</td>
<td>Synergism between methanogenic bacteria and sulfate-reducing bacteria</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>in the absence of sulfate</td>
<td></td>
</tr>
<tr>
<td>Fig. 3-1:</td>
<td>Schematic of reactor set up</td>
<td>26</td>
</tr>
<tr>
<td>Fig. 4-1:</td>
<td>Influent and effluent TOC vs ORP at COD/sulfate ratio of 10000 mg/l: 1000 mg/l</td>
<td>29</td>
</tr>
<tr>
<td>Fig. 4-2:</td>
<td>Influent and effluent sulfate vs ORP at COD/sulfate ratio of 10000 mg/l: 1000 mg/l</td>
<td>30</td>
</tr>
<tr>
<td>Fig. 4-3:</td>
<td>Effluent dissolved sulfide vs ORP at COD/sulfate ratio of 10000 mg/l: 1000 mg/l</td>
<td>32</td>
</tr>
<tr>
<td>Fig. 4-4:</td>
<td>Individual gas production rate vs ORP at COD/sulfate ratio of 10000 mg/l: 1000 mg/l</td>
<td>33</td>
</tr>
<tr>
<td>Fig. 4-5:</td>
<td>Influent and effluent TOC vs ORP at COD/sulfate ratio of 10000 mg/l: 5000 mg/l</td>
<td>35</td>
</tr>
<tr>
<td>Fig. 4-6:</td>
<td>Influent and effluent sulfate vs ORP at COD/sulfate ratio of 10000 mg/l: 5000 mg/l</td>
<td>37</td>
</tr>
<tr>
<td>Fig. 4-7:</td>
<td>Dissolved sulfide vs ORP at COD/sulfate ratio of 10000 mg/l: 5000 mg/l</td>
<td>39</td>
</tr>
<tr>
<td>Fig. 4-8:</td>
<td>Individual gas production rate vs ORP at COD/sulfate ratio of 10000 mg/l: 5000 mg/l</td>
<td>41</td>
</tr>
<tr>
<td>Fig. 4-9:</td>
<td>Influent and effluent TOC vs ORP at COD/sulfate ratio of 10000 mg/l: 3000 mg/l</td>
<td>43</td>
</tr>
<tr>
<td>Fig. 4-10:</td>
<td>Influent and effluent sulfate vs ORP at COD/sulfate ratio of 10000 mg/l: 3000 mg/l</td>
<td>44</td>
</tr>
<tr>
<td>Fig. 4-11:</td>
<td>Dissolved sulfide vs ORP at COD/sulfate ratio of 10000 mg/l: 3000 mg/l</td>
<td>45</td>
</tr>
<tr>
<td>Fig. 4-12:</td>
<td>Individual gas production rate vs ORP at COD/sulfate ratio of 10000 mg/l: 3000 mg/l</td>
<td>46</td>
</tr>
</tbody>
</table>
Fig. 4-13:  Methane production rate vs influent sulfate concentration at various ORP
Fig. 4-14:  Sulfate reducing activity vs influent sulfate concentration at various ORP
Fig. 4-15:  Flow of chemical reactions in anaerobic system under elevated ORP
Fig. A1-1:  ORP curve for COD/\(\text{SO}_4^{2-}\) ratio of 10000 mg/l: 1000 mg/l and average ORP of −233 mV
Fig. A1-2:  ORP curve for COD/\(\text{SO}_4^{2-}\) ratio of 10000 mg/l: 1000 mg/l and average ORP of −183 mV
Fig. A1-3:  ORP curve for COD/\(\text{SO}_4^{2-}\) ratio of 10000 mg/l: 3000 mg/l and average ORP of −235 mV
Fig. A1-4:  ORP curve for COD/\(\text{SO}_4^{2-}\) ratio of 10000 mg/l: 5000 mg/l and average ORP of −235 mV
Fig. A1-5:  ORP curve for COD/\(\text{SO}_4^{2-}\) ratio of 10000 mg/l: 5000 mg/l and average ORP of −185 mV
Fig. A2-1:  Gas production vs time for exploratory test
Fig. A2-2:  Methane yield and sulfate removal vs pH for second test
Fig. A4-1  Interactions between methanogens and sulfate-reducers at various ORP at an influent COD/sulfate ratio of 10000 mg/l: 5000 mg/l
# List of Tables

Table 2-1: Typical sulfate-laden wastewater 7  
Table 2-2: Reported substrates that can be used for dissimilatory sulfate reduction 12  
Table 2-3: Characteristics of some contemporary sulfate-reducing bacteria 13  
Table 3-1: Composition of substrate 23  
Table 4-1: Experimental matrix of various COD/ŞO₄²⁻ ratios and ORP 27  
Table 4-2: Influent and effluent TOC at various ORP at COD/ŞO₄²⁻ ratio of 10000 mg/l: 1000 mg/l 29  
Table 4-3: Influent and effluent sulfate at various ORP at COD/ŞO₄²⁻ ratio of 10000 mg/l: 1000 mg/l 30  
Table 4-4: Dissolved sulfide concentration at various ORP at COD/ŞO₄²⁻ ratio of 10000 mg/l: 1000 mg/l 31  
Table 4-5: Gas production rates at various ORP at COD/ŞO₄²⁻ ratio of 10000 mg/l: 1000 mg/l 33  
Table 4-6: Material balance of carbon for COD/ŞO₄²⁻ ratio of 10000 mg/l: 1000 mg/l 34  
Table 4-7: Influent and effluent TOC at various ORP at COD/ŞO₄²⁻ ratio of 10000 mg/l: 5000 mg/l 35  
Table 4-8: Influent and effluent sulfate at various ORP at COD/ŞO₄²⁻ ratio of 10000 mg/l: 5000 mg/l 37  
Table 4-9: Dissolved sulfide concentration at various ORP at COD/ŞO₄²⁻ ratio of 10000 mg/l: 5000 mg/l 38  
Table 4-10: Gas production rates at various ORP at COD/ŞO₄²⁻ ratio of 10000 mg/l: 5000 mg/l 40  
Table 4-11: Material balance of carbon for COD/ŞO₄²⁻ ratio of 10000 mg/l: 5000 mg/l 42  
Table 4-12: Influent and effluent TOC at various ORP at COD/ŞO₄²⁻ ratio of 10000 mg/l: 3000 mg/l 43  
Table 4-13: Influent and effluent sulfate at various ORP at COD/ŞO₄²⁻ ratio of 10000 mg/l: 3000 mg/l 44
<table>
<thead>
<tr>
<th>Table 4-14:</th>
<th>Dissolved sulfide concentration at various ORP at COD/SO$_4^{2-}$ ratio of 10000 mg/l: 3000 mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 4-15:</td>
<td>Gas production rates at various ORP at COD/SO$_4^{2-}$ ratio of 10000 mg/l: 3000 mg/l</td>
</tr>
<tr>
<td>Table 4-16:</td>
<td>Material balance of carbon for COD/SO$_4^{2-}$ ratio of 10000 mg/l: 3000 mg/l</td>
</tr>
<tr>
<td>Table 4-17:</td>
<td>pH at different operating conditions</td>
</tr>
<tr>
<td>Table A2-1:</td>
<td>Results of the exploratory test</td>
</tr>
<tr>
<td>Table A2-2:</td>
<td>Results of the second pH test</td>
</tr>
<tr>
<td>Table A3-1:</td>
<td>Carbon concentrations at influent and various effluent streams</td>
</tr>
<tr>
<td>Table A3-2:</td>
<td>Carbon balance</td>
</tr>
<tr>
<td>Table A3-3:</td>
<td>Henry’s constant and equilibrium constants for carbon dioxide</td>
</tr>
<tr>
<td>Table A3-4:</td>
<td>Theoretical carbon concentrations vs measured values</td>
</tr>
<tr>
<td>Table A3-5:</td>
<td>Henry’s constant and equilibrium constants for sulfide</td>
</tr>
<tr>
<td>Table A3-6:</td>
<td>Theoretical dissolved sulfide concentrations vs measured values</td>
</tr>
</tbody>
</table>
ABSTRACT

In anaerobic treatment of sulfate-laden organic wastewater, sulfate is reduced to sulfide by sulfate-reducing bacteria. This reduction is undesirable as it reduces the methane yield by substrate competition and/or sulfide toxicity on methanogenic bacteria. Moreover, the fuel value of biogas is reduced by the presence of hydrogen sulfide, which forms sulfuric acid upon combustion and causes corrosion of plant machinery.

This study investigated the effect of oxidation-reduction potential (ORP) on the competition between sulfate-reducing bacteria and methanogens. The ultimate aim was to evaluate the feasibility of developing a two-stage anaerobic system to treat the sulfate-laden wastewater. That is, the first stage is used to allow only sulfate reducers to function through a proper ORP control, and the second stage for methanogenesis. To achieve this goal, experimental tests had been carried out to evaluate the responses of both sulfate reducers and methanogens at different ORP with different combinations of COD/\(\text{SO}_4^{2-}\) ratios (with a constant influent COD of 10,000 mg/l or TOC of 3750 mg/l).

At the natural operating ORP of around −285 mV, temperature 35°C and HRT of 15 days, the activities of the sulfate reducers were not affected by the influent sulfate increase from 1,000 to 5,000 mg/l. However, the methanogenic activity was progressively reduced by the increase of influent sulfate concentration. More specifically, the methane production rate was 176 ml/L-day at 1,000 mg/l of sulfate, and the value was reduced to 108 and 96 ml/L-day, respectively, when the sulfate concentration was increased to 3,000 and 5,000 mg/l.
When the operating ORP was increased to -235 mV, the sulfate-reducing activities were not adversely affected as long as the influent sulfate concentration did not exceed 3,000 mg/l. But as the influent sulfate was increased to 5,000 mg/l, the sulfate reducing bacteria became inhibited. The level of sulfate reducers' inhibition exceeded that of methanogens at this ORP. As such, the methanogens became more competitive for the available organic carbon. This caused a substantial increase of the methane yield under such a specific operating condition due to the elimination of substrate competition from the sulfate reducers.

When the ORP was further increased to -185 mV, the methanogenic activity became almost totally inhibited while the sulfate reducers were not. Thus, for treating high-strength sulfate-laden wastewater, it seems feasible to control the operating ORP at -185 mV to arrest the methanogenic activity, yet still allowing the sulfate reducers to remove sulfate with full potential without inhibition.

Keywords: sulfate reduction, methanogenesis, substrate competition, relative bioactivities, ORP, COD/\(\text{SO}_4^{2-}\) ratio, treatment performance, two-stage anaerobic process.
Chapter 1

INTRODUCTION

1.1 Background

Anaerobic treatment is commonly used for the treatment of high organic strength wastewater. The anaerobic process has the advantages of using less energy as no aeration is required. Moreover, methane is a major product of the process, which can be used as fuel gas. Besides, the sludge yield of the anaerobic process is much lower than that of aerobic process, hence reducing the cost of sludge handling and disposal.

Nevertheless, the conventional anaerobic process cannot be applied to high sulfate content wastewater successfully because of presence of sulfate reducing activity in the treatment system. In the system, sulfate-reducing bacteria use the sulfate in wastewater as electron acceptor in the substrate oxidation process. The sulfate reducing bacteria reduce the methane yield by competing with methanogens for substrates and also inhibiting the latter through sulfide production. Also, the hydrogen sulfide so produced will lower the quality of the biogas fuel or even render it unsuitable for use, as hydrogen sulfide is oxidized to sulfuric acid upon combustion. Furthermore, hydrogen sulfide is itself a toxic and pungent smelling gas which can cause environmental nuisance if discharged into the atmosphere.

In view of such undesirable effects of the sulfate reduction process, research has been directed to studying the interaction between the methanogens and the sulfate reducing bacteria, with the ultimate aim of reducing or eliminating the undesirable effects of sulfate reduction from the anaerobic treatment process. The current study reported herein was aimed at investigating the effect of varying oxidation-reduction
potential on the relative dominance of sulfate reducing bacteria and methane producing bacteria. The ultimate goal was to assess if oxidation-reduction potential control could be used as a viable measure for separating the anaerobic treatment of sulfate-laden wastewater into two separate stages: the sulfate reduction stage and the methane production stage.

1.2 Objective

The objective of this study was to evaluate the effect of varying oxidation-reduction potential on the interaction between methanogens and sulfate reducing bacteria. The ultimate goal of this study was to develop a two-stage system that would promote the growth of both groups of bacteria in physically separated reactors, through possible manipulation of ORP in each reactor. That is, the first reactor provides an ORP condition that is only suitable for the sulfate-reducing bacteria. Upon sulfate removal in the first stage, the second stage will then concentrate on maintaining an optimum condition for the methanogens to function.

1.3 Scope

Completely mixed anaerobic reactors with mixing by the recycled biogas were used in the study. A synthetic feed consisting of glucose, minerals and soluble sulfate was used. Each reactor was fed with a specifically designed COD/SO$_4^{2-}$ ratio by varying the relative amounts of glucose and sulfate. The oxidation-reduction potential of the system was controlled by periodic oxygen dosing into the circulating gas stream. The scope of the study included:
1. evaluation of the effect of different COD/\text{SO}_4^{2-} \text{ ratio on the sulfate reduction and methane generation;}

2. evaluation of the effect of increasing oxidation reduction potential on the sulfate reduction and methane generation, COD reduction and biogas composition; and

3. analysis of the interaction between methanogenic bacteria and sulfate-reducing bacteria from the experimental data and then proposing a hypothesis on its interaction.
Chapter 2

LITERATURE REVIEW

2.1 Anaerobic Process

Anaerobic process is the microbial degradation of organics in the absence of molecular oxygen. In such a process, the organics are converted to methane (CH₄) and carbon dioxide (CO₂). Although it was originally developed for sludge digestion, it is finding more application due to improved reactor design and its advantages over the aerobic process, especially for the treatment of high strength organic waste.

2.2 Microorganisms

The degradation of organics in anaerobic process is accomplished through a number of metabolic stages mediated by different groups of microorganisms.

The earliest model for the anaerobic process is the two-stage model, which postulates it as the coordinated effort between acid fermenting bacteria, acidogens and methane producing bacteria. The former is responsible for the hydrolysis and fermentation of organic into fatty acids and alcohols. These intermediates are subsequently converted by methanogens into methane.

This initial model was further modified by two groups of investigators who proposed the three-stage (Byrant, 1979) and four-stage model (Zeikus, 1979) respectively. These two models are depicted in Fig. 2-1.

In the three-stage model, the organics are first hydrolyzed and fermented by acidogens into intermediates like propionate, butyrate, lactate and ethanol. In the second stage, the intermediates are transformed into acetate, hydrogen/carbon dioxide under the action of obligate hydrogen-producing acetogens (OHPA). In the
third stage, the methanogens convert the acetate and hydrogen/carbon dioxide into methane through two different metabolic paths. In the aceticlastic pathway the acetate is cleaved into methane and carbon dioxide while in the carbon dioxide reduction pathway carbon dioxide is reduced by hydrogen to methane. In general, aceticlastic pathway accounts for 70% of methane formed.

Fig. 2-1 Stages of Anaerobic degradation for three-stage and four-stage model.

The four-stage model is similar to that of the three-stage except it incorporates the action of homoacetogens which are capable of forming acetate from hydrogen and carbon dioxide. Nevertheless, it is believed that the action of these bacteria is not significant and accounts for less than 5% of the total acetate formation in anaerobic process (Mackie et al, 1981).

As depicted in these models, the microorganisms involved in the anaerobic process include acidogens, obligate hydrogen-producing acetogens, homoacetogens and methanogens. The organics can only be anaerobically degraded into methane and carbon dioxide effectively when there are coordinated interactions among the four
groups of microorganisms. The process is jeopardized or even stopped when certain
group of bacteria is inhibited. Since methanogens are more sensitive to
environmental conditions and have a slower growth rate, the methanogenesis is
usually the rate determining stage of the whole anaerobic process.

Apart from the four categories of microorganisms mentioned above, certain
groups of anaerobic bacteria, collectively called the sulfate-reducing bacteria, which
have the common ability of utilizing sulfate as electron acceptor in catabolic
processes, can also have a dominant presence in anaerobic environment depending on
the availability of sulfate. These bacteria can oxidize products from acid fermentation
and some can even utilize acetate as substrate. As a result of substrate competition,
coupled with the toxic effect of hydrogen sulfide produced by these bacteria,
presence of sulfate reducing activities can seriously jeopardize the performance of
methanogens. Various interactions can arise between them and this will be discussed
in later sections.

2.3 Sulfate Laden Wastewater

Sulfate-laden wastewater mainly comes from sources like papermaking, food
processing, chemical and antibiotic manufacturing. The sulfate content in these types
of wastewater can be as high as 7000 mg/l. Apart from high sulfate content, they
usually have high organic strengths. For organic degradation, anaerobic treatment is
more suitable for these wastes; however, the sulfate in these wastes will be reduced
by sulfate-reducing bacteria to hydrogen sulfide (H₂S) which is pungent and toxic or
inhibitory to the anaerobic degradation process as mentioned in the previous section.
Table 2-1 Typical sulfate-laden wastewater

<table>
<thead>
<tr>
<th>Wastewater</th>
<th>COD (mg/l)</th>
<th>BOD₅ (mg/l)</th>
<th>SO₄²⁻ (mg/l)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distillery Waste</td>
<td>120000</td>
<td>27500</td>
<td>3500</td>
<td>Hiatt, 1973</td>
</tr>
<tr>
<td>Edible oil Waste</td>
<td>1010-8200</td>
<td>-</td>
<td>3100-7400</td>
<td>Anderson et al., 1983</td>
</tr>
<tr>
<td>Mining Waste</td>
<td>100</td>
<td>-</td>
<td>1980</td>
<td>Maree et al., 1985</td>
</tr>
<tr>
<td>Yeast Waste</td>
<td>52600-88800</td>
<td>18300-27200</td>
<td>4600-6300</td>
<td>Lo et al., 1990</td>
</tr>
</tbody>
</table>

2.4 Acid-Forming Bacteria and Acetogens

Acid-forming bacteria is a collective name for different groups of bacteria which are characterized by degrading organic to different end products like ethanol, propionate, butyrate and lactate. These intermediates can then be utilized by acetogens to form acetate. By producing acetate and hydrogen/carbon dioxide, the only substrates that can be utilized by methanogens, the acid forming bacteria and acetogens are indispensable in realizing the mineralization of organics through methanogenesis.

2.4.1 Environmental Conditions

2.4.1.1 pH

The acid-forming bacteria and acetogens can tolerate a range of pH from 4.5-8.0, which is much wider than that for the methanogens. So the anaerobic process can be disrupted when the activities of the acid formers and methanogens are unbalanced with the former producing excessive acids.

2.4.1.2 Oxygen

Unlike other groups of microorganisms in the anaerobic process which are strictly anaerobic, some acid forming bacteria are facultative and can utilize oxygen as electron acceptor.
2.5 Methanogens

2.5.1 Metabolism

This group of microbes has the unique ability of producing methane as a major catabolic product. Phylogenetically, methanogens are *Archaeobacteria*, a group of microbes that are distinguished from the true bacteria by a number of characteristics, including chemical compositions of the membrane, cell wall and ribosomal RNA sequence.

The catabolic pathways of methanogens can be categorized into three groups: carbon dioxide reducing, methylotrophic and aceticlastic pathways. The carbon dioxide reducing methanogens mainly use hydrogen, formate, and to a less extent, primary and secondary alcohols as electron donors for carbon dioxide reduction. The methylotrophic methanogens catabolize compounds with methyl groups, like methanol, trimethylamine and dimethyl sulfide. The electrons for methyl reduction can be obtained by oxidation of a fraction of methyl groups or from hydrogen. The aceticlastic pathway is a special kind of methylotrophic pathway in which the acetate molecules are broken down, with the carboxyl group being oxidized to CO$_2$ and methyl group reduced to methane.

2.5.2 Taxonomy

The kingdom of *Archaeobacteria* is divided into five orders, namely *Methanobacterales, Methanococcales, Methanomicrobiales, Methanosarcinales* and *Methanopyrales.*
The order of *Methanobacteriales* consists mainly of rod-shaped methanogens that grow by CO$_2$ reduction. *Methanococcales* is an order of marine methanogens, which are slightly halophilic and grow by using hydrogen or formate to reduce CO$_2$ to CH$_4$. The order *Methanomicrobiales* is also generally halophilic and grow by using hydrogen to reduce CO$_2$ to CH$_4$. Members of the *Methanosarcinales* are mostly methylotrophic. In fact they include all methylotrophic species except *Methanosphaera* in the order of *Methanobacteriales*. They are the only group of methanogens capable of aceticlastic catabolism and metabolically most versatile in terms of substrate range. The last order is the *Methanopyrales* that consists of only one species being able to grow near 100°C with carbon dioxide reduction.

### 2.5.3 Environmental Conditions

#### 2.5.3.1 Salinity

Methanogens are present in the whole range of salinity from freshwater to hypersaline environment.

#### 2.5.3.2 Temperature

Methanogens can live in a wide range of temperature. Nevertheless, growth optima are found in the mesophilic and thermophilic ranges. In general, the thermophiles grow more rapidly than the mesophiles. In the mesophilic range, it is well known from the anaerobic digester literature that the best operating temperature is around 35°C (McCarty, 1964).

#### 2.5.3.3 pH

Most methanogens have optimal pH near neutrality. It is proposed that the inhibition of methanogens at lower pH is due to accumulation of acetic acid/acetate
inside the cell (Russel, 1991). It has been known that a high concentration of fatty acids can enhance the low pH inhibitory effect in anaerobic reaction (McCarty, 1964).

2.5.3.4 Oxygen

The methanogens have been considered as the strictest anaerobes which need an oxidation-reduction potential more negative than -300mV (Hungate, 1967). Although it is believed that methanogens cannot grow nor produce methane in the presence of oxygen, they can be tolerant to oxygen exposure (Kiener and Leisinger, 1983), albeit with different sensitivity. This suggests that methanogens can exist in transient anaerobic conditions (i.e., with periodic short-term appearance of trace dissolved oxygen in the system) or “anaerobic microenvironments” in an aerobic system.

2.5.3.5 Toxicity

Sulfide toxicity has been observed at concentrations ranging from 200 to 1500 mg/l (Stronach, 1986). It is also reported that methanogen is partially inhibited at a total ammonia concentration of 3000 mg/l and a pH of 7.1, while 4000 mg/l caused complete inhibition (Stronach, 1986).

2.6 Sulfate Reducing Bacteria

2.6.1 Metabolism

The sulfate reducing bacteria are a group of diversified anaerobic procaryotes which are unified by the shared ability of carrying out sulfate reduction, in which sulfate is used as the terminal electron acceptor in oxidizing organic or inorganic
substrates and thus becomes reduced to sulfides. Except for a small proportion, which is used for anabolism, the sulfides generated are excreted out of the cell into the environment. This kind of sulfate reduction is called dissimilatory reduction in contrast to assimilatory reduction in which all the sulfur is incorporated into cell components.

As shown in Table 2-2, a wide range of substrates for sulfate reducing bacteria has been reported, including inorganic substances like hydrogen, carbon monoxide and organic substances like straight alkanes, mono- and di-carboxylic acids, alcohols, amino acids and sugars. For the direct oxidation of sugars, however, only a few strains of sugar-utilizing sulfate-reducing bacteria have been discovered. Among them, some are thermophilic and the others grow slower than fermenting bacteria (Hansen, 1993). In view of these, the direct sugar oxidizers may not have a significant role in the mesophilic anaerobic environment.

The metabolic abilities of the sulfate-reducing bacteria can be classified into complete oxidizer and incomplete oxidizer. For the former, organic compounds are totally oxidized to CO₂, while the latter is incapable of complete oxidation. Incomplete oxidizers cannot completely oxidize to CO₂ substrates that contain at least one carbon-carbon bond and that are not more oxidized than acetate. Consequently, the end products of their oxidation can be, though not necessarily, acetate and CO₂. Nevertheless, acetate is the common substrate for the “complete oxidizers” though it is not the most preferred one for some of them (Devereux and Stahl, 1993).
Reported Substrates that can be used for dissimilatory sulfate reduction (Widdel, 1983; Hansen, 1988; Schnell et al., 1989; Trinkerl et al., 1990; Aecckersberg et al., 1991)

<table>
<thead>
<tr>
<th>Class of Compound</th>
<th>Specific Compound Utilized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inorganic</td>
<td>Hydrogen, carbon monoxide</td>
</tr>
<tr>
<td>Hydrocarbons</td>
<td>Straight alkanes (C12 to C20)</td>
</tr>
<tr>
<td>Monocarboxylic acids (aliphatic)</td>
<td>Formate, acetate, propionate, butyrate, higher fatty acids up to C20, isobutyrate, pyruvate, lactate,</td>
</tr>
<tr>
<td>Dicarboxylic acids</td>
<td>Succinate, fumarate, malate, oxalate, maleinate, glutarate, pimelate</td>
</tr>
<tr>
<td>Alcohols</td>
<td>Methanol, ethanol, propanol-1, butanol-1, butanol-2, ethylene glycol, 1,2-propanediol</td>
</tr>
<tr>
<td>Amino acids</td>
<td>Glycine, serine, alanine, cysteine, cystine, threonine, valine, leucine, isoleucine, aspartate, glutamate, phenylalanine</td>
</tr>
<tr>
<td>Sugars</td>
<td>Fructose, glucose, mannose, xylose, rhamnose</td>
</tr>
<tr>
<td>Aromatic Compounds</td>
<td>Benzoate, phenol, catechol, aminobenzene, pyrogallol, 2-aminobenzoate, 4-aminobenzoate</td>
</tr>
</tbody>
</table>

2.6.2 Taxonomy

Phylogenetic classification of the diverse group of sulfate-reducing bacteria has not been completely finalized yet. They can be grouped into 12 genera (Devereux and Stahl, 1993), including *Desulfovibrio*, *Desulfobotulus*, *Desulfobulbus* and *Thermodesulfobacterium*, all of which are incomplete oxidizers; *Desulfobacter*, *Desulfovoccus*, *Desulfoarcina*, *Desulfobacterium*, *Desulfoara* and *Desulfoarcus*, all being complete oxidizers. The genus *Desulfotomaculum* has both incomplete and complete oxidizing species. All the genera are mesophilic except *Thermodesulfobacterium* which is thermophilic and *Desulfotomaculum* which has some moderately thermophilic strains (Widdel & Hansen, 1991). The classification of the sulfate-reducing bacteria is listed below on Table 2-3:
Table 2-3 Characteristics of some contemporary sulfate-reducing bacteria
(Widdel and Hansen, 1991)

<table>
<thead>
<tr>
<th>Genus</th>
<th>Morphology</th>
<th>Cell Wall/ Membranea</th>
<th>Substrate Oxidationb</th>
<th>Spore</th>
<th>Optimal Temp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desulfovibrio</td>
<td>Vibrio</td>
<td>Gram-,Euba</td>
<td>Incomplete</td>
<td>No</td>
<td>Mesophilic</td>
</tr>
<tr>
<td>Desulfotomaculum</td>
<td>Rod</td>
<td>Gram+,Euba</td>
<td>Both</td>
<td>Yes</td>
<td>Mesophilic and moderately thermophilic strains</td>
</tr>
<tr>
<td>Desulfomicrobium</td>
<td>Oval/Rod</td>
<td>Gram-,Euba</td>
<td>Incomplete</td>
<td>No</td>
<td>Mesophilic</td>
</tr>
<tr>
<td>Desulfobulbus</td>
<td>Oval</td>
<td>Gram-,Euba</td>
<td>Incomplete</td>
<td>No</td>
<td>Mesophilic</td>
</tr>
<tr>
<td>Desulfobacter</td>
<td>Oval/vibrio</td>
<td>Gram-,Euba</td>
<td>Complete</td>
<td>No</td>
<td>Mesophilic</td>
</tr>
<tr>
<td>Desulfobacterium</td>
<td>Oval</td>
<td>Gram-,Euba</td>
<td>Complete</td>
<td>No</td>
<td>Mesophilic</td>
</tr>
<tr>
<td>Desulfococcus</td>
<td>Sphere</td>
<td>Gram-,Euba</td>
<td>Complete</td>
<td>No</td>
<td>Mesophilic</td>
</tr>
<tr>
<td>Desulfosarcina</td>
<td>Ovalc</td>
<td>Gram-,Euba</td>
<td>Complete</td>
<td>No</td>
<td>Mesophilic</td>
</tr>
<tr>
<td>Desulfomonile</td>
<td>Rod</td>
<td>Gram-,Euba</td>
<td>Complete</td>
<td>No</td>
<td>Mesophilic</td>
</tr>
<tr>
<td>Desulfonema</td>
<td>Filamentsc</td>
<td>Gram-,Euba</td>
<td>Complete</td>
<td>No</td>
<td>Mesophilic</td>
</tr>
<tr>
<td>Desulfobotulus</td>
<td>Vibrio</td>
<td>Gram-,Euba</td>
<td>Incomplete</td>
<td>No</td>
<td>Mesophilic</td>
</tr>
<tr>
<td>Desulfoarculus</td>
<td>Vibrio</td>
<td>Gram-,Euba</td>
<td>Complete</td>
<td>No</td>
<td>Mesophilic</td>
</tr>
<tr>
<td>Thermodesulfobacterium</td>
<td>Rod</td>
<td>Gram-,Euba</td>
<td>Incomplete</td>
<td>No</td>
<td>Thermophilic</td>
</tr>
<tr>
<td>Archaeoglobus</td>
<td>Sphere</td>
<td>Archaebacteria</td>
<td>Complete</td>
<td>No</td>
<td>Thermophilic</td>
</tr>
</tbody>
</table>

a Gram-/+ , Euba= typical eubacterial Gram-negative or positive cell wall and membrane

b Incomplete = organic compound oxidized to acetate; Complete = organic compound oxidized to CO₂.

cAggregates of bacteria for Desulfosarcina; Multicellular filaments for Desulfonema.

2.6.3 Environmental Conditions

2.6.3.1 Salinity

Sulfate-reducing bacteria are present in the whole range of salinity from freshwater to hypersaline environment.

2.6.3.2 Temperature

Mesophilic sulfate reducers grow best between 28 and 38°C and have an upper limit around 45°C (Widdel and Pfennig, 1984; Widdel and Hansen, 1992). The optimum growth temperature for the thermophilic genera of Desulfotomaculum and Thermodesulfobacterium ranges from 54 to 70°C.
2.6.3.3 pH

Sulfate-reducing bacteria grow better at pH close to neutrality (pH 7.0-7.8), but can tolerate pH values ranging from 5.5-9.0 (Zobell, 1958; Pfennig et al., 1981).

2.6.3.4 Oxygen

The sulfate-reducing bacteria have long been described as strict anaerobe. Nevertheless, it was shown that the sulfate-reducing bacteria are able to survive for hours or even days when exposed to molecular oxygen (Hardy and Hamilton, 1981; Cypionka et al., 1985; Fukui and Takii, 1990; Wall et al., 1990). The capability of aerobic respiration has been detected in several genera of sulfate reducers, including Desulfovibrio, Desulfobulbus, Desulfobacterium and Desulfooccus (Abdollahi and Wimpenny, 1990; Dilling and Cypionka, 1990). In light of these, the sulfate reducers can proliferate in transient aerobic conditions and at least they can tolerate such conditions.

2.6.3.5 Inhibition

It is reported that certain concentrations of Na₂AsS₄, K₂Cr₂O₇, PbCl₂, CdCl₂ and Na₂MnO₄ have inhibitory effect on sulfate-reducing bacteria (Capone et al., 1983).

2.7 Oxidation Reduction Potential

2.7.1 Theoretical Background

The oxidation-reduction potential (ORP) is an electrochemical version of ΔG°, the change of free energy, which indicates the direction of chemical reaction or the poise of the system. The oxidation reduction potential of a redox couple is
analogous to the acid strength, pKa, of an acid-conjugate base system. Therefore, the ORP of a redox couple is a measure of its electron-transfer potential. By convention, the more negative the ORP of a system, the stronger is its electron-transfer potential.

2.7.2 ORP of Bacterial Culture

The discovery by Potter in 1910 that, when a platinum electrode was immersed in a bacterial culture it registered a more negative potential than in the original uninoculated medium. Subsequently, the findings that different bacteria, when grown under similar conditions, established characteristic ORP in their cultures, had led to hope that the ORP could be one of the informative properties of bacterial culture. At a later stage, however, it was understood that the actual value of ORP in a biological system depended on the composition of the culture medium. This is due to the fact that a bacterial culture contains a considerable number of redox couples, and one cannot be sure of the relative contribution of each different couple to the overall measured ORP value. Therefore, the ORP of such system is similar to the pKa value, if measurable, of a complex mixture of different acids, bases and buffers (Morris 1975). In light of this, two bacterial cultures with the same ORP value could have different compositions and concentrations of respective species.

2.7.3 ORP and Anaerobic Systems

Although the precise significance of ORP is still not certain in many biological systems, it is known that the ORP of an anaerobic system should be lower than certain ceiling values for the growth of some particular groups of bacteria. For instance, -100 mV is the ceiling value suggested for cultivation of sulfate reducing
bacteria (Postgate, 1984) and -300mV for methanogenic bacteria (Hungate, 1967). Since the mere exclusion of oxygen from a culture is not sufficient to achieve such low ORP, some reducing agents such as cysteine, thioglycollate, dithionite or ascorbate, or a large inoculum of bacterial culture is needed to lower the ORP to desirable values (Morris, 1975).

2.7.4 Interplay of Oxygen and ORP in Anaerobic Systems

Although it is virtually impossible to define precisely the exact implication of the ORP existing in a biological system, a change of ORP does indicate a corresponding change in the balance between contending oxidizing and reducing species that are the system's prime determinants. Since oxygen is always one of the important determinants, input of oxygen tends to increase its value. It is not sure whether the suppressive or toxic effect of oxygen on anaerobic system is actually caused by the raising of ORP, or oxygen and its derivative are themselves the toxic substances (Morris, 1976). The reducing agents which are needed to lower the culture ORP as mentioned in the previous section therefore may serve as scavengers of oxygen free radical (Morris, 1976).

2.8 Interaction between Methanogens and Sulfate-Reducing Bacteria

2.8.1 General

There are many physiological and ecological similarities between these two groups of anaerobic bacteria. They are found together in a variety of anaerobic environments. Besides, they share the common substrate of hydrogen and acetate. Interactions between the two groups can be categorized as: (1) competition for limiting common substrates; (2) coexistence through use of different substrates; (3)
synergism in which members of one group supply electron donors needed by the other.

2.8.2 Competition

Since hydrogen and acetate are the common substrates for the two groups of bacteria, they have been the foci of many competition studies. When sulfate, the electron acceptor for sulfate reduction, is unlimited, it is found that the sulfate reducers are superior scavengers of hydrogen and acetate than methanogens and the growth of the latter becomes limited (Lovely et al., 1982; Robinson and Tiedje, 1984). Nevertheless, the degree of superiority of the sulfate reducer will not show up as the COD/\(\text{SO}_4^{2-}\) ratio is increased (Li et al., 1996; Choi and Rim, 1991) since the higher COD eliminates organic substrate as a limiting factor for the methanogenic activity.

Fig. 2-2 Competition between methanogenic bacteria and sulfate-reducing bacteria (Dotted line shows dissimilatory sulfate reduction)

One explanation for the general superiority of the sulfate-reducing bacteria is based on their higher affinity (or lower \(K_a\)) for substrates, where \(K_a\) is the half velocity constant in the expression of the Monod kinetics. Apparent \(K_a\) value for hydrogen uptake by methanogens is 0.006 mM \(H_2\) while that of sulfate reducing bacteria is around 0.001 mM (Kristjansson et al., 1982). Similar difference was also reported
on apparent \( K_e \) values for acetate uptake, 3.0 mM for methanogens and 0.2 mM for sulfate reducers (Schonheit et al., 1982). The other explanation is based on the comparison of free energy yields for oxidation of \( \text{H}_2 \) and acetate coupled to reduction of either \( \text{SO}_4^{2-} \) or \( \text{CO}_2 \). The energetic advantage of using \( \text{SO}_4^{2-} \) as electron acceptor is 10% for \( \text{H}_2 \) and 40% for acetate oxidation (Smith, 1993).

### 2.8.3 Coexistence

This phenomenon is observed when the two groups of bacteria use different substrates. For instance, coexistence happens when trimethylamine or methionine, which cannot be utilized by sulfate-reducing bacteria, are present as electron donor for methanogens (Smith, 1993). The other possibility for coexistence is when the common substrate for both groups of bacteria is in excess.

Fig. 2-3 Coexistence of methanogenic bacteria and sulfate-reducer bacteria, each uses a different substrate.

![Diagram of coexistence](image)

### 2.8.4 Synergism

This form of interaction is observed in the absence or very low level of sulfate. The anaerobic degradation may start with some complex organic material
whose oxidation requires different types of metabolism offered by groups of microorganisms. It has been found in laboratory that by inoculating sulfate-reducing bacteria to substrate, the sulfate reducers were able to ferment lactate to acetate and hydrogen in the absence of sulfate; the hydrogen and acetate are subsequently utilized by methanogens (Byrant et al., 1977). By doing so, the methanogens has removed hydrogen and made the system more thermodynamically favorable for further hydrogen production from lactate.

Fig. 2-4 Synergism between methanogenic bacteria and sulfate-reducing bacteria in the absence of sulfate.

\[
\text{Fatty Acids, Lactic Acid, Alcohols} \rightarrow \text{Sulfate-reducing Bacteria} \rightarrow \text{Acetate, } H_2 \rightarrow \text{Methanogenic Bacteria} \rightarrow CH_4
\]

2.8.5 Interaction in Anaerobic Treatment Process

From the previous discussion, it is known that acetate is the principal intermediate in the treatment system employed in this study. Thus, methanogenic
bacteria and sulfate-reducing bacteria have inevitably to use this common substrate. The interaction pattern in the treatment of sulfate-laden wastewater should therefore be predominated by competition.
Chapter 3

MATERIALS AND METHODS

3.1 Apparatus

The experiment was carried out in several completely mixed anaerobic reactors kept in 35°C water bath. For each reactor, mixing is achieved by recirculating reactor biogas through a cadet pump. The substrate was delivered from refrigerator at 4°C by peristaltic pump. The oxidation-reduction potential (ORP) of the reactor was monitored by ORP electrodes. In each test, the ORP was maintained at a target value by periodic, slug dosing of pure oxygen. The dosing was controlled by an electrical valve, which turned on and off according to the preset ORP target and the actual ORP input into the controller. Figure 3-1 shows the schematic arrangement of the anaerobic reactor set up.

3.1.1 Reactor

Each reactor was made of an acrylic cylinder 150 mm in diameter, covered at both ends by plastic plates held together by stainless steel fasteners. Rubber O-ring was used to seal the contact between the plates and the cylinder to prevent gas or liquid leakage. The reactor had a liquid volume of 4 liters and a headspace of 0.95 liters. The hydraulic retention time (HRT) of the reactor was 15 days. Plastic gas bag was used for the biogas collection.
3.1.2 Reactor Mixing

Mixing of the reactor was achieved by continuous biogas recirculation using an Air Cadet dual head pump (Cole Parmer model E-07530-65). The recirculation gas flow rate was 3 litres/min.

3.1.3 Oxidation Reduction Potential Measurement

Oxidation-reduction potential was measured by double junction platinum band ORP electrode (Cole Parmer model E-27006-21) and pH/ORP meter (Corning pH/ORP meter model 345). The proper functioning of the electrode was confirmed by quinhydrone standards as recommended by the instrument supplier. By comparing the meter reading of the redox potential of the Zaobell’s solution, which was prepared according to the Standard Methods for the Examination of Water and Wastewater, 18th ed., the ORP meter reading was corrected accordingly, so that the reported value was against the standard reference of hydrogen electrode. The variation in oxidation-reduction potential was recorded continuously by pen recorder (Rikadenki Multiple-pen recorder).

3.1.4 Oxidation Reduction Potential Control

The oxidation reduction potential was maintained at some targeted elevated values above the system natural ORP through the use of a pH/ORP controller (Cole Parmer model E-05656-05) which turned on an electrical solenoid valve (SMC model VX2110) when the reactor ORP was 10 mV below the targeted value. When the solenoid valve was on, pure oxygen (supplied by Chun Wang Industrial Gases) was injected into the recirculating biogas at a flow rate of approximately 1-2 ml/min until the ORP was raised to a value 10 mV above the targeted level. Then the solenoid valve
was turned off and the oxygen dosing was stopped automatically. In each dosing, it was found to last from 3 to 4 minutes. The ORP curves for various COD/SO₄²⁻ ratios and targeted ORP was shown in Appendix 1.

3.2 Substrate

The substrate is a solution containing glucose, potassium sulfate and other minerals as shown in Table 3-1. The substrate was stored at 4°C in a refrigerator and delivered to the reactor by peristaltic pump (Cole Parmer standard drive model E-07521-10) with Tygon tubing E-03409-13. The flow rate was set at 265ml/day.

Table 3-1 Composition of substrate

<table>
<thead>
<tr>
<th>Components</th>
<th>Concentration (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>9375</td>
</tr>
<tr>
<td>Potassium Sulfate</td>
<td>1707-8533</td>
</tr>
<tr>
<td>Sodium Bicarbonate</td>
<td>3750</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>955</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>85</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>170</td>
</tr>
<tr>
<td>MgCl₂•6H₂O</td>
<td>300</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>100</td>
</tr>
<tr>
<td>CoCl₂•6H₂O</td>
<td>5.5</td>
</tr>
<tr>
<td>FeCl₃</td>
<td>71</td>
</tr>
<tr>
<td>NiSO₄•6H₂O</td>
<td>80</td>
</tr>
<tr>
<td>Trisodium Citrate dihydrate</td>
<td>278</td>
</tr>
<tr>
<td>pH</td>
<td>7.3-8.0 varies with sulfates conc.</td>
</tr>
</tbody>
</table>

3.3 Chemical Analysis

3.3.1 Sample Preparation

No pretreatment was required for analyzing the influent samples except dilution to a suitable concentration range for each chemical analysis. The effluent samples to be analyzed were collected from the gas liquid separator (Fig 3-1), and then placed in air-tight centrifuge bottles for 10-minutes centrifugation at 10,000 rpm (Centrifuge by
Sorvall Instrument model RC5C), which had a centrifugal force of 7.3 g. The clear supernatants were subsequently diluted to specific concentration ranges for respective analytic equipment. The dilution water was generated by reverse osmosis (Elga Prima reverse osmosis system) plus resin adsorption (Elga Maxima ultra pure water system) and it had a specific resistance of 18.2 MΩ.

3.3.2 pH

The pH of influent and effluent were measured with a research pH meter (Orion model 420A with auto temperature compensation probe). The pH meter was calibrated with pH 4.01 and 7.00 buffers regularly to ensure proper functioning.

3.3.3 Gas Compositions

The methane, carbon dioxide and hydrogen sulfide contents of the biogas were measured by gas chromatography (Shimadzu model GC17A; Column: Alltech Hayesep 30’ X 1/8” X 0.085” S.S.). The temperatures for injector, column and thermal conductivity detector were set at 40°C, 120°C, and 120°C respectively. Helium was used as the carrier gas at a flow rate of 30 ml/min. The instrument was first calibrated with pure methane, nitrogen, carbon dioxide and hydrogen sulfide before its use.

3.3.4 Total Organic Carbon

The total organic carbon (TOC) and inorganic carbon concentration of each sample was analyzed by Total Organic Carbon Analyzer (Shimadzu TOC-5000A). Potassium phthlate and sodium carbonate/sodium bicarbonate were used as organic and inorganic calibration standards, respectively.
3.3.5 Sulfate

The sulfate was analyzed by ion chromatography (Dionex model DX500; Column: Dionex AS4A-SC 4mm). The eluent was 1.8mM sodium carbonate/1.7mM sodium bicarbonate at a flow rate of 2ml/min. Detection was made by electrochemical detector (Dionex model ED40). The equipment was calibrated by reagent grade potassium sulfate at various concentrations.

3.3.6 Sulfides

The soluble sulfides were measured by Iodometric method modified from the Standard Methods for the Examination of Water and Wastewater (18th ed.). The modification included the following:

1. Separation of soluble and insoluble sulfide was made by centrifugation at 10,000 rpm for 10 minutes rather than by aluminum chloride precipitation. This was because the former method gave a better solid separation in this particular study.

2. A sample volume of 15ml, instead of 200 ml, was used due to high sulfide concentration in the samples.

3.3.7 Suspended Solids and Volatile Suspended Solids

Suspended solids and volatile suspended solids were determined according to Standard Methods for the Examination of Water and Wastewater (1989).
Fig. 3-1 Schematic of Reactor Set Up

ORP Electrode

To gas bag

Biogas Recirculation

ORP Meter and Controller

Gas Pump

Gas

Effluent

Gas Liquid Separator

Legend

- Liquid line
- Gas line
- Electrical signal

Completely mixed Reactor at 35°C

Influent Tank at 4°C

Peristaltic Pump

Oxygen Cylinder

Needle Valve

Solenoid Valve
Chapter 4

RESULTS AND DISCUSSION

4.1 Effects of COD/\(\text{SO}_4^{2-}\) Ratios and ORP on Anaerobic System Properties

In this study, tests had been carried out to evaluate the anaerobic treatment performance with a feed substrate which had different COD/\(\text{SO}_4^{2-}\) ratios of 10000 mg/l: 1000 mg/l, 10000 mg/l: 3000 mg/l and 10000 mg/l: 5000 mg/l at a hydraulic retention time of 15 days. Such a long detention time was used so that there would be no methanogen washout problem. The anaerobic reactors had been maintained at different ORP conditions, each for at least one and half month to assess the effect of ORP increases of 0mV, 50mV and 100mV from their natural values (about –280 mV). The matrix of COD/\(\text{SO}_4^{2-}\) ratios and ORP which were employed in this study are shown in Table 4-1.

Table 4-1 Experimental matrix of various COD/\(\text{SO}_4^{2-}\) ratios and ORP

<table>
<thead>
<tr>
<th>ORP Increment</th>
<th>COD/(\text{SO}_4^{2-}) ratios (mg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10000/1000</td>
</tr>
<tr>
<td>Natural ORP</td>
<td>-283 mV</td>
</tr>
<tr>
<td>(No increment)</td>
<td></td>
</tr>
<tr>
<td>50 mV increment</td>
<td>-233 mV</td>
</tr>
<tr>
<td>100 mV increment</td>
<td>-183 mV</td>
</tr>
</tbody>
</table>

The parameters monitored during the tests included: influent and effluent total organic carbon; influent and effluent sulfate; effluent dissolved sulfides concentration;
gas production rate and gas compositions. The following sections will elaborate the changes of these parameters as ORP and COD/SO$_4^{2-}$ ratios were varied.

4.2 Effects of ORP on System Performance at a COD/SO$_4^{2-}$ Ratio of 10000 mg/l:

1000 mg/l

4.2.1 Total Organic Carbon

The influent total organic carbon was kept at a level of 3750 mg/l for all tested ORP values and it was found that the total organic carbon concentrations remaining in the treated effluent were quite similar regardless of the tested ORP. The removal efficiencies were all over 96% (Table 4-2).

At the natural system ORP of -283 mV, organic carbon removal was accomplished by conversion to methane and carbon dioxide by methanogens and sulfate reducing bacteria. As will be discussed later, both methanogenesis and sulfate reduction were inhibited considerably by the ORP increase of either +50 mV or +100 mV at this COD/SO$_4^{2-}$ ratio. As a result, their contributions to the organic carbon reduction would be decreased. However, due to the periodic injection of pure oxygen, some aerobic oxidation of organic carbon by facultative bacteria had taken place at these elevated ORPs. Thus, the overall TOC reductions were similar to that at the natural ORP of -283 mV.
Table 4-2 Influent and effluent TOC at various ORP at COD/\(\text{SO}_4^{2-}\) ratio of 10000 mg/l: 1000 mg/l

<table>
<thead>
<tr>
<th>ORP (mV)</th>
<th>Infl. TOC (mg/l)</th>
<th>Effl. TOC (mg/l)</th>
<th>TOC removal Effi.</th>
</tr>
</thead>
<tbody>
<tr>
<td>-283</td>
<td>3750</td>
<td>117.5±10.5</td>
<td>96.9%</td>
</tr>
<tr>
<td>-233</td>
<td>3750</td>
<td>144.5±85.5</td>
<td>96.1%</td>
</tr>
<tr>
<td>-183</td>
<td>3750</td>
<td>102.5±34.5</td>
<td>97.3%</td>
</tr>
</tbody>
</table>

Fig. 4-1 Influent and effluent TOC vs ORP at COD/sulfate ratio of 10000 mg/l: 1000 mg/l

4.2.2 Sulfate

The influent sulfate was maintained at 1000 mg/l for this COD/\(\text{SO}_4^{2-}\) ratio. The effluent sulfate concentration and hence the extent of sulfate reduction was taken to be the indicator of sulfate reducing activity at the three ORP levels examined. It was found that the extent of sulfate reduction was decreased with increasing ORP values.
(Table 4-3). As will be discussed in Sections 4.3 and 4.4, the rise in ORP might not have caused inhibition by itself on the sulfate reducing activity. The introduction of molecular oxygen at higher ORPs could have allowed facultative bacteria in the reactor to use oxygen for aerobic degradation, and this became a superior competitor for substrate than the sulfate-reducing bacteria. For this reason, the extent of sulfate reduction was reduced with increasing ORP while the removal of TOC was not adversely affected, as shown in Table 4-2.

Table 4-3 Influent and effluent sulfate at various ORP at COD/\(\text{SO}_4^{2-}\) ratio of 10000 mg/l: 1000 mg/l

<table>
<thead>
<tr>
<th>ORP (mV)</th>
<th>Infl. Sulfate (mg/l)</th>
<th>Effl. Sulfate* (mg/l)</th>
<th>Effl. TOC (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-283</td>
<td>1000</td>
<td>13±5</td>
<td>117.5±10.5</td>
</tr>
<tr>
<td>-233</td>
<td>1000</td>
<td>367.5±54.5</td>
<td>144.5±85.5</td>
</tr>
<tr>
<td>-183</td>
<td>1000</td>
<td>561±30</td>
<td>102.5±34.5</td>
</tr>
</tbody>
</table>

*About 4-6 observations

Fig. 4-2 Influent and effluent sulfate vs ORP at COD/sulfate ratio of 10000 mg/l: 1000 mg/l
4.2.3 Dissolved Sulfides

As no sulfide was present in the feeding substrate, all of the dissolved and gaseous sulfides in the effluent samples were from sulfate reduction. However, the dissolved sulfide concentrations at this COD/\(\text{SO}_4^{2-}\) ratio were very low (Table 4-4), and they were not stoichiometrically related to the amount of sulfate reduced. Also, the dissolved sulfide levels in this COD/\(\text{SO}_4^{2-}\) ratio were much lower when compared with those at lower COD/\(\text{SO}_4^{2-}\) ratios (as will be seen in Sections 4.3.3 and 4.4.3). As confirmed by theoretical calculation based on Henry's law and equilibrium conditions (will be discussed in Appendix 3), the dissolved sulfide at the natural ORP was found to be consistent with the hydrogen sulfide gas content measured by gas chromatography. The decreasing dissolved sulfide concentration with increasing ORP found in this phase was mainly due to the fact that most of the produced sulfides had been oxidized by molecular oxygen which was dosed to the system periodically to maintain the targeted ORP.

Table 4-4 Dissolved sulfide concentration at various ORP at COD/\(\text{SO}_4^{2-}\) ratio of 10000mg/l: 1000 mg/l

<table>
<thead>
<tr>
<th>ORP (mV)</th>
<th>Dissolved Sulfides (mg/l)</th>
<th>([S^2-/\text{SO}_4^{2-} \text{ -S}] \text{ removed ratio})</th>
</tr>
</thead>
<tbody>
<tr>
<td>-283</td>
<td>16±8</td>
<td>4.9%</td>
</tr>
<tr>
<td>-233</td>
<td>4.5±1.5</td>
<td>2.1%</td>
</tr>
<tr>
<td>-183</td>
<td>2±1</td>
<td>1.4%</td>
</tr>
</tbody>
</table>

31
4.2.4 Gases Productions

The daily production rates of methane, carbon dioxide and hydrogen sulfide were measured at different ORP values. The methane production rate was taken as the indicator for the methanogenic activity. It was observed that methane production decreased with increasing ORP, indicating a definite inhibition of the methanogenic activity (Table 4-5). For carbon dioxide, it was noted that its proportion in the biogas increased with increasing ORP. This was due to an increase of aerobic degradation by facultative bacteria due to oxygen injection at higher ORP levels. As discussed in Appendix 3, the relative amounts of dissolved inorganic carbon and gaseous carbon dioxide measured at all ORP levels were relatively close to theoretical values predicted from Henry's law and equilibrium conditions. The hydrogen sulfide content of the collected biogas dropped to zero upon the first increment of ORP. Since there were still considerable sulfate reducing activities even at the highest ORP of -183 mV (Table 4-3), some hydrogen sulfide productions should have occurred. Obviously,
most or all of the produced sulfide had been oxidized to sulfur by molecular oxygen dosed into the system at elevated ORP.

Table 4-5 Gas production rates at various ORP at COD/SO$_4^{2-}$ ratio of 10000 mg/l: 1000 mg/l

<table>
<thead>
<tr>
<th>ORP (mV)</th>
<th>Methane (ml/day)</th>
<th>CO$_2$ (ml/day)</th>
<th>H$_2$S (ml/day)</th>
<th>Total (ml/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-283</td>
<td>702</td>
<td>484</td>
<td>15</td>
<td>1201</td>
</tr>
<tr>
<td>-233</td>
<td>235</td>
<td>527</td>
<td>0</td>
<td>762</td>
</tr>
<tr>
<td>-183</td>
<td>24</td>
<td>953</td>
<td>0</td>
<td>977</td>
</tr>
</tbody>
</table>

Fig. 4-4 Individual gas production rate vs ORP at COD/sulfate ratio of 10000 mg/l: 1000 mg/l

4.2.5 Material Balance

As shown in Table 4-6, the recoveries of carbon were from 64% to 83% (refer to Appendix 3 for details). Material balance of sulfur was not carried out as no total sulfide data was measured, but it was found that the relative levels of dissolved sulfide
and gaseous hydrogen sulfide were consistent with those predicted by Henry’s law and equilibrium conditions (details will be discussed in Appendix 3).

Table 4.6 Material balance of carbon for COD/\(\text{SO}_4^{2-}\) ratio of 10000 mg/l: 1000 mg/l

<table>
<thead>
<tr>
<th>ORP (mV)</th>
<th>Infl. Carbon (mg/day)</th>
<th>Effl. Carbon (mg/day)</th>
<th>Gaseous Carbon (mg/day)</th>
<th>VSS Carbon* (mg/day)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-283</td>
<td>1144</td>
<td>214</td>
<td>635</td>
<td>100</td>
<td>83</td>
</tr>
<tr>
<td>-233</td>
<td>1144</td>
<td>197</td>
<td>408</td>
<td>119</td>
<td>64</td>
</tr>
<tr>
<td>-183</td>
<td>1144</td>
<td>196</td>
<td>523</td>
<td>136</td>
<td>75</td>
</tr>
</tbody>
</table>

* Taken as 50% of the effluent VSS.

4.3 Effects of ORP on System Performance at a COD/\(\text{SO}_4^{2-}\) Ratio of 10000 mg/l:

5000 mg/l

4.3.1 Total Organic Carbon

As in the case of 10000/1000 for the COD/\(\text{SO}_4^{2-}\) ratio, the effluent TOC was about the same at different ORP, and the removal efficiencies were all over 90%. Of course, the mineralization of organic carbon was by methanogenesis and sulfate reduction at the natural ORP of -283 mV. However, as will be shown below, at the elevated level of -233 mV, beside methanogenesis and sulfate reduction, a certain degree of aerobic degradation by facultative bacteria had also contributed to the TOC removal. When the ORP was further increased to -183 mV, methanogenesis became almost completely inhibited; at this ORP, sulfate reduction and aerobic degradation by facultative bacteria were responsible for the total organic carbon removal.
Table 4-7 Influent and effluent TOC at various ORP at COD/\text{SO}_4^{2-} \text{ ratio of 10000 mg/l: 5000 mg/l}

<table>
<thead>
<tr>
<th>ORP (mV)</th>
<th>Infl. TOC (mg/l)</th>
<th>Effl. TOC (mg/l)</th>
<th>TOC removal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-285</td>
<td>3750</td>
<td>289±90</td>
<td>92%</td>
</tr>
<tr>
<td>-235</td>
<td>3750</td>
<td>147±5</td>
<td>96%</td>
</tr>
<tr>
<td>-185</td>
<td>3750</td>
<td>134±26</td>
<td>96%</td>
</tr>
</tbody>
</table>

Fig. 4-5 Influent and effluent TOC vs ORP at COD/sulfate ratio of 10000 mg/l: 5000 mg/l

4.3.2 Sulfate

The influent sulfate concentration for this COD/\text{SO}_4^{2-} \text{ ratio was kept at 5000 mg/l. As shown in Table 4-8, high sulfate removal was observed at both ORP of -285 mV and -185 mV, effluent sulfate being around 100 mg/l and the sulfate removal efficiencies being over 96%. Nevertheless, at ORP of -235 mV, the effluent sulfate was as high as 1228 mg/l, with a removal efficiency of only 75%. The discrepancy was explained by the following hypothesis, and is illustrated by the schematic drawing in Appendix 4 (Fig. A4-1):
In elevated ORP levels, the methanogenesis were subjected to two forms of
suppression, which were:

1. Substrate competition from the more superior sulfate reducing bacteria.

2. Inhibition due to rise in ORP level.

Despite the fact that sulfate-reducing bacteria was more tolerant to ORP
increase, the sulfate removal efficiency could still be depressed at a high sulfate loading
rate, or at a low COD/\(\text{SO}_4^{2-}\) ratio such as that in this particular case. For the
methanogens, although they were also somewhat inhibited at the ORP of \(-235\) mV,
such inhibition was more or less compensated by the reduced activity of sulfate-
reducing bacteria, which was also a suppressive force on methanogenesis. This
suggested that at an ORP of \(-235\) mV, the competitiveness of methanogens against the
sulfate reducing bacteria for organic carbon was improved. This conjecture was in line
with the observation of an opposite trend in the extent of methanogenic activity with
increasing ORP (as will be shown in Table 4-10). In order to clarify this point, an
additional experimental run was carried out at a COD/\(\text{SO}_4^{2-}\) ratio of 10000mg/l: 3000
mg/l (to be discussed in Section 4.4) to confirm such a hypothesis.

When the ORP was further increased to \(-185\) mV, however, the methanogens
were more severely inhibited at this ORP than sulfate-reducers. Consequently, the
sulfate reducing bacteria faced nearly no competition from methanogens for substrate.
Although there was also some aerobic degradation by facultative bacteria, the
availability of molecular oxygen to this process was significantly reduced by the
scavenging effect of sulfide oxidation (as will be discussed in Section 4.3.3). As a
result, the sulfate reducers were able to completely reduce the influent sulfate despite
its high loading rate and inhibition by ORP increase.
Table 4-8 Influent and effluent sulfate at various ORP at COD/SO₄²⁻ ratio of 10000 mg/l: 5000 mg/l

<table>
<thead>
<tr>
<th>ORP (mV)</th>
<th>Influent sulfate (mg/l)</th>
<th>Effluent sulfate* (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-285</td>
<td>5000</td>
<td>108.5±70</td>
</tr>
<tr>
<td>-235</td>
<td>5000</td>
<td>1228±19</td>
</tr>
<tr>
<td>-185</td>
<td>5000</td>
<td>77.5±63</td>
</tr>
</tbody>
</table>

*About 4-6 observations

Fig. 4-6 Influent and effluent sulfate vs ORP at COD/sulfate ratio of 10000 mg/l: 5000 mg/l

4.3.3 Dissolved Sulfides

As discussed previously, the only source of sulfide was from sulfate reduction. The dissolved sulfide concentrations in this COD/SO₄²⁻ ratio were much higher than those found at the previous ratio, especially at the natural ORP of ~285 mV. As will be shown in Appendix 3, the relative amounts of dissolved sulfides and gaseous hydrogen sulfide measured were close to those predicted by Henry’s law and equilibrium
conditions. It was noted that the dissolved sulfide concentration decreased with increasing ORP. When the ORP was increased from −285 mV to −235 mV, a nearly 50% decrease in sulfide concentration was observed. This decrease could not be completely accounted for by the 25% reduction in sulfate-reducing activity (Table 4-8). Oxidation by molecular oxygen to sulfur was believed to be the cause of the drastic reduction of dissolved sulfide concentration. Such oxidation also had a biological significance since it reduced the availability of molecular oxygen to facultative bacteria, hence reducing the extent of facultative oxidation of the organic substrate. The effect of such oxidation was even more prominent at the ORP of −185 mV, at which the influent sulfate was almost completely reduced but only very low dissolved sulfide concentration was observed. Besides, a lot of yellow sulfur granules were observed in the mixed liquor, reactor cover and biogas recirculation tubing.

Table 4-9 Dissolved sulfide concentration at various ORP at COD/SO$_4^{2-}$ ratio of 10000mg/l: 5000 mg/l

<table>
<thead>
<tr>
<th>ORP (mV)</th>
<th>Dissolved Sulfides (mg/l)</th>
<th>$[S^{2-}] / [SO_4^{2-}]$ removed ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-285</td>
<td>432.5±32</td>
<td>26.5</td>
</tr>
<tr>
<td>-235</td>
<td>220±34</td>
<td>17.5</td>
</tr>
<tr>
<td>-185</td>
<td>8±6</td>
<td>0.5</td>
</tr>
</tbody>
</table>
4.3.4 Gases Production

For methane production rate, it was first increased when ORP was raised from $-285$ mV to $-235$ mV and then dropped to nearly zero when the ORP was further increased to $-185$ mV. Such phenomena occurred because at the ORP of $-235$ mV, the inhibition of the sulfate reducers due to high sulfate loading had reduced their substrate competition against the methanogen. The latter, though were also somewhat inhibited at this ORP, the improved substrate competitiveness resulted from inhibition of sulfate reducers was enough to compensate for the ORP inhibition. As such, the methanogens became more competitive for the available substrate than was at the natural ORP of $-285$ mV, at which the sulfate reducers experienced no inhibition. As a
result, methane production was increased. As for the CO₂ production, the increase in carbon dioxide at the ORP of −235 mV was partly caused by some improvement of the TOC removal (effluent TOC was −100mg/l lower than that at the natural ORP) as well as the occurrence of aerobic degradation. As will be shown in Appendix 3, the relative amounts of dissolved inorganic carbon and gaseous carbon dioxides measured were consistent with values predicted by Henry's law and equilibrium conditions. For hydrogen sulfide, although the gaseous sulfide was decreased as ORP was increased, this did not mean that less hydrogen sulfide was generated at the high ORP than at low ORP. As having been pointed out before, considerable amounts of hydrogen sulfide were oxidized to sulfur as the ORP was increased.

The different responses of methanogens and sulfate-reducers with respect to the ORP increase from −285 mV to −235 mV at this low COD/SO₄²⁻ ratio (10000 mg/l: 5000 mg/l) could have some practical significance. It showed that for a reactor treating sulfate-laden wastewater, a controlled injection of oxygen or air to increase the ORP to −235 could suppress sulfate reducers and allow the methanogens to have a better environment to convert organic carbon to methane gas. This would in effect improve the fuel quality of the biogas.

Table 4-10 Gas production rates at various ORP at COD/SO₄²⁻ ratio of 10000 mg/l: 5000 mg/l

<table>
<thead>
<tr>
<th>ORP (mV)</th>
<th>Methane (ml/day)</th>
<th>CO₂ (ml/day)</th>
<th>H₂S (ml/day)</th>
<th>Total (ml/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-285</td>
<td>382</td>
<td>353</td>
<td>58</td>
<td>793</td>
</tr>
<tr>
<td>-235</td>
<td>516</td>
<td>517</td>
<td>36</td>
<td>1069</td>
</tr>
<tr>
<td>-185</td>
<td>6</td>
<td>291</td>
<td>0</td>
<td>297</td>
</tr>
</tbody>
</table>
4.3.5 Material Balance

As shown in Table 4-11, the recoveries of carbon in the three tested ORP's were from 65% to 86% (refer to Appendix 3 for details). Material balance of sulfur was not carried out as no total sulfide data was measured, but it was found that the relative levels of dissolved sulfide and gaseous hydrogen sulfide were consistent with those predicted by Henry's law and equilibrium conditions (details will be discussed in Appendix 3).
Table 4.11 Material balance of carbon for COD/SO$_4^{2-}$ ratio of 10000mg/l: 5000 mg/l

<table>
<thead>
<tr>
<th>ORP (mV)</th>
<th>Infl. Carbon (mg/day)</th>
<th>Effl. Carbon (mg/day)</th>
<th>Gaseous Carbon (mg/day)</th>
<th>VSS Carbon* (mg/day)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-285</td>
<td>1144</td>
<td>386</td>
<td>394</td>
<td>83</td>
<td>75</td>
</tr>
<tr>
<td>-235</td>
<td>1144</td>
<td>327</td>
<td>553</td>
<td>102</td>
<td>86</td>
</tr>
<tr>
<td>-185</td>
<td>1144</td>
<td>484</td>
<td>159</td>
<td>105</td>
<td>65</td>
</tr>
</tbody>
</table>

* Taken as 50% of effluent VSS.

4.4 Effects of ORP on System Performance at a COD/SO$_4^{2-}$ Ratio of 10000mg/l:

3000 mg/l

In order to explain the response of the sulfate reducing and methanogenic activities to the ORP increase at the COD/SO$_4^{2-}$ ratio of 10000 mg/l: 5000 mg/l, it was necessary to hypothesize that the sulfate reducing bacteria were somewhat inhibited at a high influent sulfate conc. (5000 mg/l) when the operating ORP was −235 mV (Section 4.3.2). An additional experimental test was carried out at a lower influent sulfate level (3000 mg/l) which provided a COD/SO$_4^{2-}$ ratio of 10000 mg/l: 3000 mg/l, to see if the degree of suppression of sulfate reducers could be reduced as compared to that at the COD/SO$_4^{2-}$ ratio of 10000 mg/l: 5000 mg/l. The 3000 mg/l sulfate concentration was selected because in the previous study at −235 mV ORP, it was found that the sulfate reducers were able to remove 3772 mg/l of sulfate (i.e., 5000 mg/l − 1228 mg/l = 3772 mg/l).
4.4.1 Total Organic Carbon

The influent TOC was kept at 3750 mg/l and the effluent TOC was measured after one and half month of operation each at the natural ORP of -285 mV and also at the elevated ORP of -235 mV. The effluent TOC at these two ORP's were in the same range. Since methanogenic activity was suppressed (as will be seen in Section 4.4.4) to some extent at the elevated ORP, the low effluent TOC indicated that aerobic degradation had occurred at the elevated ORP.

Table 4-12 Influent and effluent TOC at various ORP at COD/SO\(_4^{2-}\) ratio of 10000 mg/l: 3000 mg/l

<table>
<thead>
<tr>
<th>ORP (mV)</th>
<th>Influent TOC (mg/l)</th>
<th>Effluent TOC (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-285</td>
<td>3750</td>
<td>158±9</td>
</tr>
<tr>
<td>-235</td>
<td>3750</td>
<td>295±90</td>
</tr>
</tbody>
</table>

Fig. 4-9 Influent and effluent TOC vs ORP at COD/sulfate of 10000 mg/l: 3000 mg/l
4.4.2 Sulfate

The influent sulfate to the system was kept at 3000 mg/l. The effluent sulfate concentration, as predicted, was at very low levels at both ORP values. The sulfate removal efficiency was greater than 92% for both ORP. This suggested that a rise in ORP would not cause an increase in effluent sulfate concentration if the sulfate loading was suitable. The suitable sulfate loading means that on one hand the sulfate loading was high enough so that sufficient sulfide are generated to scavenge molecular oxygen which was dosed into the system, thus reducing the quantity of substrate oxidation by the facultative bacteria. On the other hand, the loading cannot be too high for the sulfate reducers to suffer too much suppression on their activities at the elevated ORP.

<table>
<thead>
<tr>
<th>ORP (mV)</th>
<th>Influent sulfate (mg/l)</th>
<th>Effluent sulfate* (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-285</td>
<td>3000</td>
<td>29±2</td>
</tr>
<tr>
<td>-235</td>
<td>3000</td>
<td>33.5±6.5</td>
</tr>
</tbody>
</table>

*About 4-6 observations

Fig. 4-10 Influent and effluent sulfate vs ORP at COD/sulfate ratio of 10000 mg/l: 3000 mg/l
4.4.3 Dissolved Sulfides

As in the case of COD/\(\text{SO}_4^{2-}\) ratio 10000 mg/l: 5000 mg/l, the dissolved sulfides in this case decreased with increasing ORP. Since the extent of sulfate reduction at both ORP were similar (see Table 4-14), some of the produced sulfide at the \(-235\) mV ORP should have been oxidized by oxygen to sulfur.

Table 4-14 Dissolved sulfide concentration at various ORP at COD/\(\text{SO}_4^{2-}\) ratio of 10000mg/l: 3000 mg/l

<table>
<thead>
<tr>
<th>ORP (mV)</th>
<th>Dissolved sulfides (mg/l)</th>
<th>([\text{S}^{2-}]/[\text{SO}_4^{2-} - \text{S}] ) removed ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-285</td>
<td>504.5±35</td>
<td>50.9</td>
</tr>
<tr>
<td>-235</td>
<td>155±20</td>
<td>15.7</td>
</tr>
</tbody>
</table>

Fig. 4-11 Dissolved sulfide vs ORP at COD/sulfate ratio of 10000 mg/l: 3000 mg/l

4.4.4 Gas Productions

As expected, the methane production rate and the hydrogen sulfide content of the biogas decreased with increasing ORP. Of significance was the fact that at \(-235\)
mV ORP, the methane production in this particular case was much less than that of the previous test, i.e., 293 vs 516 ml/day (Table 4-10). This reflected that once the sulfate reducers’ suppression was removed, the methanogens’ competitiveness for the organic substrate was drastically reduced. The increase of carbon dioxide at the higher ORP was mainly due to aerobic oxidation by facultative bacteria. It must be pointed out that at the natural ORP, the methanogenic activity was reduced by about 38% (from 702 ml/day to 432 ml/day) when the influent sulfate level was increased from 1000 mg/l to 3000 mg/l. This reflected that a higher sulfate loading could substantially reduce the activity of methanogens.

Table 4-15 Gas production rates at various ORP at COD/SO$_4^{2-}$ ratio of 10000 mg/l:
3000 mg/l

<table>
<thead>
<tr>
<th>ORP (mV)</th>
<th>Methane (ml/day)</th>
<th>CO$_2$ (ml/day)</th>
<th>H$_2$S (ml/day)</th>
<th>Total (ml/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-285</td>
<td>432</td>
<td>378</td>
<td>54</td>
<td>864</td>
</tr>
<tr>
<td>-235</td>
<td>293</td>
<td>591</td>
<td>40</td>
<td>924</td>
</tr>
</tbody>
</table>

Fig. 4-12 Individual gas production rate vs ORP at COD/sulfate ratio of 10000 mg/l: 3000 mg/l
4.4.5 Material Balance

As shown Table 4-16, the recoveries of carbon were 77% for both ORPs (refer to Appendix 3 for details). Material balance of sulfur was not carried out as no total sulfide data was measured, but it was found that the relative levels of dissolved sulfide and gaseous hydrogen sulfide was consistent with those predicted by Henry’s law and equilibrium conditions (details will be discussed in Appendix 3).

Table 4-16 Material balance of carbon for COD/\(\text{SO}_4^{2-}\) ratio of 10000 mg/l: 3000 mg/l

<table>
<thead>
<tr>
<th>ORP (mV)</th>
<th>Infl. Carbon (mg/day)</th>
<th>Effl. Carbon (mg/day)</th>
<th>Gaseous Carbon (mg/day)</th>
<th>VSS Carbon (mg/day)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-285</td>
<td>1144</td>
<td>382</td>
<td>434</td>
<td>62</td>
<td>77</td>
</tr>
<tr>
<td>-235</td>
<td>1144</td>
<td>400</td>
<td>474</td>
<td>34</td>
<td>79</td>
</tr>
</tbody>
</table>

4.5 Effects of COD/\(\text{SO}_4^{2-}\) Ratio on System Performance at Same ORP Level

It is apparent from the previous discussion that systems at different COD/\(\text{SO}_4^{2-}\) ratios had responded differently to the same ORP increase. Some of these variations are highlighted in this section to demonstrate the importance of COD/\(\text{SO}_4^{2-}\) ratio in determining the system responses.

4.5.1 At Natural ORP

All reactors ran at the three different COD/\(\text{SO}_4^{2-}\) ratios gave nearly complete removal of TOC and sulfate at this ORP. Yet there is a gradual decrease of methanogenic activity as the ratio was decreased (i.e. an increase in sulfate concentration), as shown in Fig. 4-13. It is noteworthy that at a high influent sulfate
concentration of 5000 mg/l, the methanogenic activity was still quite high, amounting to about 54% of the system having an influent sulfate of 1000 mg/l.

It is also noted that the dissolved sulfide concentration increased markedly when the influent sulfate was increased from 1000 mg/l to 3000 mg/l and then to 5000 mg/l. As having been discussed, a high dissolved sulfide concentration was able to scavenge some of the added molecular oxygen, thereby limiting the extent of aerobic oxidation by facultative bacteria.

Fig. 4-13 Methane production rate vs influent sulfate concentration at various ORP

![Graph showing methane production rate vs influent sulfate concentration at various ORP](image)
4.5.2 ORP Increment of 50 mV

This ORP increase was considered mild as a preliminary exploratory test (not reported in this thesis) had shown that an ORP increase of 20 mV did not cause any noticeable effect on the behaviour of anaerobic system. The high TOC removal was maintained at all ratios at this ORP increase. A decrease in sulfate reduction, however, was observed in reactor operating at a high COD/SO$_4^{2-}$ ratio of 10000mg/l: 1000 mg/l and a low ratio of 10000mg/l: 5000 mg/l (Fig. 4-14). These decreases were attributed to different reasons. For the high ratio, aerobic degradation of organic by facultative bacteria had taken place and the microbial competition was no longer the one between only sulfate reducing bacteria and methanogens. The more competitive facultative bacteria exerting aerobic metabolism (though limited in extent by limitation in molecular oxygen supply) was also involved. As the low ratio (or a high sulfate concentration of 5000 mg/l) is concerned, the extent of aerobic degradation should be limited. This was because the total contribution to organic mineralization by both methanogenesis and sulfate reduction did not change much. The slight decrease in sulfate reduction (25%) was caused by a decrease in sulfate utilization rate due to
some inhibition from the increased ORP of $-235$ mV. This had resulted in improved substrate competitiveness for methanogens at this ORP (as previously discussed in Section 4.3.2). As a result, the sulfate reducers were unable to use up all of the influent sulfate within the hydraulic retention time of 15 days. Moreover, as seen from Fig. 4-14, the sulfate utilization rate of the sulfate reducers at this influent sulfate level of 5000 mg/l (at the ORP of $-235$ mV) was still higher than that at the influent sulfate of 3000 mg/l at the natural ORP. It would be expected, therefore, the ORP increase of 50 mV would not have a noticeable effect on the sulfate removal efficiency if the sulfate level was less than 3000 mg/l. This was supported by the observation at the third test which was operated at a COD/SO$_4^{2-}$ ratio of 10000mg/l: 3000 mg/l. No inhibition of sulfate reducing activity was found at this lower sulfate loading.

As shown in Fig. 4-13, methanogenic activity at $-235$ mV ORP was suppressed strongly at the COD/SO$_4^{2-}$ ratio of 10000 mg/l: 1000 mg/l (from 702 ml/day to 235 ml/day), but increased at the 10000 mg/l: 5000 mg/l ratio and then slightly suppressed at the ratio of 10000mg/l: 3000 mg/l. A hypothesis has been proposed in Sections 4.3.2, 4.3.3 to explain these unique observations. Furthermore, the higher methane production rate observed at the 10000 mg/l: 5000 mg/l ratio with $-235$ mV than the 10000 mg/l: 3000 mg/l ratio at $-285$ mV could be caused by a higher dissolved sulfide concentration (505 mg/l) in the latter case than in the former (220 mg/l). Methanogens were subjected to stronger sulfide inhibition at the 10000 mg/l: 3000 mg/l ratio. For the higher methane activities at $-235$ mV for the 10000 mg/l: 5000 mg/l ratio than for the 10000 mg/l: 3000 mg/l ratio, it could be caused by some aerobic oxidation by facultative bacteria, which competed with the methanogens, in the latter case. The presence of aerobic oxidation in this COD/SO$_4^{2-}$ ratio was implicated by a constant TOC removal in spite of a 30% decline in the methanogenic activity at $-235$ mV.
4.5.3 ORP Increment of 100 mV

At this ORP increase, the methanogenic activity at both high and low COD/SO\(_4^{2-}\) ratios were nearly completely suppressed. The sulfate reduction of the high ratio was inhibited significantly (56% decrease from the natural ORP condition) while that of the low ratio was unaffected. Since a low effluent TOC was maintained in spite of the suppression of methanogenic activity, aerobic degradation was responsible for part of the organic removal. The extent of this aerobic process was smaller in the low ratio condition as evident from its smaller contribution to carbon dioxide formation. This was probably attributed to the fact that there were more sulfides available to scavenge the added molecular oxygen. Besides, hydrogen sulfide was not detected in the biogas of both reactors having the high and low COD/SO\(_4^{2-}\) ratios.

4.6 Effects of pH Change on Properties of Systems

As shown in Table 4-17, the pH change in the media was very little with respect to changing ORP. In view of this, pH change would not have a significant impact on the response of the test systems. Batch tests on different pH were carried out to confirm this conjecture.
Table 4-17 pH at different operating conditions

<table>
<thead>
<tr>
<th>ORP (mV)</th>
<th>COD/SO₄²⁻ ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10000/1000</td>
</tr>
<tr>
<td>Natural</td>
<td>~6.8</td>
</tr>
<tr>
<td>ΔV=+50 mV</td>
<td>~6.6</td>
</tr>
<tr>
<td>ΔV=+100 mV</td>
<td>~6.4</td>
</tr>
</tbody>
</table>

The batch tests were carried out on serum bottles seeded with sludge from one of the reactor. The bottles were purged with nitrogen after seeding and addition of substrate. Afterwards, they were kept at water bath of 35°C for 20 hours. The residual sulfate and methane production was analyzed after the incubation to investigate the effect of pH on both methanogenic and sulfate reducing activities. Details of the test are recorded in Appendix 2.

The data showed that pH did have a significant effect on the performance of methanogenic and sulfate reducing activities (refer to Appendix 2 for details). The suppression on methanogenic activity and sulfate reduction increased with increasing deviation from neutral pH. Nevertheless, a drop of pH from 7.5 to 6.1 did not cause as much reduction in methane production as for the case when ORP was increased by 50 mV at the COD/SO₄²⁻ ratio of 10000/1000, which had a pH variation of only from 6.8 to 6.6. Therefore, the decrease in methane production was not induced by the slight pH decrease. Similarly, the decline in sulfate reduction when pH was reduced from 7.5 to 6.1 was about the same as that of the 100 mV ORP rise at the COD/SO₄²⁻ ratio 10000/1000, which once again just had a pH drop of 6.8 to 6.4. Hence pH did not have a significant effect on sulfate reduction in this case.
4.7 Conceptual Model

A conceptual model has been formulated to explain the behaviour of the system under different COD/SO$_4^{2-}$ ratios and ORP. Figure 4-15 below shows the flow of substrate, intermediates, products and oxygen postulated by the model. The box arrows are paths of organic carbon and the dotted line are paths of molecular oxygen dosed into the system for raising the ORP. The thicker dotted line of oxygen in the sulfide direction just indicates that oxygen reacts more readily with sulfide to form sulfur than with organic carbon.

In this model, three groups of bacteria, namely facultative bacteria, sulfate reducing bacteria and methanogens can mineralize organic carbon to either carbon dioxide or methane. The relative contribution of these three groups to mineralization depends on the following three factors:

1. Molecular oxygen

Molecular oxygen will react with sulfide more readily than with organic carbon because it is more thermodynamically favorable. If there is not enough sulfide to consume the molecular oxygen, the latter will be utilized by facultative bacteria to carry out aerobic oxidation at some random locations where oxygen molecules were made available (micro-aerobic metabolism). Since aerobic catabolism of organic generates more energy than its anaerobic counterpart, the facultative bacteria will grow faster than both the methanogen and sulfate reducing bacteria. The former will capture as much organic as molecular oxygen is available to them. Since the oxygen dosing rate is dependent on the ORP value of the system, the higher the ORP the faster the dosing rate. Besides, if excessive molecular oxygen is present, it may exert toxicity on
both methanogens and sulfate reducers, rendering them even less competitive.

2. Sulfate loading of the system

The higher the sulfate loading, the greater the contribution to organic degradation by sulfate reducing bacteria provided that the sulfate level is not so high as to impose "substrate inhibition". The sulfate reducing activity will reduce substrate availability to methanogens. Moreover, a higher sulfate loading will produce more dissolved sulfides in the medium, which will limit the availability of molecular oxygen to the facultative bacteria for aerobic metabolism. If the produced sulfides are not quickly oxidized to sulfur element by molecular oxygen, they may impose toxicity to methanogens.

3. The ORP of the system

In general, the sulfate reducing bacteria are more resistant to ORP increase than the methanogen. An increase in ORP will generally suppress the methanogens more strongly than the sulfate reducing bacteria. However, under a very high sulfate loading condition (such as the one with an influent sulfate of 5000 mg/l), coupled with an ORP of −235 mV, the activity of the sulfate reducers was suppressed more than that of the methanogens. Under such a situation, the competitiveness of methanogens became stronger than the sulfate reducers (as previously discussed in Section 4.3.2).
Fig. 4-15 Flow of chemical reactions in anaerobic system under elevated ORP

- Glucose
- Fermenting bacteria (including facultative species)
- Acetate, hydrogen
- Facultative Bacteria
- CO$_2$, H$_2$O
- Sulfate Reducers
- CO$_2$
- Sulfide
- CH$_4$, CO$_2$
- Sulfur
Chapter 5

CONCLUSIONS

From the experiment results observed in this study, the following conclusions can be drawn:

1. For anaerobic treatment of a glucose waste having a COD of 10,000 mg/l (TOC=3750 mg/l) and a sulfate content of 1,000 mg/l (i.e. a COD/\(SO_4^{2-}\) ratio of 10:1), the following treatment results are obtained when the HRT is 15 days, temperature 35°C:

   At ORP = -283 mV:
   - Effluent \(SO_4^{2-}\) = 13 mg/l
   - CH\(_4\) production = 176 ml/L-day
   - Effluent TOC = 118 mg/l

   At ORP = -233 mV:
   - Effluent \(SO_4^{2-}\) = 368 mg/l
   - CH\(_4\) production = 59 ml/L-day
   - Effluent TOC = 145 mg/l

   At ORP = -183 mV:
   - Effluent \(SO_4^{2-}\) = 561 mg/l
   - CH\(_4\) production = 6 ml/L-day
   - Effluent TOC = 103 mg/l

2. For the same waste under the same operating conditions, except that the influent sulfate was increased to 3,000 mg/l, the following results have been observed:

   At ORP = -285 mV:
   - Effluent \(SO_4^{2-}\) = 29 mg/l
   - CH\(_4\) production = 108 ml/L-day
   - Effluent TOC = 158 mg/l

   At ORP = -235 mV:
   - Effluent \(SO_4^{2-}\) = 34 mg/l
   - CH\(_4\) production = 73 ml/L-day
   - Effluent TOC = 295 mg/l
3. When the influent sulfate was further increased to 5,000 mg/l, for the same waste under the same operating conditions, the following results are obtained:

At ORP = -285 mV:

\[ \text{Effluent } SO_4^{2-} = 109 \text{ mg/l} \]

(Natural ORP)

\[ \text{CH}_4 \text{ production} = 96 \text{ ml/L-day} \]
\[ \text{Effluent TOC} = 289 \text{ mg/l} \]

At ORP = -235 mV:

\[ \text{Effluent } SO_4^{2-} = 1228 \text{ mg/l} \]

(periodic O\textsubscript{2} injection)

\[ \text{CH}_4 \text{ production} = 129 \text{ ml/L-day} \]
\[ \text{Effluent TOC} = 147 \text{ mg/l} \]

At ORP = -185 mV:

\[ \text{Effluent } SO_4^{2-} = 78 \text{ mg/l} \]

(periodic O\textsubscript{2} injection)

\[ \text{CH}_4 \text{ production} = 1.5 \text{ ml/L-day} \]
\[ \text{Effluent TOC} = 134 \text{ mg/l} \]

4. For anaerobic treatment of sulfate-laden wastewater with its ORP being regulated to different levels through periodic oxygen injection, the impact of the ORP on the treatment performance cannot be determined from the TOC reduction since a certain amount of TOC can be oxidized “micro-aerobically” by facultative bacteria instead of being utilized by either sulfate reducers or methane producers. Neither can the effect of ORP on the sulfate reducers be determined from the production of sulfide since a large fraction of the produced sulfides can be quickly oxidized to sulfur element by the injected oxygen. For this reason, the impact of different ORP on such an anaerobic system has to be determined from both the extent of sulfate reduction and the production of methane gas. They represent the biological activities of sulfate reducers and methanogens, respectively.

5. At the natural operating ORP of -285 mV, the activities of sulfate reducers were not adversely affected by the influent sulfate increase from 1,000 to 5,000 mg/l. However, the methanogenic activity was progressively reduced by the increase of
influent sulfate concentration. More specifically, the methane production rate was 176 ml/L-day at 1,000 mg/l of SO$_4^{2-}$, and the value was reduced to 108 and 96 ml/L-day, respectively, when the sulfate concentration was increased to 3,000 and 5,000 mg/l.

6. When the operating ORP was increased to -235 mV, the sulfate-reducing bacterial activities were not adversely affected as long as the influent sulfate concentration did not exceed 3,000 mg/l. But as the influent sulfate was increased to 5,000 mg/l, the sulfate reducing bacteria become inhibited to some degree, possibly due to substrate inhibition. The level of sulfate reducers' inhibition exceeded that of methanogens at this ORP (-235 mV). As such, the methanogens became more competitive for utilizing the available organic carbon. This had caused a substantial increase of the methane yield under such a specific operating condition.

7. When the ORP was further increased to -185 mV, the methanogenic activity became almost totally inhibited while the sulfate reducers' were not. Thus, for treating high-strength sulfate-laden wastewater, it seems feasible to control the operating ORP at -185 mV to arrest the methanogenic activity, yet still allowing the sulfate reducers to remove sulfate with full potential without inhibition.
Appendix 1

PHOTOS OF EXPERIMENT SET UP AND ORP CURVES

Photos of experiment set up

Plate 1. Whole set up with oxygen cylinder, ORP controller and plotter in the foreground.

Plate 2. ORP meters (top) and gas/liquid separators (below).
Plate 3. Front view of whole set up

Plate 4. Gas bags and water bath
Typical ORP Curves

The following scanned images show the variation of ORP with time:

Scale:
Horizontal: 1 division = 1 hour (Time increases from right to left)
Vertical: 1 minor division = 5 mV

Fig. A1-1 COD/\(SO_4^{2-}\) ratio = 10000 mg/l: 1000 mg/l; Average ORP = -233 mV

Fig. A1-2 COD/\(SO_4^{2-}\) ratio = 10000 mg/l: 1000 mg/l; Average ORP = -183 mV

Fig. A1-3 COD/\(SO_4^{2-}\) ratio = 10000 mg/l: 3000 mg/l; Average ORP = -235 mV
Fig. A1-4 COD/\( \text{SO}_4^{2-} \) ratio = 10000 mg/l: 5000 mg/l; Average ORP = -235 mV

Fig. A1-5 COD/\( \text{SO}_4^{2-} \) ratio = 10000 mg/l: 5000 mg/l; Average ORP = -185 mV
Appendix 2

pH TEST

The tests were conducted with 50-ml serum bottles in a batch mode. At first, an exploratory test was carried out to study the effects of pH on the methane production and sulfate reduction. After this, a second test was conducted under modified testing conditions devised from information collected from exploratory test.

Exploratory Test

Each 50-ml serum bottle was seeded with sludge from a reactor operating at its natural ORP, substrate was then added. The pH of each bottle was adjusted to a predetermined value by addition of either hydrochloric acid or sodium hydroxide. The bottle was purged with nitrogen before capping. Eight serum bottles with different pH from 5 to 9 were then incubated at 35°C for 40 hours. Their gas production rates were monitored continuously by a respirometer (Challenge AER 100). The produced gas was collected by gas bags for subsequent methane analysis. After the incubation period, their residual sulfate and TOC were analyzed. The test pH and results are all shown in Table A2-1.

From the exploratory test result, the following difficulties were experienced:

1. The incubation time of 40 hours seemed too long. The pH of the bottle might have changed so much during this period. So the effect of difference pH on bacterial activities was not significant. From the gas production record (Fig. A2-1), it seemed that an incubation time of 20 hours would better show the effect of pH.

2. Gas collection by gas bag might make the methane analysis unrepresentative as there was no mixing in the bottle headspace.

3. The pH interval in this test may be too narrow to give discernable difference.
Table A2-1 Results of the exploratory test

<table>
<thead>
<tr>
<th>Cell no.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial pH</td>
<td>4.97</td>
<td>5.46</td>
<td>6.01</td>
<td>6.52</td>
<td>7.06</td>
<td>7.61</td>
<td>8.68</td>
<td>9.06</td>
</tr>
<tr>
<td>Orig. TOC</td>
<td>852</td>
<td>852</td>
<td>852</td>
<td>852</td>
<td>852</td>
<td>852</td>
<td>852</td>
<td>852</td>
</tr>
<tr>
<td>Final TOC</td>
<td>585</td>
<td>311</td>
<td>242</td>
<td>205</td>
<td>266</td>
<td>568</td>
<td>244</td>
<td>195</td>
</tr>
<tr>
<td>TOC removal</td>
<td>31%</td>
<td>63%</td>
<td>72%</td>
<td>76%</td>
<td>68%</td>
<td>33%</td>
<td>71%</td>
<td>77%</td>
</tr>
<tr>
<td>Orig. SO$_4^{2-}$</td>
<td>2112</td>
<td>2112</td>
<td>2112</td>
<td>2112</td>
<td>2112</td>
<td>2112</td>
<td>2112</td>
<td>2112</td>
</tr>
<tr>
<td>Final SO$_4^{2-}$</td>
<td>2028</td>
<td>1701</td>
<td>1714</td>
<td>1732</td>
<td>1658</td>
<td>1653</td>
<td>1585</td>
<td>1620</td>
</tr>
<tr>
<td>SO$_4^{2-}$ removal</td>
<td>4%</td>
<td>19%</td>
<td>19%</td>
<td>18%</td>
<td>21%</td>
<td>22%</td>
<td>25%</td>
<td>23%</td>
</tr>
<tr>
<td>Methane vol. (ml)</td>
<td>0.35</td>
<td>0.26</td>
<td>0.63</td>
<td>1.06</td>
<td>1.07</td>
<td>-</td>
<td>0.45</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Fig. A2-1 Gas production vs time for exploratory test

Second Test

In light of the information collected from the exploratory test, a second test was carried out with the following modifications on the experimental conditions.
1. The incubation time was shortened to 20 hours.

2. The serum bottle was not connected to gas bag, so gas sample for methane analysis were collected directly by syringe punctured into the headspace after mixing of headspace gas by inverting the bottles 10 times.

3. The serum bottles were first kept at 35°C for 15 minutes, then their headspace pressure was equalized to atmospheric by a syringe. Actual incubation began after this operation.

4. The test pH range was increased.

The results of this test are listed on Table A2-2 below:

Table A2-2 Result of second pH test

<table>
<thead>
<tr>
<th>Cell no.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial pH</td>
<td>3.05</td>
<td>3.87</td>
<td>5.03</td>
<td>6.09</td>
<td>7.51</td>
<td>7.96</td>
<td>8.96</td>
<td>9.92</td>
</tr>
<tr>
<td>Final pH</td>
<td>3.25</td>
<td>4.30</td>
<td>5.40</td>
<td>6.23</td>
<td>7.23</td>
<td>7.18</td>
<td>7.42</td>
<td>9.90</td>
</tr>
<tr>
<td>Init. TOC</td>
<td>845</td>
<td>845</td>
<td>845</td>
<td>845</td>
<td>845</td>
<td>845</td>
<td>845</td>
<td>845</td>
</tr>
<tr>
<td>Final TOC</td>
<td>808</td>
<td>790</td>
<td>749</td>
<td>451</td>
<td>319</td>
<td>319</td>
<td>416</td>
<td>839</td>
</tr>
<tr>
<td>TOC removal</td>
<td>4%</td>
<td>7%</td>
<td>11%</td>
<td>47%</td>
<td>62%</td>
<td>62%</td>
<td>51%</td>
<td>1%</td>
</tr>
<tr>
<td>Init. $SO_4^{2-}$</td>
<td>2130</td>
<td>2130</td>
<td>2130</td>
<td>2130</td>
<td>2130</td>
<td>2130</td>
<td>2130</td>
<td>2130</td>
</tr>
<tr>
<td>Final $SO_4^{2-}$</td>
<td>2077</td>
<td>2037</td>
<td>2012</td>
<td>1881</td>
<td>1578</td>
<td>1554</td>
<td>1716</td>
<td>2071</td>
</tr>
<tr>
<td>$SO_4^{2-}$ removal</td>
<td>2%</td>
<td>4%</td>
<td>6%</td>
<td>12%</td>
<td>26%</td>
<td>27%</td>
<td>19%</td>
<td>3%</td>
</tr>
<tr>
<td>Methane vol. (ml)</td>
<td>0.08</td>
<td>0.02</td>
<td>0.28</td>
<td>1.13</td>
<td>3.04</td>
<td>4.27</td>
<td>2.76</td>
<td>0.26</td>
</tr>
</tbody>
</table>

From the results, the following conclusions can be drawn:

1. The optimal pH for both methanogenesis and sulfate reduction is between 7.5-8.0.

2. Both methanogenesis and sulfate reduction are inhibited by pH deviation from neutral pH. It appears that methanogens are more sensitive than sulfate reducing bacteria to pH change.
Fig. A2-2 Methane yield and sulfate removal vs pH for Second Test
Appendix 3

MATERIAL BALANCE AND GAS/LIQUID EQUILIBRIUM

Material Balance of Carbon

The total carbon in the effluent, biogas produced and effluent volatile suspended solids was balanced against the influent total carbon. The carbon content of the volatile suspended solids was assumed to be 50%. The various carbon concentrations/amounts are as shown in Table A3-1.

Table A3-1 Carbon concentrations at influent and various effluent streams

<table>
<thead>
<tr>
<th>COD/ SO$_4^2$ (mg/l)</th>
<th>ORP (mV)</th>
<th>Influent (mg/l)</th>
<th>Effluent (mg/l)</th>
<th>Biogas (ml)</th>
<th>V.S.S. (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TOC</td>
<td>IC</td>
<td>TOC</td>
<td>IC</td>
</tr>
<tr>
<td>10000mg/l</td>
<td>-283</td>
<td>3750</td>
<td>535</td>
<td>118</td>
<td>695</td>
</tr>
<tr>
<td>: 1000mg/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-233</td>
<td>3750</td>
<td>535</td>
<td>145</td>
<td>603</td>
</tr>
<tr>
<td></td>
<td>-183</td>
<td>3750</td>
<td>535</td>
<td>103</td>
<td>643</td>
</tr>
<tr>
<td>10000mg/l</td>
<td>-285</td>
<td>3750</td>
<td>535</td>
<td>158</td>
<td>1273</td>
</tr>
<tr>
<td>: 3000mg/l</td>
<td>-235</td>
<td>3750</td>
<td>535</td>
<td>295</td>
<td>1202</td>
</tr>
<tr>
<td></td>
<td>-285</td>
<td>3750</td>
<td>535</td>
<td>289</td>
<td>1157</td>
</tr>
<tr>
<td></td>
<td>-235</td>
<td>3750</td>
<td>535</td>
<td>158</td>
<td>1068</td>
</tr>
<tr>
<td></td>
<td>-185</td>
<td>3750</td>
<td>535</td>
<td>134</td>
<td>1680</td>
</tr>
</tbody>
</table>

Table A3-2 Carbon Balance

<table>
<thead>
<tr>
<th>COD/ SO$_4^2$ (mg/l)</th>
<th>ORP (mV)</th>
<th>Influent (mg)</th>
<th>Effluent (mg)</th>
<th>Biogas Carbon (mg)</th>
<th>V.S.S. Carbon (mg)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TOC</td>
<td>IC</td>
<td>TOC</td>
<td>IC</td>
<td>Carbon</td>
</tr>
<tr>
<td>10000mg/l</td>
<td>-283</td>
<td>1144</td>
<td>217</td>
<td>635</td>
<td>101</td>
<td>83</td>
</tr>
<tr>
<td>: 1000mg/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-233</td>
<td>1144</td>
<td>199</td>
<td>408</td>
<td>121</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>-183</td>
<td>1144</td>
<td>199</td>
<td>523</td>
<td>138</td>
<td>75</td>
</tr>
<tr>
<td>10000mg/l</td>
<td>-285</td>
<td>1144</td>
<td>382</td>
<td>434</td>
<td>62</td>
<td>77</td>
</tr>
<tr>
<td>: 3000mg/l</td>
<td>-235</td>
<td>1144</td>
<td>400</td>
<td>474</td>
<td>34</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>-285</td>
<td>1144</td>
<td>386</td>
<td>394</td>
<td>83</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>-235</td>
<td>1144</td>
<td>327</td>
<td>553</td>
<td>102</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>-185</td>
<td>1144</td>
<td>484</td>
<td>159</td>
<td>105</td>
<td>65</td>
</tr>
</tbody>
</table>

Equilibrium of gaseous carbon dioxide and dissolved inorganic carbons

For this equilibrium, gaseous carbon dioxide dissolves in water according to Henry's law to form aqueous carbon dioxide and carbonic acid. The latter is in acid/base equilibrium with bicarbonate and carbonate. The relation can be depicted by the following formulae:

\[ \text{CO}_2 (g) + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 (aq) \]  \hspace{1cm} (1)

\[ \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^- \]  \hspace{1cm} (2)
\[ \text{HCO}_3^- \quad \leftrightarrow \quad \text{H}^+ \text{ + CO}_3^{2-} \] 

(3)

Equilibrium (1) is governed by the Henry's constant, \( K_H \), and the last two equilibria are governed by equilibrium constants \( K_{a1} \) and \( K_{a2} \), respectively. Values of these constants at 35°C are listed below in Table A3-3. The total dissolved inorganic carbon concentrations are calculated from the measured partial pressure of the gaseous carbon dioxide (by gas chromatography). Then these calculate values are compared with the measured dissolved inorganic carbon levels (as shown in Table A3-4).

Table A3-3 Henry's constant and equilibrium constants for carbon dioxide

<table>
<thead>
<tr>
<th>( K_H ) (mol/l-atm)</th>
<th>( K_{a1} )</th>
<th>( K_{a2} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( 10^{-3.583} )</td>
<td>( 10^{-6.317} )</td>
<td>( 10^{-10.257} )</td>
</tr>
</tbody>
</table>


Table A3-4 Theoretical carbon concentrations vs measured values

<table>
<thead>
<tr>
<th>COD/ ( \text{SO}_4^{2-} )</th>
<th>ORP (mV)</th>
<th>pH</th>
<th>( P_{\text{CO}_2} ) (atm)</th>
<th>Theoretical dissolved carbon (mg/l)</th>
<th>Measured Value (mg/l)</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>10000mg/l : 1000mg/l</td>
<td>-283</td>
<td>6.8</td>
<td>0.38</td>
<td>120</td>
<td>362</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>-233</td>
<td>6.6</td>
<td>0.64</td>
<td>201</td>
<td>385</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>-183</td>
<td>6.4</td>
<td>0.88</td>
<td>277</td>
<td>336</td>
<td>0.05</td>
</tr>
<tr>
<td>10000mg/l : 3000mg/l</td>
<td>-285</td>
<td>7.1</td>
<td>0.45</td>
<td>141</td>
<td>856</td>
<td>0.597</td>
</tr>
<tr>
<td></td>
<td>-235</td>
<td>7.0</td>
<td>0.62</td>
<td>194</td>
<td>937</td>
<td>0.518</td>
</tr>
<tr>
<td></td>
<td>-185</td>
<td>7.0</td>
<td>0.85</td>
<td>267</td>
<td>1284</td>
<td>0.71</td>
</tr>
</tbody>
</table>

Equilibrium of gaseous hydrogen sulfide and dissolved sulfides

For this equilibrium, gaseous hydrogen sulfide dissolves in water according to Henry's law to form dissolved hydrogen sulfide. The latter will be in acid/base equilibrium with \( \text{HS}^- \) and \( \text{S}^{2-} \). The relation can be depicted by the following formulae:

\[ \text{H}_2\text{S (g)} \quad \leftrightarrow \quad \text{H}_2\text{S (aq)} \] 

(4)

\[ \text{H}_2\text{S (aq)} \quad \leftrightarrow \quad \text{H}^+ \text{ + HS}^- \] 

(5)
Equilibrium (4) is governed by the Henry’s constant, $K_{HS}$, and the last two equilibria (5 and 6) are governed by equilibrium constants $K_{S_{a1}}$ and $K_{S_{a2}}$, respectively. Values of these constants at 35°C are listed below in Table A3-5. The $K_{S_{a1}}$ and $K_{S_{a2}}$ values listed in the table were calculated from 25°C values by Van’t Hoff equation. The total dissolved sulfides concentrations are calculated from the measured partial pressure of the gaseous hydrogen sulfide (by gas chromatography). Then these calculate values are compared with the measured dissolved sulfide levels. (as shown in Table A3-6).

Table A3-5 Henry’s constant and equilibrium constants for sulfide

<table>
<thead>
<tr>
<th>$K_{HS}$ (atm/mol. fraction)</th>
<th>$K_{S_{a1}}$</th>
<th>$K_{S_{a2}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^{-2.831}$</td>
<td>$10^{-6.93}$</td>
<td>$10^{-19}$</td>
</tr>
</tbody>
</table>


$K_{S_{a1}}$ and $K_{S_{a2}}$ at 25°C from D.R. Lide “Handbook of Chemistry and Physics”, Section 8-43, CRC Press, 1995.

Table A3-6 Theoretical dissolved sulfide concentrations vs measured values

<table>
<thead>
<tr>
<th>COD/ SO$_4^{2-}$</th>
<th>ORP (mV)</th>
<th>pH</th>
<th>$P_{HS}$ (atm)</th>
<th>Theoretical dissolved sulfides (mg/l)</th>
<th>Measured Value (mg/l)</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[H$_2$S] [HS$^-$] [S$^{2-}$] Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10000mg/l : 1000mg/l</td>
<td>-283</td>
<td>6.8</td>
<td>0.0115</td>
<td>30 22 0</td>
<td>53</td>
<td>16</td>
</tr>
<tr>
<td>10000mg/l : 3000mg/l</td>
<td>-285</td>
<td>7.1</td>
<td>0.055</td>
<td>145 214 0</td>
<td>358</td>
<td>505</td>
</tr>
<tr>
<td>10000mg/l : 5000mg/l</td>
<td>-235</td>
<td>7.0</td>
<td>0.030</td>
<td>82 97 0</td>
<td>179</td>
<td>155</td>
</tr>
<tr>
<td>10000mg/l : 5000mg/l</td>
<td>-285</td>
<td>7.0</td>
<td>0.065</td>
<td>171 201 0</td>
<td>372</td>
<td>433</td>
</tr>
<tr>
<td>10000mg/l : 5000mg/l</td>
<td>-235</td>
<td>7.0</td>
<td>0.0313</td>
<td>82 97 0</td>
<td>179</td>
<td>220</td>
</tr>
</tbody>
</table>
Appendix 4

INTERACTION BETWEEN METHANOGENS AND SULFATE-REDUCERS AT VARIOUS ORP AT AN INFLUENT COD/SULFATE RATIO OF 10000mg/l:5000mg/l

Fig. A4-1 Interactions between methanogens and sulfate-reducers at various ORP at an influent COD/Sulfate ratio of 10000/5000

-285 mV

-235 mV

-185 mV

Some inhibition due to ORP rise

Serious inhibition due to both ORP rise and “high sulfate inhibition” at 5000 mg/l SO₄²⁻

Total inhibition due to excessively high ORP

Inhibition decreased due to elimination of methanogenic competition

Acetate H₂/CO₂
References


Mackie, R.L. and Bryant M.P., 1981. Metabolic activity of fatty acid oxidizing bacteria and the contribution of acetate, propionate, butyrate and CO2 to methanogenesis in cattle waste at 40°C to 60°C. *Applied Environmental Microbiology* 40 1363-1373.


Zeikus, J.G. 1979 Microbial Populations in Digesters *Anaerobic Digestion* 66-89