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Addition of ferric chloride in anaerobic digesters to enhance sulphide removal and methanogenesis

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ABSTRACT

A feasibility study on the addition of ferric chloride ($FeCl_3$) as selective regulator to reduce the sulphide reduction activities of sulphate reducing bacteria (SRB) in anaerobic digester was investigated. A lab-scale up-flow anaerobic sludge blanket (UASB) fed with synthetic sulphate enriched wastewaters were operated continuously for 56 d. The influent sulphate concentrations with an addition of potassium sulphate (K_2SO_4) were operated at corresponding COD/SO_4^{2-} ratios of 5.3, 2.5 and 1.3, respectively. An amount of $FeCl_3$ at dosages of 10.2, 22.2 and 44.5 mM were added on days 66, 86 and 108, respectively (i.e. 8–10 d after each COD/SO_4^{2-} ratio was introduced). Results showed that sCOD removal efficiencies averaged at 78%, 80% and 70%, respectively while methane yield averaged at 0.35, 0.32 and 0.27 $L\ CH_4.gCOD_{destroyed}^{-1}$ when $FeCl_3$ dosage were added at 10.2, 22.2 and 44.5 mM, respectively. Furthermore, the scanning electron microscopy (SEM) examinations demonstrated that UASB operated at COD/SO_4^{2-} ratio of 1.3 were dominated by several filamentous rod-shaped bacteria attached to the structural matrix of the digesting sludge as compared to COD/SO_4^{2-} ratio of 2.5. However, when UASB was supplemented with $FeCl_3$ at dosages of 22.2 mM and 44.5 mM, the sludge contained no rod-shape bacterium and the morphology of sludge showed the presence of iron sulphide precipitation.

Keywords: Upflow anaerobic sludge blanket; Sulphate-reducing bacteria (SRB); Ferric chloride ($FeCl_3$); COD to sulphate ratio (COD/SO_4^{2-}); Methane productivity

1. Introduction

The primary discharge sources of sulphate-enriched wastewaters are mostly from industrial activities. It is essential to effectively treat these types of wastewaters found in industrial effluents to prevent environmental pollution. Furthermore, it also helps to protect public health by safeguarding water supplies. Many sulphate enriched wastewaters with high sulphate contents are generated from food processing (molasses, seafood, edible oil, etc.), pharmaceutical, petroleum and pulp and paper industries. For the past years, the major problem associated with the anaerobic treatment of sulphate-rich wastes is the sulphate reduction process. The result from the reduction of oxidized sulphur compounds to H_2S leads to a variety of problems in anaerobic digesters. These problems include the production of poor quality biogas, reduction in the COD removal efficiencies, toxicity to cohabiting bacteria, generation of H_2S, low methane content and metal corrosion. Fundamentally, sulphate is a common constituent of many natural waters and wastewaters, and is sometimes present in high concentrations. During the anaerobic treatment of high sulphate content wastewater, large amounts of sulphide are produced. It is well-known that sulphate reducing bacteria (SRB) can compete with methane producing bacteria (MPB) for substrate (i.e.: acetate and hydrogen) under sulphate rich nutrient conditions [1,2].

In order to achieve stable operation of anaerobic treatments for sulphate-containing wastewater, it is necessary to suppress or inhibit the metabolic activities of SRB. There are many technologies being used by wastewater industries to minimize the adverse effect of hydrogen sulphide formed

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in anaerobic digester. Control of oxidation-reduction (redox) potential by injecting pure oxygen, air or nitrate salt to prevent sulphide formation [3–5] is one of the predominant technique used to reduce the toxicity of sulphide in anaerobic digesters. On another note, oxygenation in anaerobic digesters may lead to change in microbial community structure and hence methane yield could be reduced. Addition of $Mg(OH)_2$ has been performed by Gutierrez et al. [6] to elevate pH thus minimizing the transfer of molecular H_2S to the gas phase. Moreover, several heavy metals including iron, molybdate, zinc, lead and copper salts can precipitate sulphide by forming highly insoluble metallic sulphide precipitates [7–12]. It is also reported that the inhibitory or toxicity H_2S to anaerobic microorganisms including sulphate-reducing bacteria, depends on sulphate concentration [12,13]. Some heavy metals have tendency to deactivate enzymes because of their reaction with functional groups, such as sulfhydryl (–SH), and can replace cofactors such as Cu(II), Zn(II), Co(II), Ni(II) causing a negative effect over the growth and metabolic activity of SRBs.

Iron can interact with sulphides in anaerobic digesters, a sulphide scavenging tools for treating sulphate-rich wastewaters [14]. In previous studies [15,32], it was observed that the addition of $FeCl_3$ solution to sulphide containing wastewater during anaerobic process causes the reduction of ferric to ferrous ion and precipitates as iron sulphide. Thus, addition of iron can diminish toxic effects associated with H_2S in anaerobic digesters. Even though previous studies have proven the effectiveness of $FeCl_3$ in controlling dissolved sulphides, however a very limited number of studies were reported on the effect addition of Fe^{3+} on the activity of sulphate enriched wastewater under anaerobic settings. Besides that, the addition of Fe^{3+} to remove sulphide from raw municipal sewage in the absence of sewer biofilms was conducted by [16].

The interactions between Fe^{3+} additions on sulphate reducing bacteria activities during the anaerobic treatment of sulphate enriched wastewater have not been investigated. It is also well known that metal ions have tendencies to inhibit the activities of pure strains and mixed cultures of SRBs [17]. This indicates that supplementation of iron into an anaerobic system enables Fe^{3+} to serve as final electron acceptor for SRBs, thus changing the outcome of substrate competition between SRBs and methanogens. The alteration in metabolic affinity for SRB and other anaerobes can be determined by monitoring concentration of sulphide and the susceptibility of anaerobes itself to sulphide.

Therefore, the main goal of this study is to better understand the effectiveness of iron chloride addition to suppress the metabolic activities of SRB during the anaerobic treatment of sulphate-enriched wastewater. The impact of Fe^{3+} on SRBs was investigated by comparing the sulphate reduction and methane content and yield rates within the UASB reactor. It was envisaged that addition of ferric chloride will ultimately increase the inorganic content of the sludge blanket and consequently enhance methanogenesis while hindering sulphate reduction. Therefore, the strategy adopted in this study intermittently induces ferric chloride to decrease the inorganic content of the sludge blanket and generate high methane outputs.

2. Materials and methods

2.1. Upflow anaerobic sludge blanket (UASB)

In this study, the anaerobic bioreactor was made up of Perspex materials, approximately 402 mm in height, 140 mm in internal diameter and surrounded by an outer layer providing hot water flow to accommodate the desired mesophilic temperature. The active volume of the UASB system is 4 L. The operational reactor set-up and schematic diagram are illustrated in Fig. 1. The reactor had

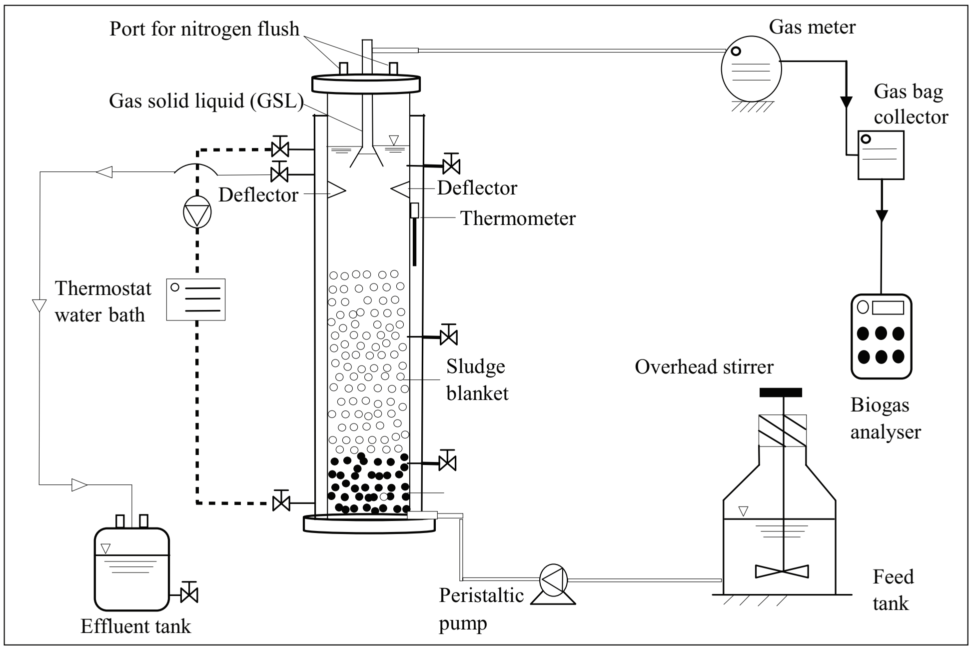


Fig. 1. Schematic diagram of the experimental set-up.

two deflectors, angled at 45° and placed 50 mm below the effluent ports to prevent sludge washout during effluent discharges.

The UASB system was equipped with a gas-solids-liquid (GSL) separator at the top of the reactor. The GSL separator is basically an inverted cone with an intermediate overlap to facilitate the three-phase separation of the treated waste. The generated biogas was collected in the void space of the funnel and channelled for quantitative analysis with the aid of an optical gas bubble counter which had a measurement range of 0–1.5 L·h^{-1} and a precision of ± 1% [18]. For biogas collection, a Tedlar gas bag was connected to the hood of the reactor. The reactor was maintained at temperature (37 ± 1°C) using jacket heating controllers and monitored by thermometer attached inside the first reactor layer. Peristaltic pumps (Masterflex L/S 07523–80) were used to control the influent feeding schedule. After the reaction phase, the discharge effluents were collected in an effluent tank for qualitative analyses. The UASB system had three sampling ports namely the bottom sampling port, middle sampling port and top sampling port. Each sampling port was designed in 120 mm intervals, in order to allow mixed liquor and liquid samples to be withdrawn from sludge bed. The bottom sampling port was used for sampling sludge to be taken at intervals during the operation of the reactor and the two others were used for sampling liquors inside the reactor.

2.2. Synthetic wastewater

The UASB reactor was supplied with sulphate enriched wastewater and glucose as the carbon source. In this study, the synthetic wastewater materials were prepared by using the mixture of peptone, glucose and meat extract, whereas several nutrients and trace elements, such as nitrogen, phosphorous, sulphur, calcium, iron and magnesium, contributed approximately 38%, 33% and 29% of the soluble chemical oxygen demand (sCOD) content of the substrate, respectively. The composition of the synthetic wastewater used as substrate for the UASB in this study includes 4080 mg·L^{-1} glucose (carbon source), 1200 mg·L^{-1} bacteriological peptone, 840 mg·L^{-1} meat extract (Lab-Lemco powder), 3000 mg·L^{-1} sodium bicarbonate ($NaHCO_{3)}$, 57 mg·L^{-1} calcium chloride ($CaCl_2{\cdot}2H_20$), 63 mg·L^{-1} magnesium sulphate ($MgSO_4{\cdot}7H_20$), 240 mg·L^{-1} ammonium chloride (NH_4Cl), 48 mg·L^{-1} ferrous sulphate ($FeSO_4{\cdot}7H_2O$), and 120 mg·L^{-1} potassium dihydrogen ortho-phosphate (KH_2PO_4). At COD concentrations of 8000 mg·L^{-1}, the substrate was supplemented with three varying concentrations of dipotassium sulphate – 1500 mg·L^{-1}, 3200 mg·L^{-1} and 6400 mg·L^{-1} K_2SO_4 as sulphate source, respectively.

Nutrients and trace elements were added in order to provide a balanced feed to the system at approximately COD:N:P ratio of 200:5:1. Three distinct COD to sulphate ratio (COD/SO_4^{2-}) were investigated i.e. 5.3, 2.5 and 1.3, respectively by adding an appropriate quantity of K_2SO_4 on days 1, 21 and 41 while the organic matter concentrations were fixed. Fe^{3+} was added from a stock solution of $FeCl_3$ with dosages of 10.2, 22.2 and 44.5 mM, respectively on days 11, 31 and 52 to investigate the ability of dosage $FeCl_3$ to precipitate sulphate as sulphides from sulphate enriched wastewater. 0.1 M NaOH was added with each addition of $FeCl_3$ to maintain a pH between 7.0 and 8.0 while minimizing the toxic effects of sulphides. Equilibrium reactions for the precipitation of iron sulphides by adding Fe(III) into the digester are defined as follows:

$$2Fe^{3+} + HS^{-} \longrightarrow 2Fe^{2+} + S^{0} + H^{+} \tag{1}$$

$$Fe^{2+} + HS^{-} \longrightarrow FeS_{(S)} + H^{+} \tag{2}$$

2.3. Reactor operation

UASB was seeded with anaerobic sewage sludge (Indah Water Konsortium (IWK) Sewerage Treatment Plant, Ulu Tiram, Johor). Sample was sieved using a 2.0 mm mesh to remove debris and to obtain a denser fraction for granulation. The solid contents of the sewage sludge was 51,400 mg TSS·L^{-1} and 26,700 mg VSS·L^{-1}. The reactor was filled with 1.3 L of sludge (30% of the reactor working volume). Meanwhile, the remaining volume of the reactor was filled with tap water before the reactor was clamped and closed tighthly with head plates in order to achieve air-tight condition. The left headspace of the reactor was flushed with the purified nitrogen gas to displace any residual air in the system. The reactor was allowed to stabilize at 37±1°C for 24 h without any alteration before introducing the feed. Table 1 shows the summary of reactor's operating conditions.

Table 1
Summary of reactor operating conditions of UASB system

OLR[a]	[b]COD/SO_4^{2-} (approximate)	Duration (d)	HRT (d)	Influent COD[c,d] (approximate)	Influent sulphate[c]	Dosage of iron[e]
2.0	5.3	0–11	4.0	8000	1500	–
2.0	5.3	12–21	4.0	8000	1500	10.2
2.0	2.5	22–31	4.0	8000	3200	–
2.0	2.5	32–41	4.0	8000	3200	22.2
2.0	1.3	42–50	4.0	8000	6400	–
2.0	1.3	51–64	4.0	8000	6400	44.5

[a]= average value (kgCOD·m^{-3}·d^{-1}), [b]= g COD^{-1} g SO_4^{2-}, [c]= mg·L^{-1}, [d]= average COD, [e]=milliMolar.

Table 2
Summary of reactor performance during treatment of sulphate enriched wastewater

Day	COD/SO_4^{2-} [a]	Dosage of Iron*	pH	Soluble COD removal[b]	Methane content[c]	Methane Yield[d]
0–11	5.3	–	6.96	80.3	60.1	0.25
12–21	5.3	10.2	6.82	80.1	64.6	0.35
22–31	2.5	–	7.65	77.5	61.9	0.32
32–41	2.5	22.2	7.56	78.6	65.1	0.32
42–50	1.3	–	7.88	58.8	55.8	0.24
51–64	1.3	44.5	7.60	65.8	65.3	0.27

[a]= g COD^{-1} g SO_4^{2-}, [b]= average COD, [c]= average %, [d]= average value L CH_4·gCODdestroyed^{-1}, *= milliMolar.

2.4. Sampling and analysis

The supernatant liquor, gas and sludge collected from different UASB sampling ports were qualitatively analysed. The reactor performance was evaluated based on the influent and effluent analyses of soluble chemical oxygen demand (sCOD), sulphate, Volatile Fatty Acids (VFA) according to Standard Methods [19] and GF/A grade microfibre filters were used. The sCOD, total Volatile Fatty Acids (VFA) and sulphate (SO_4^{2-}) were analysed using a HACH DR 5000 UV-Vis Spectrophotometer. Meanwhile, reactor gas compositions CH_4 and CO_2 was determined using Biogas Analyzer (GeoTech 5000, UK) under operating conditions set-up by the manufacturer. For scanning electron microscopy (SEM), a scanning electron microscope (Carl Zeiss EVO50 XVP) was used and pictures were taken at several magnifications. Sludge's cell pellets were retrieved by centrifugation at 16,000 rpm, 4°C for 20 min and were fixed for 1–2 h in 2.5% (v/v) gluteraldehyde by 0.1 M phosphate buffer, pH 7.4 prior to being washed twice with deionized water. The cell pellets were dehydrated using increased concentration of ethanol (25%, 50%, 70%, 90% and 100%) for 10 min at each concentration, and then left overnight in freeze dryer. Aqueous carbon was used to fix the specimens onto SEM mounts before coating with gold splutter (30 mA for 2.5 min, vacuum 0.2 Torr).

3. Results and discussion

3.1. pH

The pH level in the reactor was increased gradually to pH 7.1 on day 11 when sulphate was added at concentration of 1500 mg·L^{-1} (COD/SO_4^{2-} ratios of 5.3). With the addition of $FeCl_3$ 10.2 mM into the system on days 12–21, the pH levels were gradually decreased within the reactor and in the discharge effluent to pH 6.9 and pH 6.7, respectively until day 14. Thereafter, an eventual increase in pH to 7.4 was observed until day 21. When sulphate concentration was increased to 3200 mg·L^{-1} (COD/SO_4^{2-} ratios of 2.5), a similar pattern was observed with when the pH levels in the reactor was increased gradually to pH 8.1 on day 31, but then decreased and stabilized at pH 7.6–7.7 both in reactor and effluent on day 41 (when $FeCl_3$ was added between day 31 to 41 with iron dosage of 22.2 mM). Fig. 2 shows the pH profile of the UASB system at different COD/SO_4^{2-} and $FeCl_3$ concentrations.

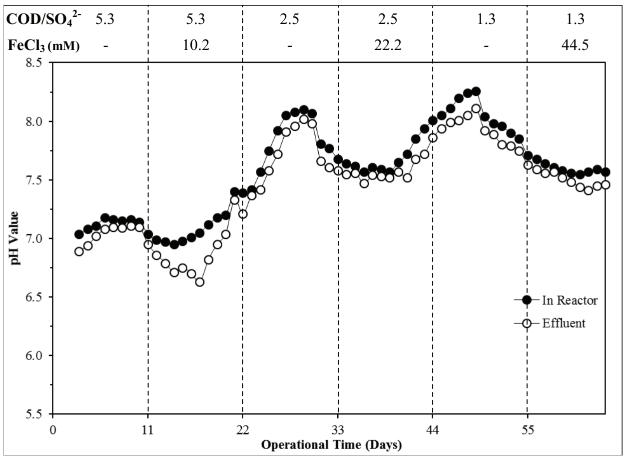


Fig. 2. pH profile of the UASB system at different COD/SO_4^{2-} and $FeCl_3$ concentrations.

Decreasing the COD/SO_4^{2-} ratio further to 1.3 (sulphate concentration of 6400 mg·L^{-1}) brought about a rapid increase in pH levels from pH 7.5±0.1 to pH 8.2±0.1 both in reactor and effluent, thereby causing accumulation of alkalinity (OH^{-1}) in the system. However, the pH levels both in reactor and effluent were decreased to pH 7.7±0.1 before stabilizing at pH 7.5±0.1 (day 52–64) when reactor was fed with $FeCl_3$ (iron dosage of 44.5 mM) on day 52. Similarly, a study by [20] reported that the pH levels were in the range of 7.9 and 9.2 when investigating sulphate removal from industrial wastewater using a packed-bed anaerobic reactor. It was presumed that the above pH alkaline in the current reactor suggests that bicarbonate ions (HCO_3^{2-}) is produced from the by-product of sulphate reduction reactions. Furthermore, [21] also investigated the increase of bicarbonate alkalinity due to sulphate reduction when the higher pH value was observed in a packed-bed reactor treating wastewater containing 500 mg·L^{-1} sulphate.

However, the effects of sulphate on anaerobic degradation of benzoate pointed out that sulphide toxicity had an effect when the operating pH values were 7.0 and 8.1 [22,23]. From the pH data, it can be concluded that the treatment of sulphate could lead to increasing the pH value (from pH 6.6±0.1 to 8.2±0.1 both in reactor and effluent) due to sulphate reduction being dominant over the anaerobic degradation process.

3.2. Soluble COD (sCOD) removal

Fig. 3 shows the temporal changes in the sCOD removal efficiencies and total COD removal (%) for each iron dosage addition. When sulphate was added at concentration of 1500 mg·L^{-1} (COD/SO$_4^{2-}$ ratios of 5.3), the soluble chemical oxygen demand sCOD removal efficiency of the UASB was reduced from 87 to 78% (day 0–11). The sCOD removal efficiency showed a slight decrease from 78% to an average removal of 73% (day 14) before gradually increasing to 82% (day 20) when FeCl$_3$ was added at an iron dosage of 10.2 mM on day 11. The above results are comparable with observations made by [24], in a Jet-loop anaerobic fluidized bed treating high-sulphate wastewater, when they found out that the COD removal efficiencies were between 80% to 90% in range at COD/SO$_4^{2-}$ ratio exceeding 1.2 at HRT of 6.0 and 13.0 h.

However, when the COD/SO$_4^{2-}$ was further reduced to 2.5, (sulphate was added at concentration of 3200 mg·L^{-1}), the sCOD removal efficiency showed slight drop to an average value of 75% (day 23) but then increased and became stable at an average value of 79% (day 24–30). Although the sCOD removal was eventually high, the reduced fraction of COD eliminated via methanogenesis pathway was compensated (high sulphate reduction, in Fig. 5 by a higher degree of sCOD removal from the sulphate reducing activity. Even though UASB was fed with FeCl$_3$ (iron dosage of 22.2 mM) after reactor was operated at COD/SO$_4^{2-}$ ratio of 2.5, the sCOD removal efficiency showed a constant value of 80% removal (day 40) before dropping rapidly to average value of 58% removal on day 52) as the COD/SO$_4^{2-}$ was further reduced to 1.3.

The sCOD removal observed (average removal efficiencies of 58%–60%) at COD/SO$_4^{2-}$ ratio of 1.3 was generally comparable with other reported values. According to [25], the COD removal remained high despite the lower COD/SO$_4^{2-}$ with a minimum removal of 75% and COD was removed via sulphate reduction up to 38.5 % at COD/SO$_4^{2-}$ of 1. Furthermore, [20] observed COD/SO$_4^{2-}$ at 1.3 and below, a maximum COD removal efficiency of 78% was observed when they investigated sulphate removal from industrial wastewater using packed-bed anaerobic reactor. Other than that, operation at iron dosage (i.e.: 10.2, 22.2 and 44.5 mM) resulted in the sCOD increase and slightly affected its removal efficiency. Despite the slight changes in COD removal when UASB was operated with iron dosage, further observations were noted for VFA and methane content.

3.2.1.Volatile fatty acids

The volatile fatty acid (VFA) concentrations increased to 1300 mg·L^{-1} when the reactor was operated at COD/SO$_4^{2-}$ ratio of 5.3 (Fig. 4). After addition of 10.2 mM of FeCl$_3$ on day 11, the total VFA concentration was reduced rapidly to a minimum of 420 mg·L^{-1} on day 21. Further reduction of the COD/SO$_4^{2-}$ ratio from 5.3 to 2.5 on day 21, the total VFA concentration increased the average VFA concentration to 1500 mg·L^{-1} on day 31. In contrast, when FeCl$_3$ was added (at 22.2 mM dosage) on day 31, the amount of total VFA concentration was decreased to 900 mg·L^{-1}. Further lowering COD/SO$_4^{2-}$ ratio to 1.3 on day 41, the total VFA concentration peaked transiently at 2000 mg·L^{-1} (day 43). However, on day 52, the total VFA concentration was reduced gradually to 1600 mg·L^{-1}.

With the addition of 44.5 mM FeCl$_3$, the VFA concentration reduced gradually to an average value of 1100 mg·L^{-1} on day 64. It was assumed that the recovery process was almost immediate when the total VFA concentration decreased from 1600 mg·L^{-1} (day 52) to 1100 mg·L^{-1} (day 64) within 12 operation days at 44.5 mM of FeCl$_3$. In order to have clearer understanding of total VFA concentration, it is essential to analyse some prior biochemical reactions which involve simple acids. Compared to previous studies by others researches [9,11,26,27], the accumulation of total VFA concentration (mainly acetic acid) appears when molybdate (MoO$_4^{2-}$) is used as a selective inhibitor for sulphate reducing bacteria (SRBs).

The colourless sulphur bacteria consisting of very diverse bacterial groups can oxidize reduced sulphur (sulphide, elemental sulphur, thiosulphate, or organic sulphur) to gain energy and support growth. Besides reduced sulphur compounds, some groups of bacteria can use ferrous ion as an electron donor. While a majority of the colourless sulphur

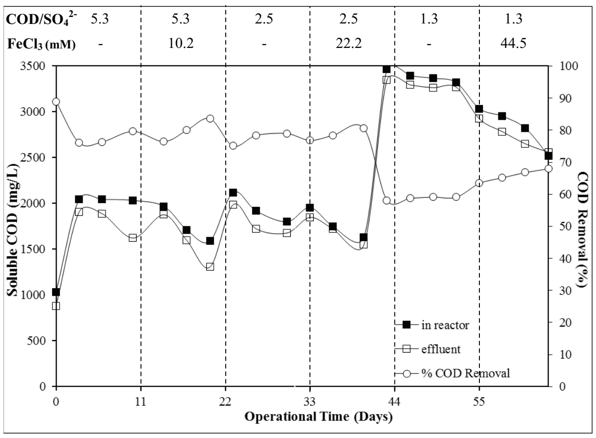


Fig. 3. Contribution of soluble sCOD removal efficiencies within the reactor and effluent (mg·L^{-1}) and total COD removal (%) of the UASB at different COD/SO$_4^{2-}$ and FeCl$_3$ concentrations.

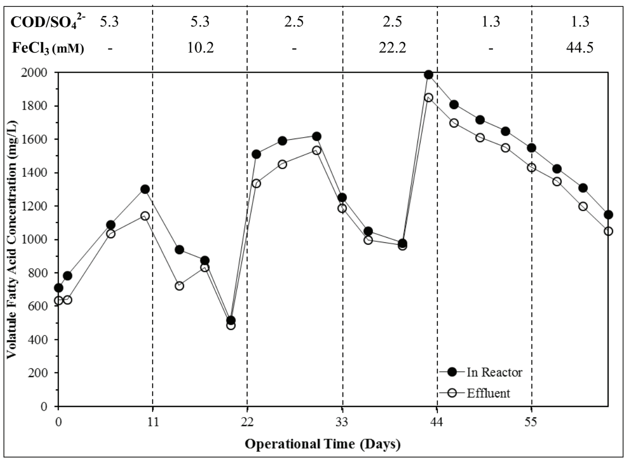


Fig. 4. Total VFA concentration profile in reactor and effluents of UASB at different COD/SO$_4^{2-}$.

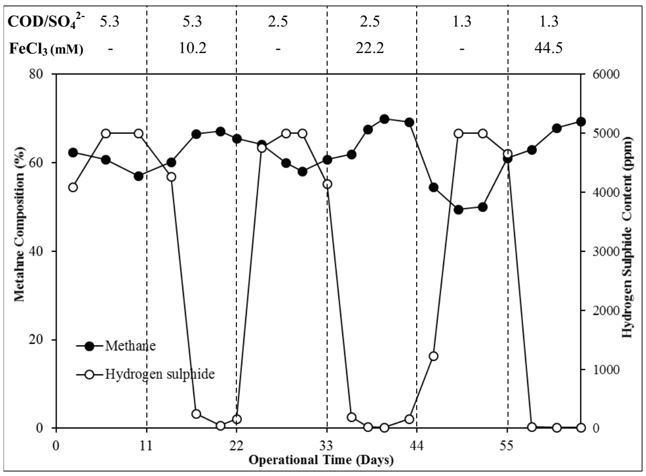


Fig. 5. Proportion of methane, CH_4 (%) and hydrogen sulphide, H_2S (ppm) in the biogas at different COD/SO_4^{2-}.

bacteria use oxygen as an electron acceptor, others can use nitrate in the process known as denitrification [28]. Among the colourless bacteria studied, *Thiobacillus* most frequently appears in sulphur-related wastewater treatment plants.

3.2.2. Methane composition and yield

Methane composition showed increased level when the reactor was operated at COD/SO_4^{2-} ratios of 5.3, 2.5 and 1.3 (Fig. 5), respectively. Meanwhile, the hydrogen sulphide (H_2S) content peaked to 5000 ppm when the system was supplemented with 1500 mg·L^{-1} sulphate concentration (given corresponding COD/SO_4^{2-} ratio of 5.3). It was observed that the profiles of methane composition of the UASB system reduced from an average value of 66% to 57% when the reactor was operated at COD/SO_4^{2-} ratio of 5.3.

When UASB was operated with $FeCl_3$ at iron dosage of 10.2 mM (day 11), the composition of methane increases from 57% to 67%. Overall, the methane production was observed to improve after each time $FeCl_3$ was added at dosage of 10.2 mM, 22.2 mM and 44.5 mM into the system on days 11, 31 and 41, respectively. In similar trend, the H_2S contents reduced rapidly from an average value of 5000 ppm to 55 ppm, 16 ppm and 11 ppm when $FeCl_3$ was added at dosage of 10.2 mM, 22.2 mM and 44.5 mM, respectively. Further decrease in COD/SO_4^{2-} ratio to 1.3 caused the UASB system settings to become favourable to SRBs. At this ratio, methane composition decreased from 69% (day 96) to 49.5% (day 52). However, methane production increased back with higher yields in the system when UASB was operated with dosage $FeCl_3$ at 44.5 mM on day 52. Furthermore, the addition of $FeCl_3$ as a regulatory agent for the reduction of hydrogen sulphide (H_2S) in UASB resulted in high methane composition.

Another useful parameter to assess the performance of anaerobic reactor is methane yield (L CH_4·gCOD$_{destroyed}^{-1}$). Fig. 6 shows the average values of methane yield decreasing gradually when UASB was operated at COD/SO_4^{2-} ratio of 5.3, 2.5 and 1.3 (day 0, 21 and 41 were 0.25, 0.22 and 0.21 L CH_4·gCOD$_{destroyed}^{-1}$, respectively. Methane yield was observed when UASB was operated with $FeCl_3$ dosages of 10.2 mM, 22.2 mM and 44.5 mM of $FeCl_3$, respectively. The trend in methane yield attained its peak when the reactor was fed with COD/SO_4^{2-} of 5.3, 2.5 and 1.3 (average values of .35, 0.32 and 0.27 L CH_4·gCOD$_{destroyed}^{-1}$) on day 16, 36 and 58, respectively.

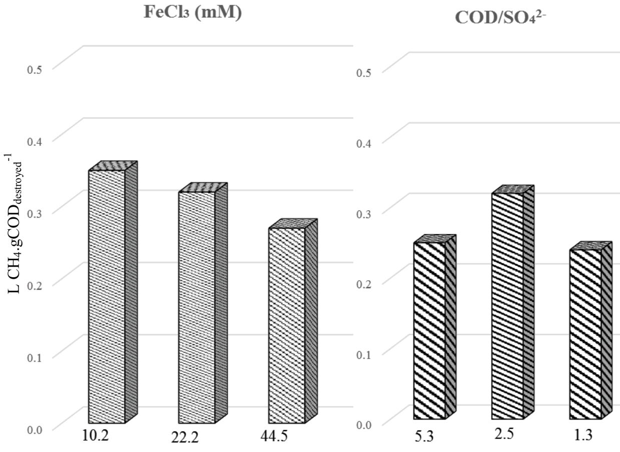


Fig. 6. Methane yield in each COD/SO_4^{2-} ratios and with the addition of ferric chloride $FeCl_3$ in the system.

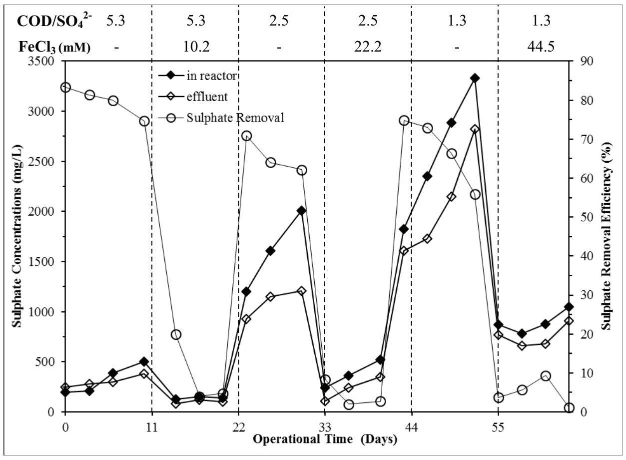


Fig. 7. Contribution of sulphate in effluent (mg·L^{-1}) and sulphate removal efficiencies (%) in upflow anaerobic sludge blanket (UASB) at different COD/SO_4^{2-}.

It can be concluded that methane yield was affected when UASB operated with sulphate enriched wastewater. The effectiveness of using $FeCl_3$ at certain dosage leads to enhanced methane yield as methane yield observed in this study increased after a period of 9–10 days' operation at each dosage.

3.2.3. Sulphate reduction

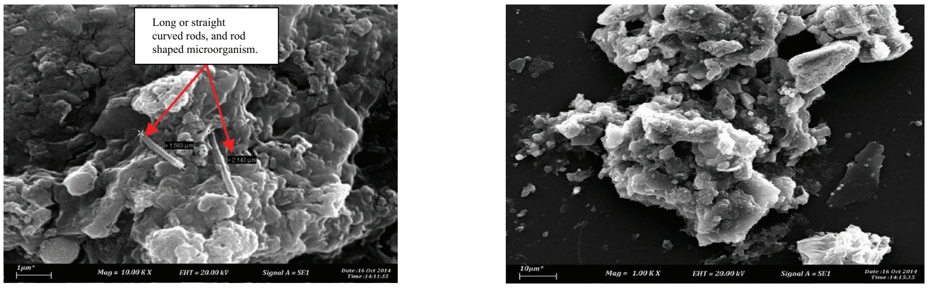
The sulphate removal efficiencies showed slight decline when UASB was operated at COD/SO_4^{2-} of 5.3 (80 to 70% removal from days 0 to 11) as shown in Fig. 7. Further decrease in COD/SO_4^{2-} ratios from 5.3 to 2.5 (on day 21) and COD/SO_4^{2-} of 1.3 (on day 41) caused the sulphate removal efficiencies to attain 60–70% and 55–75%, respectively. The fluctuation in sulphate degradation when treating sulphate enriched wastewater on first day onwards was caused by two factors. Firstly, the major contribution of influent chemical oxygen demand (COD) was removed via sulphate reduction. Secondly, substantial sulphide production could have been toxic to SRBs and MPBs in the form of dissolved sulphide and undissociated H_2S concentration.

However, a study conducted by [25] on anaerobic treatment of sulphate-laden wastewater in anaerobic baffled reactor (ABR) showed that majority of the sulphate reduction occurred at lower COD/SO_4^{2-}. It was also observed that such anaerobic system attained low pH, high VFA concentrations and high H_2S biogas content. These results indicated that more than 80% sulphate were removed at a COD/SO_4^{2-} of 2.5 as compared to only 65% sulphate removal achieved in the current study. Furthermore, [24] showed that when COD/SO_4^{2-} ratio was less than 3.0, the sulphate removal decreased gradually when treating high sulphate wastewater. It was later observed that the total sulphate removal was less than 80% at COD/SO_4^{2-} ratio of 1.7.

In contrast, [20] also showed that lower COD/SO_4^{2-} had the ability to reduce the sulphate removal. It investigated the sulphate removal from industrial wastewater using a packed-bed anaerobic reactor and noticed that COD/SO_4^{2-} of 0.8 and 0.5 resulted in sulphate removal efficiency of 69% and 65%, respectively. Therefore, similar observations were also recorded in the current study (i.e. drop in pH values, high VFA concentration in each COD/SO_4^{2-} ratios, as shown in Figs. 2 and 4), and sulphate reduction is likely to occur in each COD/SO_4^{2-} ratio. In this study, sulphate removal efficiencies take place at lower COD/SO_4^{2-} which is at ratio of 1.3 (average sulphate removal efficiencies 66%).

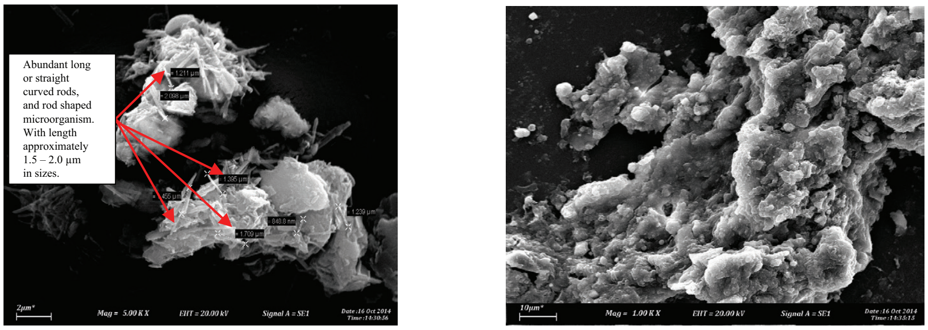
3.2.4. Characteristics of sludge morphology

In the study, the morphology of anaerobic sludge was identified. After 10 days of operation with COD/SO_4^{2-} of 2.5, the sludge consisted mainly of rod-shaped bacterium which presumably referred to a few common shapes of SRBs species (Fig. 8a). The sizes were between 1.5–2.0 µm. Similar



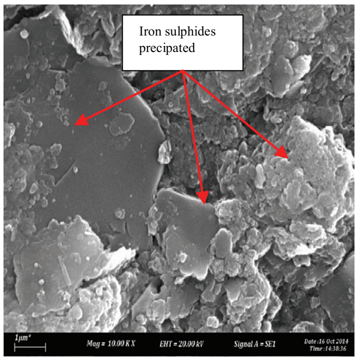
(a) X 10 000 (Day 31)

(b) X 1 000 (Day 41)



(c) X 5 000 (Day 50)

(d) X 1 000 (Day 64)



(e) X 10 000 (Day 64)

Fig. 8. SEM photographs of the sludge in UASB.

observation was found when UASB was operated at COD/SO$_4^{2-}$ of 1.3 ratio, with more rod-shaped bacterium attached to sludge. The sludge sampled at different COD/SO$_4^{2-}$ ratio shows the presence of heterogeneous microbial population at the surface and [30] suggested that these bacteria are of *Desulfovibrio* genus.

Furthermore, a variety of single rods, filamentous rods and long rod-shaped bacterium were dominant morphotypes in the sampled sludge (Fig. 8c). The identification and verification were SRBs and share similar properties with common SRBs such as being motile, contains a short or straight curved rods, and rod-shaped (i.e.; *Bacilli, Desulfovibrio sp*). A study conducted by [31] revealed that this microbial diversity was retained at lower COD/SO$_4^{2-}$ ratios when they investigated the effect of influent COD/SO$_4^{2-}$ ratios in mesophilic expanded granular sludge bed (EGSB) reactors.

After UASB was successfully operated with FeCl$_3$ at iron dosage of 22.2 mM and 44.5 mM, the surface analysis was further observed (day 41 and 64). The SEM observation of Fig. 8b and 8d were taken from the middle and centre parts of sludge, respectively. These pictures revealed that the sludge contained no rod-shaped bacterium. A more detailed SEM image of sample from Fig. 8d taken from different angles on the surface sludge showed the presence of iron sulphide precipitates (Fig. 8e). From this current SEM photographs, it is well interpreted that more iron is captured by sulphide to form iron-sulphide precipitated in the UASB reactor.

4. C.onclusion

The influence of FeCl$_3$ as a regulatory agent for the reduction of hydrogen sulphide (H$_2$S) in the anaerobic treatment sulphate enriched wastewater was studied. At 22.2 mM and 44.5 mM dosage, the addition of ferric chloride attained 89% and 91% reduction in dissolved sulphides, with a respective methane yield of 0.32 and 0.27 L $CH_4 \cdot gCOD_{destroyed}^{-1}$. Thus, FeCl$_3$ additions for the reduction of H$_2$S through formation of ferric and ferrous sulphides (Fe$_2$S$_3$, FeS) rapidly adapted with the microbial community structure to significantly reduce SRB activity and this approach can be used for successful treatment of wastewaters containing high sulphate concentrations. Therefore, the methane yield from an anaerobic digester after the addition of ferric chloride substantially promotes increased methanogenesis.

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