INHIBITION OF SULPHATE REDUCING BACTERIA TO ENHANCE METHANE YIELD IN ANAEROBIC DIGESTER

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Dedicated to my beloved mother, Allahyarham father, and supportive family.

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ABSTRACT

Sulphate is a common constituent of industrial or domestic effluent and sometimes presents in high concentration. In anaerobic treatment, the reduction of sulphate to sulphide by sulphate reducing bacteria (SRB) leads to a number of problems. One of the major problems is low methane production. In order to retrieve successful anaerobic treatment of sulphate enriched wastewater, it is essential to control sulphate reduction in anaerobic digestion processes. Therefore, the aim of this research is to investigate the inhibition of SRB using ferric chloride ($FeCl_3$) in an Upflow Anaerobic Sludge Blanket (UASB) reactor treating sulphate enriched wastewater. The UASB performance was observed based on pH, soluble chemical oxygen demand (sCOD) removal efficiency, total volatile fatty acids (VFAs), methane production, composition and yield. A 4 litres UASB was operated under anaerobic conditions using synthetic wastewater for 120 days. The study was carried out in two phases. Phase 1 investigated the performance of UASB during start-up period. Phase 2 investigated the ability of $FeCl_3$ to inhibit SRB in anaerobic treatment of sulphate enriched wastewater at COD/SO_4^{2-} ratios of 5.3, 2.5 and 1.3. Results showed that sCOD removal efficiency during the start-up period was more than 80% and methane production and composition observed were 9 $L.day^{-1}$ and 67(±2%), respectively. Results of Phase 2 showed that sCOD removal efficiency average values were 78%, 80% and 70%. Methane yield average values were 0.9, 1.2 and 1.3 L $CH_4.gCOD_{destroyed}^{-1}$ when $FeCl_3$ dosage of 10.2, 22.2 and 44.5 mM respectively were used. Scanning electron microscopy (SEM) was used to observe the microbial morphology of anaerobic sludge. The result showed that when UASB was operated at COD/SO_4^{2-} ratio of 1.3, abundant of filamentous rods and long-rod shape bacterium were dominantly attached to the sludge compared to COD/SO_4^{2-} ratio of 2.5. The sludge consisted mainly of rod-shape bacteria which presumably referred to the common shape of SRBs species. However, when UASB was added with $FeCl_3$ at dosage of 22.2 mM and 44.5 mM, the sludge contained no rod-shape bacteria and the morphology of sludge showed the presence of iron sulphide precipitated. The addition of $FeCl_3$ promotes substantially to an even higher methane yield production.

ABSTRAK

Sulfat adalah komponen lazim di dalam effluen perindustrian atau domestik dan kadang-kadang hadir dalam kepekatan yang tinggi. Di dalam rawatan secara anaerobik, pengurangan sulfat kepada sulfida oleh bakteria pengurangan sulfat (SRB) akan membawa kepada beberapa masalah. Salah satu dari masalah utama adalah penjanaan metana yang rendah. Langkah mengawal pengurangan sulfat di dalam proses pencernaan anaerobik adalah amat penting. Oleh itu, tujuan kajian ini dijalankan adalah untuk menyiasat perencatan SRB dengan menggunakan ferik klorida ($FeCl_3$) dalam reaktor anaerobik enapcemar alir naik (UASB) bagi merawat air sisa yang mengandungi kandungan sulfat yang tinggi. Prestasi UASB dinilai berdasarkan pH, permintaan oksigen kimia (sCOD) terlarut, jumlah asid lemak meruap (VFAs), komposisi dan penjanaan gas metana. Sebuah UASB yang berisipadu sebanyak 4 liter telah beroperasi dalam keadaan anaerobik dengan menggunakan air sisa sintetik selama 120 hari. Kajian ini telah dijalankan dalam dua fasa. Fasa 1 dijalankan bagi menilai prestasi UASB pada tempoh permulaan. Fasa 2 pula dijalankan untuk menyiasat keupayaan $FeCl_3$ untuk merencat SRB dalam kaedah rawatan secara anaerobik bagi air sisa yang mengandungi kandungan sulfat pada nisbah COD/SO_4^{2-} sebanyak 5.3, 2.5 dan 1.3. Keputusan menunjukkan bahawa kecekapan penyingkiran sCOD adalah lebih daripada 80% dan pengeluaran dan komposisi gas metana dicatat adalah 9 $L.hari^{-1}$ dan 67 (± 2%). Keputusan kajian Fasa 2 pula menunjukkan bahawa purata kecekapan penyingkiran sCOD adalah 78%, 80% dan 70%, manakala purata penghasilan gas metana adalah sebanyak 0.9, 1.2 dan 1.3 L $CH_4.gCOD_{termusnah}^{-1}$ apabila dos $FeCl_3$ ditambah masing-masing pada 10.2, 22.2 dan 44.5 mM. Mikroskop imbasan elektron (SEM) telah digunakan untuk melihat morfologi mikrob dalam enapcemar anaerobik. Keputusan menunjukkan apabila UASB beroperasi pada nisbah COD/SO_4^{2-} 1.3, lebih banyak bakteria rod berfilamen dan rod panjang yang dominan telah melekat pada enapcemar berbanding pada nisbah COD/SO_4^{2-} 2.5. Enapcemar tersebut yang terdiri daripada bakteria berbentuk rod yang sama dengan bentuk kebiasaan spesies SRBs. Walau bagaimanapun, apabila UASB ditambah dengan $FeCl_3$ pada dos 22.2 mM dan 44.5 mM, enapcemar didapati bebas daripada kandungan bakteria berbentuk rod dan kehadiran ferik sulfida dapat dilihat pada morfologi enapcemar. Penambahan $FeCl_3$ didapati dapat menggalakkan penjanaan gas metana yang lebih tinggi.

TABLE OF CONTENTS

CHAPTER	TITLE	PAGE
	DECLARATION	ii
	DEDICATION	iii
	ACKNOWLEDGEMENT	iv
	ABSTRACT	v
	ABSTRAK	vi
	TABLE OF CONTENTS	vii
	LIST OF TABLES	xi
	LIST OF FIGURES	xiii
	LIST OF ABBREVATIONS	xvii
1	**INTRODUCTION**	**1**
	1.1 Introduction	1
	1.2 Problem Statements	3
	1.3 Objective of the Study	3
	1.4 Scope of Study	4
	1.5 Significances of Study	4
2	**LITERATURE REVIEW**	**5**
	2.1 Introduction	5
	2.2 Anaerobic Digestion	5
	2.3 Advantages of Anaerobic Digestion	7
	2.4 Limitations of Anaeobic Digestion	9

2.5 Stages of Anaerobic Digestion 10

2.6 Microbiology of anaerobic digestion 11

2.7 Anaerobic digestion variants 14

2.8 Types of anaerobic reactors 15

2.9 Upflow anaerobic sludge blanket 17

2.9.1 Treatment principles and concept 17

2.9.2 Granular sludge 20

2.10 Environmental conditions in anaerobic digestion 21

2.10.1 pH and alkalinity 21

2.10.2 Temperature 22

2.10.3 Nutrient requirements 22

2.10.4 Toxicity and inhibitions 23

2.11 Important process parametes 26

2.11.1 Organic loading 26

2.11.2 Hydraulic loading 27

2.11.3 Mixing 27

2.11.4 Feed characteristic 27

2.12 Sulphate enriched wastewater 28

2.12.1 Competition between sulphate reducing bacteria and methane producing bacteria in sulphate rich anaerobic treatment 29

2.12.2 Sulphate reduction 33

2.12.3 Sulphide toxicity 36

2.12.4 Inhibition of sulphate reducing bacteria sulphide toxicity 37

2.13 Summary of literature review 40

3	**MATERIALS AND METHODS**	**42**
	3.1 Introduction	42
	3.2 Experimental Design	42
	3.3 Experimental Materials	43
	3.3.1 Details of Upflow Anaerobic Sludge Blanket (UASB)	43
	3.4 Methods	47
	3.4.1 Feeds and nutrients	47
	3.4.2 Feeding during start-up phase	49
	3.4.3 Feeding during treatment sulphate enriched wastewater	49
	3.4.4 Seeding the Upflow Anaerobic Sludge Blanket (UASB)	50
	3.4.5 Sampling and analysis	51
	3.4.5.1 Detection of sulphate reducing bacteria using Sani - Check SRB	52
	3.4.5.2 Hungate's rolling tube method for isolation sulphate reducing bacteria	53
	3.4.5.3 Cell fixation for scanning electron microspopy (SEM)	54
	3.4.5.4 Soluble chemical oxygen demand (sCOD)	56
	3.4.5.5 pH	58
	3.4.5.6 Total suspended solid and volatile suspended solid	58
	3.4.5.7 Volatile fatty acid	60
	3.4.5.8 Sulphate	61
	3.4.5.9 Bubble counter meter	61
	3.4.9.10Biogas analysis	62
4	**OPERATIONAL START-UP OF UASB TREATING SYNTHETIC WASTEWATER**	**64**
	4.1 pH	64

	4.2 Soluble COD removal	65
	4.3 Total volatile fatty acid (VFAs)	67
	4.4 Methane production and composition	69
	4.5 Total suspended solid and volatile suspended solid	70
5	**TREATMENT OF SULPHATE ENRICHED WASTEWATER AND SULPHATE ENRICHED WASTEWATER AND INHIBITION OF SULPHATE REDUCING BACTERIA USING FERRIC CHLORIDE IN UPFLOW ANAEROBIC SLUDGE BLANKET**	**72**
	5.1 pH	72
	5.2 Soluble COD removal	73
	5.3 Total volatile fatty acids (VFAs)	75
	5.4 Methane production and composition	77
	5.5 Total suspended solid and volatile suspended solid	81
	5.6 Sulphate reduction	82
6	**MICROBIOLOGICAL STUDY FOR BACTERIA IN UPFLOW ANAEROBIC SLUDGEBLANKET**	**85**
	6.1 Detection of sulphate reducing bacteria using Sani-Check SRB kit	85
	6.2 Isolation of sulphate reducing bacteria	87
	6.3 Microscopic study using scanning electron microscopy (SEM)	89
7	**CONCLUSIONS AND RECOMMENDATIONS**	94
	7.1 Conclusions	94
	7.2 Recommendations	96
	REFERENCES	**98**
	APPENDICES A-B	115-118

LIST OF TABLES

TABLE NO.	TITLE	PAGE
2.1	Types of anaerobic bioreactor	15
2.2	Anaerobic bioreactor treating high strength wastewater.	16
2.3	Typical characteristics of sulphate-enriched industrial wastewaters.	29
2.4	Stoichiometry and Standard Free Enthalpy Change for Sulfate-Reducing, Acetogenic, and Methanogenic Reactions (Thauer *et al.*, 1977)($\Delta G^{\circ'}$ at 37°C in kJ/reaction).	35
2.5	Measures to reduce the reactor sulphide concentration. Thus allowing the integration of methanogenesis and sulphate reduction (Pol et al., 1989).	40
3.1	Composition of synthetic wastewater for UASB anaerobic bioreactor.	48
3.2	Summary of the UASB operating conditions during start-up	49
3.3	Summary of the UASB operating conditions during treatment of sulphate enriched wastewater	50
3.4	Analysis and sampling schedule.	51
3.5	Composition of Postgate'C medium for growth and isolation of sulphate reducing bacteria (SRB)	53
3.6	Summary of reactor sampling schedule of UASB system for SEM study on day 50, 85, 96, 105 and 120	55
3.7	General fixation schedule for sludge cell	55
4.1	Summary of the UASB operating conditions during start-up	66

6.1	Consortium of sulphate reducing bacteria (SRB) in anaerobic sludge taken from Indah Water Konsortium (IWK), sewerage treatment plant.	86
6.2	Total numbers of identified colonies from serially diluted anaerobic sludge sample using Rolling Tube Technique (Hungate, 1969).	87

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE
2.1	Carbon flow to methane in anaerobic digesters with the microorganism responsible for each step. Sources from Guher and Zehnder, 1983.	13
2.2	Schematic Diagram of an Upflow Anaerobic Sludge Blanket (UASB) reactor (Sources from Chernicharo, 2007).	18
2.3	Scheme of anaerobic degradation of organic compounds in the presence of sulphate.	30
3.1	Experimental framework	44
3.2	Schematic Experimental set-up	45
3.3	Close-up of Upflow Anaerobic Sludge Blanket (UASB) during start-up	45
3.4	Detail design of Anaerobic Sludge Blanket (UASB)	46
3.5	Sani-Check sulphate reducing bacteria test kit	52
3.6	a) Fresh Postgate'C medium; b) Clear medium turn to black indicates the SRBs growth; c) Solidified agar	54
3.7	HACH DRB 200	57
3.8	HACH DR 5000 UV-Vis Spectrophotometer	57
3.9	pH meter	58
3.10	Dual Head Pump	59
3.11	Oven	59
3.12	Digital Muffle Furnace	60
3.13	Bubble counter meter	62

3.14	Portable Biogas Analyzer, GeoTech 5000	63
4.1	Influent and effluent pH profile in UASB during reactor start-up.	65
4.2	Contribution of soluble chemical oxygen demand (sCOD) within in reactor and effluent (mg.L-1) and total COD removal (%) of the UASB reactor at different organic loading rate (OLR).	66
4.3	Total volatile fatty acids (VFAs) concentration in reactor and effluent at different organic loading rate (OLR).	68
4.4	Methane production and composition at different organic loading rate (OLR).	70
4.5	Total Suspended Solid (VSS) and Total Suspended Solid (TSS)	71
5.1	pH profiles in UASB and effluent in each OLR at different COD/SO_4^{2-}. Legends above the graph represent the COD/SO_4^{2-} ratio and dosage of $FeCl_3$ added during the operational reactor shown by the dashed line.	72
5.2	Contribution of soluble COD profile within in reactor and effluent (mg.L^{-1}) and total COD removal (%) of the UASB at different COD/SO_4^{2-}. Legends above the graph represent the COD/SO_4^{2-} ratio and dosage of $FeCl_3$ added during the operational reactor shown by the dashed line.	74
5.3	Total VFAs concentration profile in reactor and effluents of UASB at different COD/SO_4^{2-}. Legends above the graph represent the COD/SO_4^{2-} ratio and dosage of $FeCl_3$ (mM) added during the operational reactor shown by the dashed line.	76
5.4	Methane productions in each OLR of UASB at different COD/SO_4^{2-}. Legends above the graph represent the COD/SO_4^{2-} ratio and dosage of $FeCl_3$ (mM) added during the operational reactor shown by the dashed line.	77
5.5	Proportion of CH_4 (%) and H_2S (ppm) in the biogas at different COD/SO_4^{2-}. Legends above the graph represent the COD/SO_4^{2-} ratio and dosage of $FeCl_3$ (mM) added during the operational reactor shown by the dashed line.	78
5.6	Methane yield in each COD/SO_4^{2-} ratio of UASB at different OLR. Legends above the graph represent the	80

	COD/SO_4^{2-} ratio and dosage of $FeCl_3$ (mM) added during the operational reactor shown by the dashed line.	
5.7	Total Suspended Solid (TSS) and Volatile suspended solid (VSS) of mixed liquor in UASB throughout the experiments. Legends above the graph represent the COD/SO_4^{2-} ratio and dosage of $FeCl_3$ (mM) added during the operational reactor shown by the dashed line.	82
5.8	Contribution of sulphate within in reactor and effluent ($mg.L^{-1}$) and sulphate removal efficiencies (%) in Upflow Anaerobic Sludge Blanket (UASB) at different COD/SO_4^{2-}. Legends above the graph represent the COD/SO_4^{2-} ratio and dosage of $FeCl_3$ (mM) added during the operational reactor shown by the dashed line.	83
6.1	Growth of SRB colonies isolated from 10^{-3} and 10^{-4} dilution factor. SRB were inoculated and grown on Postgate's C medium using Hungate's techniques.	89
6.2	Scanning electron micrographs (SEM) showing the microstructure looks like shape of granules during start-up on day 50 (1500 x magnification).	91
6.3	Scanning electron micrographs (SEM) showing the microstructure of sludge of at COD/SO42- ratio of 2.5 on day 76 (10 000 x magnification).	91
6.4	Scanning electron micrographs (SEM) showing the microstructure of sludge treatment with FeCl3 dosage of 22.2 mM on day 86 (1000 x magnification).	92
6.5	Scanning electron micrographs (SEM) showing the microstructure of sludge at COD/SO42- ratio of 1.3 on day 96 (5000 x magnification).	92
6.6	Scanning electron micrographs (SEM) showing the microstructure of sludge after treatment with FeCl3 dosage of 44.5 mM on day 120 (1000 x magnification).	93
6.7	Scanning electron micrographs (SEM) was taken from different angles of Figure 6.6a showed iron sulphide precipitated on day 120 (10 000 x magnification).	93

LIST OF ABBREVIATIONS

UASB	-	Upflow Anaerobic Sludge Blanket
APHA	-	American Public Health Association
COD	-	Chemical Oxygen Demand
sCOD	-	Soluble Chemical Oxygen Demand
SO_4^{2-}	-	Sulphate
S^{2-}	-	Sulphide
COD/SO_4^{2-}	-	COD to sulphate ratio
$K_2SO_4^{2-}$	-	Potassium Sulphate
$FeCl_3$	-	Ferric Chloride
mM	-	milimolar
CH_4	-	Methane
H_2S	-	Hydrogen Sulphide
VFA	-	Volatile Fatty Acids
OLR	-	Organic Loading Rate
HRT	-	Hydraulic Retention Time
TSS	-	Total Suspended Solid
VSS	-	Volatile Suspended Solid
SMP	-	Soluble Microbial Poduct
SRB	-	Sulphate Reducing Bacteria
MPB	-	Methane Producing Bacteria
SEM	-	Scanning Electron Microscopic

CHAPTER 1

INTRODUCTION

1.1 Introduction

Nowadays, residential and industrial sector in Malaysia generates a large amount of wastewater which may affect the environmental life. Wastewater from these sources often contains chemicals which need a proper treatment before discharged to the receiving water such as river or stream. Wastewater treatment processes are divided into three major types: physical, chemical and biological. Biological treatment processes have found wide application in the treatment of domestic and industrial wastewater. Biological processes are substantially cheaper compared to physical or chemical methods. There are two major types of the biological methods used, namely aerobic and anaerobic processes. Aerobic process was very popular back in 1960s (Visser, 1995). However, due to energy crisis in the early 1970s, anaerobic digestion processes was almost exclusively used for the digestion of sewage sludge.

Anaerobic system has more advantages compared to aerobic proces such as a low investment technology, warranting no aeration equipment, reduced sludge disposal facility, and the prime advantage of methane (CH_4) recovery. Traditionally, anaerobic technologies were used for treatment of wastewater especially those derived from wastewater treatment plant. Sludge is categorized as organic material, which could be used to produce useful energy by product which is methane gas. Anaerobic digestion is a natural biological process which involves the breakdown of organic matter by microorganism under anaerobic conditions. Anaerobic digestion mainly produces two main products which are biogas and digestate. The biogas produced

consists of CH_4 and carbon dioxide (CO_2). Meanwhile, the digestate resulting from this process was nutrient-rich and can be directly used as fertilizer.

Moreover, previous research shows high rate of anaerobic treatment system which can retain high amount of biomass, even at low hydraulic retention time. Therefore, this contributes to the development of a suitable reactor design, troubleshooting the treatment problem and solving the errors. Upflow Anaerobic Sludge Blanket (UASB) introduced by Lettinga and Vinken (1980) was able to treat high-strength wastewater. It has been installed across countries to treat a wide range of industrial wastewater (Metcalf and Eddy, 2003). The design is simple, easy to construct and low in maintenance and operating cost and has the ability to withstand fluctuations in pH, temperature and influent substrate concentration (Alvarez *et al.*, 2006; Tiwari *et al.*, 2006). Moreover, the formations of anaerobic granular sludge submerged beneath the reactor are considered as the key features of successful UASB reactor concept for anaerobic treatment of industrial effluent. A dense sludge bed is established at the bottom of the reactor, where all biological transformation takes place (Stronach *et al.*, 1986). Basically, it takes several months to develop the granulated sludge, and sometimes, seed is supplied from other facilities to accelerate the start-up process. As a result, the development of range reactor design led to any suitable for treatment of low, medium and high strength wastewater.

Sulphate enriched wastewater was generated from many industries such as food processing (molasses, seafood, edible oil, etc.), pharmaceutical, petroleum and pulp and paper. The common problems related to sulphate enriched wastewater are associated to the production of H_2S, a reduction of CH_4 yield, low COD removal efficiency, toxicity to other bacteria, odours and corrosion problems. In Malaysia, the domestic or industry sewage and wastewater need proper treatment in order to meet the strict water quality legislation for environmental protection according to Environmental Quality Act 1974. In addition, the emitted amount of sulphide allowed by the Department of Environmental Malaysia (DOE) under Enviromental Quality (Industrial Effeluents) Regulations 2009 is only 0.5 $mg.L^{-1}$.

Therefore, this study was performed to evaluate the ability of Ferric Chloride ($FeCl_3$) to inhibit activity of SRB and treatment efficiency in laboratory-scale systems operating on synthetic wastewater. The impact of COD/SO_4^{2-} at HRT constant and

variable organic substrate loading by varying feed concentration in the UASB was examined. Furthermore, the influence of addition of $FeCl_3$ on microbial community inside UASB was also investigated. Due to time constraint, it was decided to conduct intermittent feed of COD/SO_4^{2-} and $FeCl_3$ alternately because in this study, there is only one peristaltic pump for feeding process. In order to alleviate chemical reaction occuring in the feed tank, UASB were operated in intermittent feeding, with either COD/SO_4^{2-} or $FeCl_3$ at certain time.

1.2 Problem Statement

An approach towards appropriate technology for the treatment of recalcitrant sulphate enriched wastewater has become imperative due to strict water quality legislation for environmental protection. Due to high sulphate content in wastewater, and is sometimes present in high concentrations, the production of high level of sulphide is toxic to both sulphate reducing bacteria (SRB) and methane production bacteria (MPB). Its accumulation in the digestion reactors usually causes inhibition effects on organic removal and methane production, and can even result in system failure. Even though there are many publications on the treatment of high-sulphate wastewaters, there are no published reports on anaerobic treatment of wastewater enrich with sulphate in conjunction to anaerobic sludge as well as resulting higher methane yield at the end of the process. The SRB will be the key factor of success of the study. SRB will either inhibit due to addition of iron, or MPB will be predominant towards the ability to enhance methane yield, which will be investigate well. This study attempts to evaluate how these two microbes compete for substrate, addressing the usage of $FeCl_3$ which may affect the inhibition of SRB in the anaerobic reactor technology in Malaysian industries to treat sulphate enriched wastewaters.

1.3 Objective of the Study

The objectives of the study are as follows:

i. To investigate the performance of Upflow Anaerobic Sludge Blanket (UASB) during start-up period using synthetic wastewater.
ii. To investigate the treatment of sulphate enriched wastewater and inhibition of sulphate reducing bacteria (SRB) using ferric chloride ($FeCl_3$) in UASB.
iii. To study the microbial populations of the sludge under Scanning Electron Microscopic (SEM).

1.4 Scope of the study

The current study mainly focuses on treatment of sulphate enriched wastewater and inhibition of sulphate reducing bacteria. At the starting point of this project, the UASB had only been run on synthetic wastewater for 2 months, before introduced to treatment of COD/SO_4^{2-} ratios of 5.3, 2.5 and 1.3. The performance of UASB was evaluated based on soluble COD removal efficiency, pH stability, volatile fatty acids (VFAs), sulphate removal and methane production, composition and yield.

1.5 Significance of study

This study will contribute to better understanding on the application of iron as reducing bacteria agent and could provide a new approach to control negative impacts by SRB approach on controlling quantity and quality of effluents characteristics. The supplementation of dosage iron as electron acceptor may change the outcome of substrate competition between SRB and methanogens bacteria. These iron sources can be examined to be utilized in anaerobic system to inhibit SRBs. Furthermore, it was a preliminary investigation to provide a new approach to control negative impacts by SRBs in order to be implemented to by other Malaysian wastewater treatment plant systems as well.

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