BIOLOGICAL REMOVAL OF SULFUROUS POLLUTANTS OF SKIM LATEX WASTEWATER

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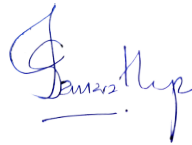
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ABSTRACT

Skim Latex Wastewater (SLW) contains high concentrations of sulfate, together with organic matter and nitrogenous compounds such as Ammonia and protein. High concentrated sulfuric acid is added in coagulation process to recover rubber particles and ammonia is used for preservation of rubber latex. Under anaerobic digestion, sulfate breakdown into hydrogen sulfide which is one of the highly toxic, corrosive and odorous gas which causes severe threat to the environment and health Nevertheless, it degrades the commercial value of biogas as a renewable energy source causing severe corrosion in connected components of equipment. Conventional biological process to treat sulfate rich wastewater consists of two processes, sulfate reduction to sulfide by Sulfate Reducing Bacteria (SRB) and Sulfide oxidation to elemental sulfur by Sulfide Oxidation Bacteria (SOB) in separate reactors. Major objectives of this research study are to investigate the effect of ammonia rich SLW on sulfate reduction and Hydrogen sulfide emission reduction under anaerobic condition and develop strategies for enhancement of sulfate reduction for subsequent elementary sulfur formation under different micro-aeration techniques. Optimum conditions for both sulfate reduction as well as elementary sulfur formation are also investigated.

In previous studies, various reactor configurations have been developed by integrating both the SRB and SOB into a single reactor. In this study SRB and SOB integrated suspended growth reactor for SLW which is not only rich in sulfate, but also ammonia and protein which ultimately breakdown to produce more ammonia is introduced. This new reactor is termed as Single-stage Sulfate-removal Micro-aerated Anaerobic Digester (SSMAD). It is hypothesized that this SRB and SOB integrated micro-aerated anaerobic reactor approach can be applied to enhance removal of sulfurous pollutants from SLW.

To achieve the research objectives, seven experiments were conducted. All experiments were conducted semi batch wise using 3 litres airtight completely mixed anaerobic reactors which were maintained at $35 \pm 1°C$. From the results, it can be concluded that, Single-stage Sulfate-removal Micro-aerated Anaerobic Digester (SSMAD) simultaneously reduced high concentrated influent sulfate of SLW, while hydrogen sulfide been transformed to reusable elemental sulfur. To achieve the optimum sulfate reduction as well as maximum elemental sulfur yield, bulk liquid of the SSMAD was micro-aerated with air at rate of 1.6 ml/hr for two hours following half an hour of feeding SLW. It was found that yield and the stability of the generated elemental sulfur improved at O_2/S ratio 1.0-1.2, after 18-24 hours of feeding. At this range, specific H_2S formation was less than 0.2 mmol/mmol while the sulfate reduction was 95.8%. The COD/SO_4^{-2} ratio of SLW was nearly 3 and it was increased to 5 adding an external electron donor for efficient sulfate reduction but further increased up to 10, reduced the sulfate reduction as Methanogens dominate than SRB. Although ethanol enhances the sulfate reduction than acetate, excess ethanol adversely affected on the micro-aerobic systems degrading generated elemental sulfur back to gaseous H_2S faster. Thus, the elemental sulfur yield reduced by 69% when the COD/SO_4^{-2} ratio was increased from 5 to 10. However, sufficient precautions were taken to increase the C/N ratio from 3.8 to 6.9, by maintaining pH of the reactor at 7.5-8.0 and volumetric loading at $50\ l/m^3.d$ to minimize ammonia inhibition in the reactor. Developed novel approach through Single-stage Sulfate-removal Micro-aerated Anaerobic Digester (SSMAD) can be successively used to recover sulfurous pollutants from SLW.

Key words: Skim latex wastewater, Sulfate reduction, sulfide oxidation, Ammonia inhibition, Micro aeration

ACKNOWLEDGEMENTS

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LIST OF ABBREVIATIONS

Abbreviation	Description
AD	Anaerobic Digestion
BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
sCOD	Soluble Chemical Oxygen Demand
tCOD	Total Chemical Oxygen Demand
DO	Dissolved Oxygen
DRC	Dry Rubber Content
EDX	Energy Dispersive X-ray
FAN	Free Ammoniacal Nitrogen
GC	Gas Chromatograph
HRT	Hydraulic Retention Time
MB	Methanogenic Bacteria
ORP	Oxidation Reduction Potential
SME	Scanning Electron Microscope
SRB	Sulfur Reducing Bacteria
SOB	Sulfur Oxidizing Bacteria
SLW	Skim Latex Wastewater
SSMAD	Single-stage Sulfate-removal Micro-aerobic Anaerobic Digester
TDS	Total Dissolved Sulfide
TAN	Total Ammoniacal Nitrogen
TKN	Total Kjeldahl Nitrogen
TMTD	Tetra Methyl Thiuram Disulfide
TS	Total Solid
TSS	Total Suspended Solid
VFA	Volatile Fatty Acid

LIST OF APPENDICES

1. INTRODUCTION

1.1 Background

The wastewater discharged from skim latex industry is one of the major source of air and water pollution, because of an improper management and treatment [1]. Skim Latex Wastewater (SLW) contains high concentrations of sulfate, organic matter and nitrogenous compounds like ammonia and protein[2]. High concentrated sulfuric acid is added in coagulation process to recover rubber particles and ammonia is used for preservation of rubber latex [2].

1.1.1 Wastewater generation in skim latex production

Two sub processes contribute for total wastewater generated from skim latex processing industry:

- Skim latex coagulation (rubber skimming) process and
- Washing process taken place in other unit operations. These two processes are shown in Figure 1.1.

Wastewater generated from skim latex coagulation process is highly acidic, i.e. pH 2.0-4.5, high in Chemical Oxygen Demand (COD) and sulfate. which is around 14,911±1,819 mg/l and 6,506 ± 1,038 mg/l respectively[1]. On the other hand, pH of the wastewater generated from the washing process is slightly alkali i.e. pH 7.9 and has low COD and the sulfate at 500±126 and 275±82 mg/l respectively[3]. As a result of mixing, both wastewater streams of skim latex coagulation and washing processes, final discharged COD and sulfate concentration are intermediate.

Even though the combination of both wastewater steams reduces the COD and sulfate to some extent, still the reported COD and sulfate values for final wastewater generated from skim latex industry is reported to be high compared to set values in the environmental standards.

Field latex

Receiving → Rubber
- - - → Wash water

DAP →
NH_4ClO_3 → Preservation → Sludge
- - - → Wash water

Centrifug → Bowl rubber Sludge
- - - → Wash water

Concentrated latex   Skim   latex

NH_3 →

Storage

Concentrated Latex

H_2SO_4 → Rubber Skimming
Wastewater

Crushing
- - - → Wash water

Cutting
- - - → Wash water

Drying

Pressing and Packing

Skim Block

Rubber trap
Wastewater pond rubber
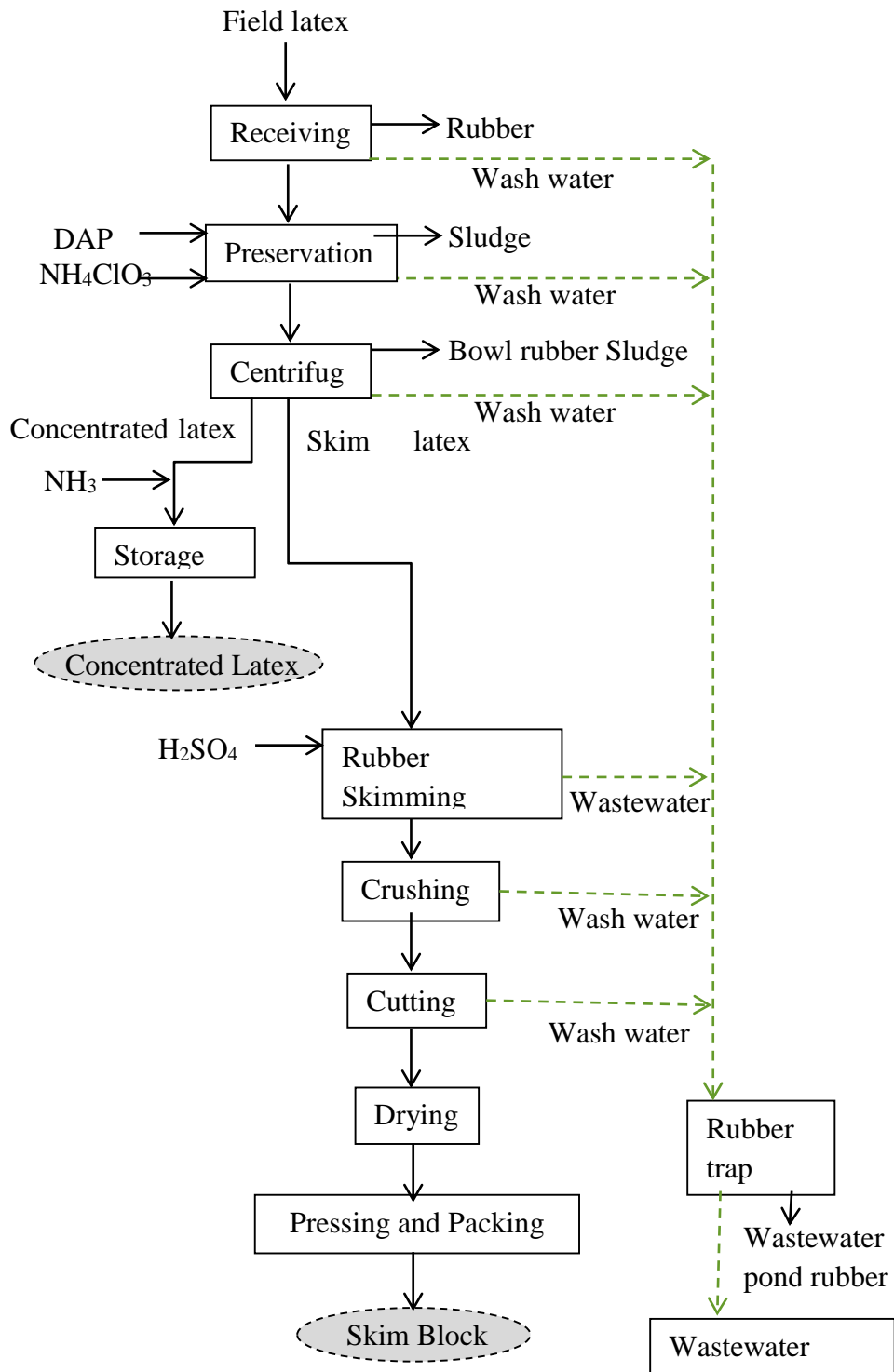
Wastewater

Figure 1.1: Block diagram of wastewater generation points at Concentrated and Skim latex rubber processing

1.1.2 Characteristics of typical wastewater discharged from skim rubber latex industry

According to the records of Rubber Research Institute Sri Lanka, there are over 160 raw rubber processing factories producing various rubber products such as Ribbed Smoked Sheet rubber (RSS), latex crepe rubber, scrap crepe, all grades of Technically Specified Rubber (TSR), centrifuged latex and specialty rubbers[4]. The effluent characteristics are different from one another depending on the type of the raw rubber manufactured. Effluents from centrifuged latex which produces by product skim rubber using sulfuric acid contains the highest concentrations of undesirable non-rubber pollutants[4]. Although some average values of effluent constituents are shown in the Table 1.1, in some industries the observed wastewater discharged concentrations are much higher with the variation of dilution factor of wash water.

Table 1.1: Discharge wastewater quality of main rubber products. (All figures are in mg/l except pH)

Parameter	RSS	Crepe	TSR	Centrifuged Latex		Dipped products	CEA tolerance limits
				Skim	Average		
pH	4.9	5.0	5.7	3.7	4.5	7.2	6.5-8.5
COD	3300	3500	2740	25,000	8201	2011	400
BOD	2630	2500	1747	-	2192	1336	50/60*
TSS	140	130	237	1000	190	241	100
TS	3745	3500	1915	13,000	7576	2457	1500*/1000
Ammoniacal Nitrogen	75	80	66	-	401	126	300*/40
Total Nitrogen	500	550	147	900	816	180	300*/60
Sulfate	-	374	-	11,300	5610	72	1000
Sulfide	-	15	-	-	-	-	-
Sulfite	-	190	-	-	-	-	-

(Extracted from Hand Book of Rubber Processing Technology (2003)[4])

As per the past literature, the most of the pollutants of rubber effluent consists of biodegradable organic matter such as volatile organic acids, sugar, protein, lipids and mineral salts[5].

1.1.3 Adverse effects of sulfurous pollutants of SLW

As shown in Table 1.1, SLW is rich in sulfate. Sulfate doesn't cause any direct impact on the environment as it is a nontoxic, non-volatile and chemically inert compound [6]. But sulfate is transformed biologically into hydrogen sulfide through anaerobic degradation by Sulfur Reducing Bacteria (SRB)[7]. H_2S is one of the highly toxic compounds which causes severe threat to the environment and health. Nevertheless, it degrades commercial value of the biogas to be used as a renewable energy source directly with gaseous H_2S, because it causes severe corrosion on connected components of equipment as well as buildings when the biogas is directly used as a renewable energy source.

Hydrogen sulfide is a highly toxic, reactive and flammable gas with unpleasant odour of rotten egg smell in between threshold value of 3-5 ppm. The density of the Hydrogen sulfide gas is higher than air. Hence it accumulates near the ground level. Following limits are identified as the limits for odour and health related effects (Table 1.2). H_2S is a highly corrosive gas[8].

Table 1.2: Limits for hydrogen sulfide in the atmosphere for odour and health related issues [9].

Description of effects on human	Concentrations in atmosphere/ ppm
Odour limit	0.1 - 0.2
Unpleasant smell	3 - 5
Recommended criterion for workday	10
Effect on eyes	50 - 100
Inactivation of smell	150- 250
Serious water accumulation in lungs	300 - 500
Deadly impact on nervous system	500 - 1000
Immediate cessation of respiration	1000 - 2000

As a result of high concentrated sulfate presence in skim latex wastewater, H_2S emits when treated in anaerobic reactors. However separate H_2S removal units with various physical-chemical processes are required to be installed in biogas streams, and this add extra cost and burden on the system. Sulfate reduction process by Sulfate Reducing Bacteria (SRB) and generated sulfide inhibits the methanogenic microorganisms which degrade organic matter [3], [10]. Free hydrogen sulfide ions are the main form of sulfide which causes the most toxicity. This toxicity affects the anaerobic reactor causing many problems within the wastewater treatment facilities such as significant reduction in COD treatment efficiency or complete failures in anaerobic process, decrease in methane yield, reduction of biogas quality and inhibition of methanogenic bacteria.

As explained above, the SLW treatment which is rich in sulfate is challenging for environmental Engineers in achieving the desired quality compatible with recommended Environment standards. High proteins and nitrogenous compound of SLW makes more difficult to treat these pollutants. Because protein compounds breakdown to Ammonia under anaerobic conditions[11] which is toxic to the micro-organisms at elevated concentrations. It is vital to implement an effective and sustainable method to reduce influent sulfate concentration of SLW as well as to reduce toxic hydrogen sulfide gas emission under anaerobic digestion at ammonia rich environment and reach the standards of environmental regulations. As a result, it will reduce the health and environmental impact as well.

1.1.4   Non biological treatment methods for sulfurous pollutants removal

Influent sulfate can be removed from wastewaters by chemical precipitation or desalination processes such as reverse osmosis and ion exchange. But they are significantly expensive[12]. Physio-chemical treatment process to remove H_2S from the biogas are Adsorption using Activated carbon, Iron oxide, Molecular sieve, Zinc oxide, Alkaline solids, Absorption using Water, physical solvents without water, alkaline solutions, Zinc oxide slurries, Iron oxide slurries, Iron salt chelated or not chelated, Quinone and Vanadium salts, Chemical oxidants (H_2O_2, $KMnO_4$, Hypochlorite), Amines, membrane purification[13]. These systems lead many other drawbacks such as considerable energy requirement, high operation, labour and

maintenance cost, high chemical and disposal cost and disposing the spent chemicals[12]. Recently biological sulfide oxidation methods became most popular all around the world as an clean alternative for H2S removal[14].

1.2 Biological sulfurous pollutants removal through Micro aeration inside anaerobic reactor for ammonia rich wastewater

Biological technologies to remove sulfurous compounds in wastewater becoming more popular due to its economic viability and sustainability. Among many techniques, Micro aeration is suggested to be a better alternative solution for simultaneous reduction of influent aqueous sulfurous compounds like sulfide and sulfate while preventing emission of gaseous H2S , finally producing reusable elemental sulfur [15]. There are several researches conducted for direct sulfide conversion to elemental sulfur using different reactor configurations and various types of wastewater but only very few literatures were recorded for biological removal of influent sulfate via elemental sulfur which is a two steps process. In first step, sulfate have to be reduced to sulfide by sulfate reducing bacteria (SRB) and in second step generated sulfide Biologically oxidized to elemental sulfur (S^0) by Sulfur Oxidizing Bacteria (SOB) whereas the this undissolved elemental sulfur can be separated by physical separation method [14].

First step: Sulfate reduction[16]

$$SO_4^{-2}(aq) + 2C(Organic) + 2H_2O(l) \leftrightarrow H_2S(aq) + 2HCO_3^-(aq) \quad \text{......} (1)$$

$$H_2S(aq) \leftrightarrow H_2S(g) \qquad \text{......} (2)$$
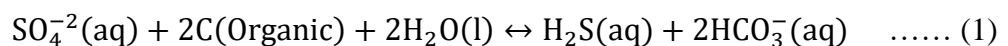
Sulfate is an electron acceptor in sulfate reduction process. SRB compete with acetogens and MB for simple intermediate products of organic matter degradation such as products of acidogenesis stage and acetogenesis stage, which provide the electron donor for sulfate reduction[17]. Therefore, influent COD/SO_4^{-2} ratio, Gibb's free energy, kinetic reactions, sensitivity for sulfide inhibition of each species, types of substrate, relative micro-organisms, temperature and pH are the major factor affecting the sulfate reduction.

Second Step: Sulfide Oxidation

- Under controlled micro-aeration (DO concentration < 0.1 mg/l) elemental sulfur is the final end product[18], [19], [20]. This reaction is performed by SOB.

$$2HS^- + O_2 \leftrightarrow 2S^0 + 2OH^-; \Delta G^0 = -169.4 \text{ kJ/mol} \qquad \ldots\ldots\ldots (3)$$
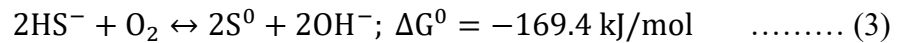
- But if oxygen supply is higher, sulfide oxidized more forming sulfate, sulfite or thiosulfate[21].

$$2HS^- + 4O_2 \leftrightarrow 2SO_4^{-2} + 2H^+; \Delta G^0 = -732.6 \text{ kJ/mol} \ldots\ldots\ldots (4)$$

SRB are strict anaerobic bacteria which are inhibited in high aerobic condition and SOB require limited oxygen for conversion of sulfide to elemental sulfur. Previous research studies have been conducted using above two steps in two separate reactors maintaining one reactor in strict anaerobic condition for sulfate reduction to sulfide and the other subsequent reactor at micro-aerobic condition for conversion of transformed sulfide to elemental sulfur or gaseous H_2S to elemental sulfur. But with new technology development, it is more focused to maintain suitable condition for both the SRB and SOB, promoting both processes to be taken place in a single reactor, while enhancing elemental sulfur formation.

The other main component i.e. influent ammonia in the reactor will increase with the time due to breakdown of protein to ammonia. Although TAN (Total Ammoniacal Nitrogen)is an essential nutrient for microorganisms' high concentrations of TAN and FAN (Free Ammonia Nitrogen) adversely affect the micro-organisms including SRB and MB[22]. FAN found to be the most toxic compound. Strategies for controlling the ammonia inhibition are acclimation of microflora, pH control, temperature control, adjustment of C:N ratio of feed stock, dilution of reactor contents and immobilizing the micro-organisms[23]. F. Straka[24] also observed ammonia inhibition in integrated SRB and SOB reactor, when the C/N ratio was lower than 10. He found out that the best C/N ratio lies between 20-40.

Since ammonia present in SLW, it can be assumed that ammonia nitrifies, but nitrification of ammonia to nitrate would only take place at high DO concentrations

greater than 1 mg/l whereas the desired DO concentration for elemental sulfur formation which is less than 0.1 mg/l and at DO greater than 0.3 mg/l SRB inhibited with completely failure of the single reactor which SRB and OB takes place[18]. S. Luostarinen et al[25] found out that at least 1-2 mg/l require for nitrification whereas B. Rusten[26]et al. has explained that for complete nitrification, required DO concentration was 2-3.5mg/l in Moving Bed Biofilm Reactor (MBBR). However DO concentration of 2 ± 0.83 mg/l was maintained in a reactor, in which ammonia oxidation process and partial nitrification achieved simultaneously in a single reactor[27]. However, Z. Zheng[28] and his team has found that at DO concentrations of 3.50, 1.45 and 0.7 mg/l the inorganic nitrogen removal with nitrification were 93.4%, 87.5% and 92.7%. Therefore, it is convinced that in the range of DO< 0.1 mg/l, ammonia nitrification is impossible whereas only biological conversion of sulfide to elemental sulfur is encouraged.

1.3 Micro-aeration for sulfurous pollutant removal

The desired micro-aeration condition inside integrated SRB and SOB reactor for major end product of sulfide oxidation to be elemental sulfur generation is oxygen concentration below 0.1 mg/l[29]. Beyond 0.1 mg/l oxygenation level, sulfate will be the major end product and obligate anaerobic bacteria will be inhibited with oxygen. In the past, micro-aeration for sulfide removal has taken place in different oxygenation levels and in various types of reactor configurations.

At the very beginning, two separate reactors for SRB and SOB activities had been applied. Firstly, usage of single reactor for both SRB and SOB reported for removal of H_2S from biogas for agricultural waste[29]. However, this technique was then widely experimented and utilized including full scale operation for digestion of sludge from wastewater treatment plants. Single stage Micro-aerated Anaerobic Digesters were able to remove H_2S from biogas of 2500-34000 ppm with efficiency higher than 97%[30],[31]. Initially developed full scale micro aerobic CSTR used to treat agricultural waste only removed H_2S by 68%-88% probably due to low residence time of biogas in the head space in comparison to anaerobic sludge digester. Later on, micro-aeration was applied to Up-flow Anaerobic Sludge Blanket (UASB) reactors, Expanded Granular Sludge Bed (EGSB) reactors and Fluidized Bed Reactors (FBR)

for the treatment of industrial wastewaters with high sulfurous pollutant load such as discharge from brewery, sugar and paper. These reactors were able to reduce H_2S with $70 - 82\%$ from biogas streams containing 20,000 to 67,000 ppm[15]. On the other hand, Camiloti et al. [32] has reported the application of silicone tubes for micro-aeration of liquid phase without bubble formation of AD in which sulfide oxidation was mainly performed by the SOB in the biofilm.

Although, initially the main focus of research and development was to reduce H_2S in biogas using micro-aeration technique, recently it has been focused to recover elemental sulfur for reuse. Theoretically 0.5 mol O_2 /mol S^{-2} is required for oxidation of sulfide to elemental sulfur as per Eq(1)[19]. Janssen et al. (1995) were able to gain maximum sulfur recovery of $73\pm10\%$ at O_2/S^{-2} ratio of 0.6 to 1.0 with 0.7 as the optimum. S. Alcantara et al. found that elemental sulfur production at steady state were achieved at O_2/S^{-2} ratio ranging from 0.5 to 1.5, while maximum sulfur recovery of 85% was occurred at O_2/S^{-2} ratio of 0.5 while all the elemental sulfur was completely transformed to sulfate at O_2/S^{-2} ratio of 2. 9. G. Munz et al.[29] has observed contradictory results to existing findings which researchers observed 91, 87 and 85% of sulfide conversion to elemental sulfur at O_2/S^{-2} ratios of 0.0015, 0.005 and 0.03.

There are only very few experiments conducted for SRB and SOB in ammonia rich environment. Micro-aeration was carried out by F. Straka [24] and his team for both sulfate and nitrogenous compound rich wastewater i.e. pig manure and H_2S in the biogas decreased from 4000 mg/m^3 to 220 mg/m^3. However, they have found that the ammonium nitrogen of the reactor increases steeply from 1.2 g N/l at 7.0-7.35 to 3.0 gN/l at 8.5-9.0 reducing the methane production from 15 to 20%. Thus, they suggested that for micro-aerophilic system even ammonia inhibition can be minimized by initially increasing the C/N ratio higher than 10 by co-digestion with low ammonia waste and reducing the loading rate. W. Mulbry et al. [33] also utilized miro-aeration successfully in plug flow reactors to reduce H_2S from 3500 ppm to less than 100 ppm, for diary waste which is another protein rich waste, but analysis were not carried out on nitrogenous compounds.

1.4   Research Problem

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                    ┌─────────────────────┐
                    │ Microaeration directly│
                    │     inside AD        │
                    └─────────────────────┘
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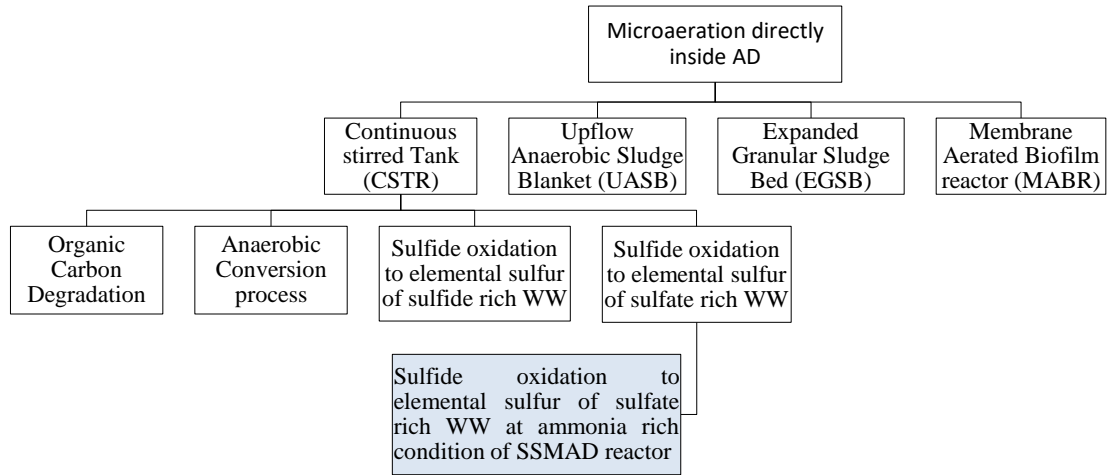
Figure 1.2: Developed SSMAD in the context of existing SRB and SOB integrated reactor technologies

As discussed earlier, development of sustainable treatment method is essential for recovering sulfurous pollutants of SLW using most preferable, biological method. Conventional biological process to treat sulfate rich wastewater consists of two processes; sulfate reduction to sulfide by Sulfate Reducing Bacteria (SRB) and sulfide oxidation to elemental sulfur by sulfide oxidation bacteria (SOB)[18] in separate reactors. A common drawback of these processes is the need of additional treatment unit that increases capital and operational cost.

With recent technological advancement, various reactor configurations for several types of wastewater were experimented integrating both SRB and SOB in a single reactor as explained in section 1.3, But no studies conducted for   SRB and SOB integrated suspended growth single reactor for SLW which is not only rich in sulfate, but also high concentrations in ammonia and protein. Therefore, it is hypothesized that this approach can be enhanced to minimize the influent high concentrated sulfate, emitted toxic hydrogen sulfide by producing optimum reusable elemental sulfur via micro-aerated anaerobic digester for nitrogen rich wastewater. Through a series of experiments, sulfate reduction as well as sulfide oxidizing mechanisms were tested for developing novel approach for SLW treatment. Nevertheless, variation of major Sulfurous compounds with time in the Single-Stage Sulfate-Removal Micro-aerated

Anaerobic Digester (SSMAD) was investigated through extensive product analysis. It is essential for enhancement of both sulfate reduction step and elemental sulfur yield. Nevertheless, investigations on SR and SO for SLW under ammonia rich environment add more value to this research, which other researchers still not carried out. The mapping of the current research with the existing knowledge gap is presented in Figure 1.2.

1.5   Research Objectives

This research investigates the possibility of conducting the micro aeration technique for SLW as it is still not utilized to recover sulfurous pollutants via elemental sulfur. The practical difficulties and limitations of applying the following technology to minimize discharge effluent sulfate and emitted gaseous H_2S during anaerobic digestion also identified. However, maintaining suitable balanced condition inside the SSMAD reactor which integrate both SRB and SOB in a single reactor, not to inhibit with oxygen or ammonia is important to optimize the sulfate reduction, H_2S reduction and optimize generation of reusable elemental sulfur when treating SLW. Nevertheless, with this research, extensive study on variation of the sulfurous compounds inside the reactor; influent sulfate, generated sulfide, hydrogen sulfide and elemental sulfur with time in the SSMAD reactor will be investigated. More importantly this research investigates on enhancement of sulfate reduction to elemental sulfur formation and optimize the elemental sulfur yield under ammonia rich environment. Objectives of this research study are to:

i.   Investigate the effect on sulfate reduction and Hydrogen sulfide emission reduction of ammonia rich SLW under anaerobic condition.

ii. Develop strategies for enhancement of sulfate reduction for subsequent elementary sulfur formation.

iii. Apply different micro-aeration techniques and conditions to enhance elemental sulfur formation.

1.6   Conceptual framework of the research

The conceptual diagram of the research strategy as applied to this study is shown in Figure 1.3.

Development of SSMAD reactor for simultaneous reduction of sulfate, gaseous hydrogen sulfide and elemental sulfur formation under ammonia and protein rich SLW

Sulfide Oxidation to elemental sulfur

Micro-aeration

Influent Sulfate Reduction

Wastewater treatment

Aerobic digestion

Ammonia rich environment

Anaerobic digestion
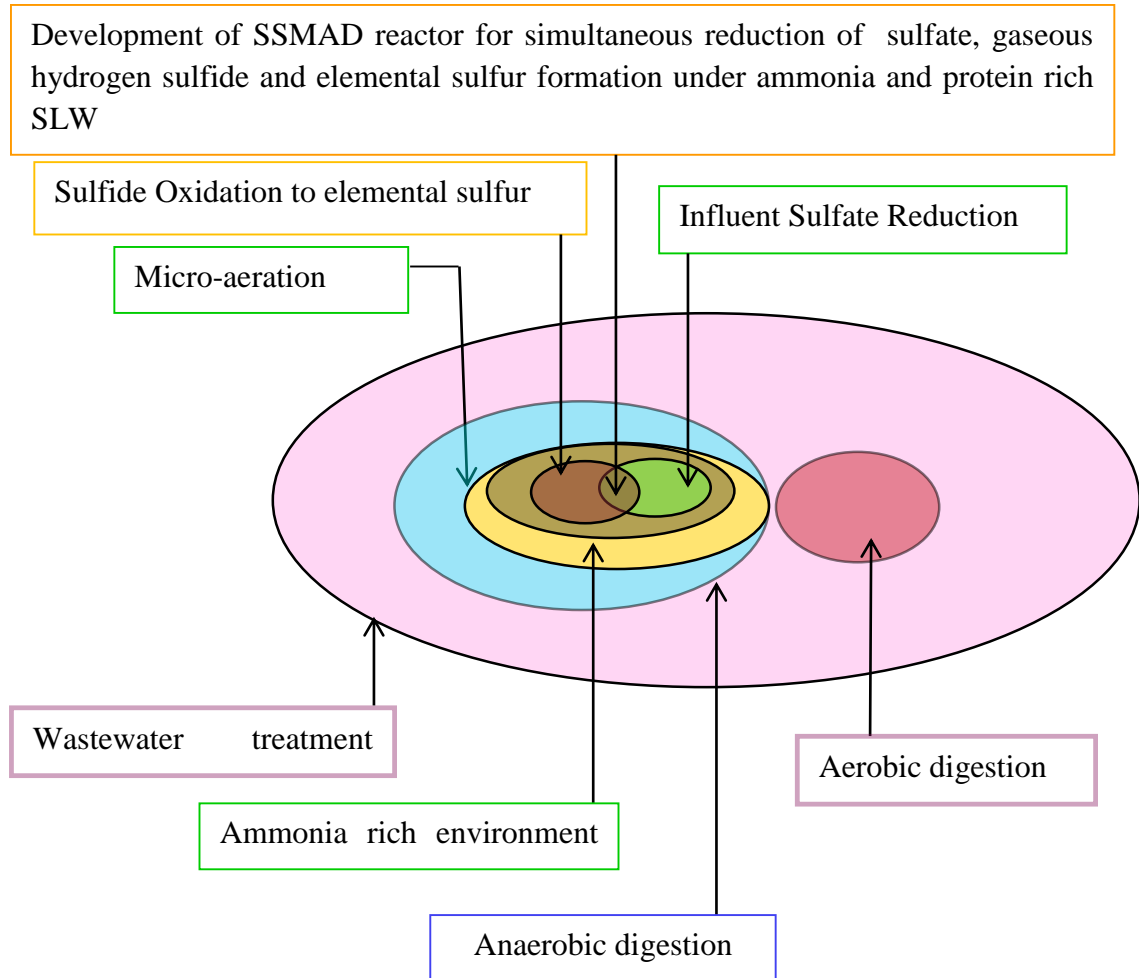
Figure 1.3:Conceptual diagram of the proposed research methodology

1.7 Outline of the Thesis

Chapter 1 of this thesis overview the background of the research problem, SLW generation and its characteristics, research problem, application of micro aeration technique for sulfate and hydrogen sulfide pollution prevention and fundamental concept of SSMAD reactor development. Chapter 2 presents Literature survey of anaerobic digestion of major constituent sulfate, organic matter and nitrogenous compounds, effect of micro aeration in anaerobic digester on sulfate and hydrogen sulfide. Chapter 3 presents the methodology. Chapter 4 discuss the results of all the experiments whereas Chapter 5 present conclusions drawn from results and recommend directions for further research work.

2   LITERATURE REVIEW

The purpose of this literature survey is to review the Skimmed Latex Wastewater (SLW) generation, the anaerobic digestion of its major constituents; sulfate, protein and organic matter, application of bioreactors to remove sulfate via micro aeration with the objective to develop Single-Stage Sulfate-removal Micro-aerated Anaerobic Digester (SSMAD) to remove oxidised sulfur pollutant by transforming them in to elemental sulfur via micro-aeration for SLW treatment which is rich in both sulfate and ammonia. Micro-aeration of anaerobic reactors to remove pollution caused due to oxidized sulfurous compounds is an advancing technology. From this technology, oxidized sulfur compounds are biologically converted to elemental sulfur which can be reused after further processing. But with this research study, knowledge contribution for further development of use of micro-aeration to remove sulfate to elemental sulfur under high strength ammonia concentrations focused. Therefore, the first section of this chapter describes skimmed latex industry, wastewater generation and its characteristics. Later sections introduce basic principles and existing knowledge based on the specific areas which directly related to the research understanding, such as anaerobic digestion of oxidized sulfurous compounds, protein degradation, sulfate removal non biological techniques available, draw backs of such techniques, various biological reactor configurations used for sulfate reduction and elemental sulfur formation, main products exist in micro-aerated anaerobic reactors and parameters affect the sulfate reduction and elemental sulfur formation and etc.

2.1   Skimmed latex wastewater generation

Main theme of this research study is treatment of skim latex wastewater. SLW generates when skim rubber which is a by-product of the concentrated latex industry is manufactured. Therefore, as the first step to the research study, better understanding of the natural rubber latex, skimmed rubber production, SLW generation and its characteristics are essential and emphasised as the first section of this chapter.

Concentrated latex is consumed as the raw material to produce some of the secondary dipped rubber products such as balloons, gloves for different commercial purposes, condoms, diaphragms infant pacifiers and toys [34].

2.1.1   Natural rubber latex

Fresh field latex is a sticky milky colloid exuded off by making an incision (tapped) in to the bark of the rubber tree[35]. Natural rubber latex consists of 20%-35% Dry Rubber Content (DRC) and Total Solid Content around 36%. The latex of Hevea Brasiliensis or natural rubber tree is a stable dispersion of polymeric substances in aqueous medium with two major phases; disperse phase and dispersion medium[36] . Disperse phase is a discontinues phase of rubber molecules. Disperse phase consists of rubber particles surrounded by aqueous emulsion made up of non-rubber compounds. Disperse phase is of 86% rubber hydrocarbons. Chemical composition of rubber hydrocarbon is of polyisoprene[37]. Predominantly, cis 1,4 - polyisoprene configuration exists. On the other hand, Dispersion medium or the Aqueous continuous phase of serum. The dispersion medium contains Carbohydrates, Proteins, Amino acids (Glycine, tyrosine and 12 others), Free nitrogenous bases like methylamine, organic acids (other than Amino acids), Metal ions (K, Mg, Fe, Na, Cu, etc.), complex enzymes and water [36]. This aqueous emulsion is known as serum [38].

Table 2.1: Chemical composition of latex

Material	Percentage by weight (%)
Dry Rubber Content (DRC)	30 – 35
Protein substances	1-1.5
Lipids	1-2.5
Inorganic ions	1.0
Sugar	1.0
Water	60-75

(Extracted from Hand Book of Rubber Processing Technology (2003)[4])

2.1.2 Natural Rubber Latex preservation

Preservative addition into latex should be carried out at the earliest possible time, because putrefaction of latex starts from the time that the latex leaves the latex vessels of the tree. Therefore, anti-coagulants are added to the tapping cups and collecting baskets in order to increase the pH to avoid premature coagulation. The most popular anti-coagulants are Ammonia, Ammonium Hydroxide, whereas Sodium sulfite, formalin and Tetra Methy Thiurum Disulfide (TMTD) and Zinc oxide.

The most preferable preservative use in natural rubber latex process is Ammonia and it is referred as the primary preservative as well. Because it is cheap, and Ammonia keeps the Volatile Fatty Acid (VFA) number of latex low while increasing the stability on storage. It produces complexes with metal ions like Zn and Mg in the latex, avoiding precipitation as insoluble salts and it inhibits bacterial growth increasing the pH. Ammonia has no effect on rubber molecule, and It has the added advantage of easy de-ammonization without adding chemicals. However, the main disadvantages of Ammonia are strong mal odour, cause environmental pollution and slight tendency to discolour the rubber produced.

The amount of Ammonia added is determined according to the season and the distance from collection site to the processing factory, longer the transport, higher the amount of ammonia. Fresh latex collected from the rubber farmers are transported to the factory to the factory by trucks.

2.1.3 Concentrated latex production through centrifugation and skim latex production process

Centrifugation is the widely used method for concentrated latex production. The pre-treated latex from the field is centrifuged to separate the latex cream from the serum. Concentrated latex cream contains around 60-70% Dry Rubber Content (DRC) which is the main product and the serum. Since, separated serum still contains 4 – 8% DRC, sulfuric acid is added to coagulate the remaining rubber particles[39] . The coagulated rubber is further processed by crushing, cutting and drying in order to make by product called skim rubber blocks. A schematic diagram of concentrated latex production together with skim latex production is presented in Figure 1.1. The SLW generation and the characteristics of SLW also presented in detail in chapter 1.

2.2 Anaerobic digestion of major compounds of skim latex wastewater

SLW is high in sulfate, organic matter and protein. Therefore, under this section, anaerobic degradation of those major pollutants is discussed to understand the inter relationship of each other, This enables the understanding of the strategies for sulfate reduction to sulphide, subsequently converting into elemental sulfur in micro aeration. In section 2.4, micro-aeration for elemental sulfur formation and oxygen shielding effect of micro-organism are discussed.

2.2.1 Anaerobic digestion of organic matter

Anaerobic digestion (AD) is a combination of biochemical and physiochemical processes which convert organic matter to gaseous end products with the occurrence of microorganisms at an oxygen depleted environment. These end products mainly composed of methane and carbon dioxide known as the biogas[40].

Anaerobic digestion (AD) takes place naturally in the environment, such as beneath the layers of soil or waste landfills, in water sediments like lakes, rivers and ocean [41]. On the other hand, AD process is utilized purposefully by engineers in order to treat biodegradable solid and wastewater. It is practiced in dedicated facility known as anaerobic digester. Currently Anaerobic digestion has become a well-established technology for treating multiple waste categories including domestic/municipal, agricultural and industrial origins [42], [43], [41].

There are five basic processes identified in the anaerobic conversion process. They are Disintegration, Hydrolysis, Acidogenesis (Fermentation), Acetogenesis (Acetate generation) and Methanogenesis (Methane generation) [40], [44]. These conversion steps are shown in Figure 2.1.

2.2.1.1 Disintegration

Disintegration is the breakdown of large particulate material by means of physical and chemical methods. For the hydrolysis process to be efficient, breakdown of particulate matter is (disintegration) important as it increases the accessibility area for enzymic reaction. This stage is especially important in solid wastes, slurries and wastewaters with high suspended solids [45].

2.2.1.2 Hydrolysis

Micro-organisms are unable to consume particulate organic materials. During this step, particulate organic materials are broken down into small soluble molecules by extra cellular enzymes which facilitate transfer across cell membranes. Hence, hydrolysis is physical, chemical and biochemical conversion of long chain organic material such as lipids, Carbohydrates (polysaccharides), protein and fats into soluble monomers which can be easily utilized by the microorganisms at subsequent acidogenesis stage.

2.2.1.3 Acidogenesis

At Acidogenesis stage, hydrolysed products are converted to simpler compound of volatile fatty acids such as acetic, propionic, butyric, valeric, caproic, and heptanoic and H_2, CO_2 and ethanol. Acid forming bacteria metabolized these intermediate products intracellular. These fermentative microorganisms are called acidifying or acidogenic microorganisms.

2.2.1.4 Acetogenesis

However, Acetogenesis is the conversion of the end products of the acidogenesis (volatile fatty acids and alcohols) to acetate, H_2 and CO_2 by group of strict anaerobic organisms called acetogenic bacteria.

2.2.1.5 Methanogenesis

There are two distinct microbial pathways which can be identified in Methanogenesis. At this stage, Acetate is converted to methane by acetoclastic methanogens, whereas generated CO_2 and H_2 are utilized by another group of methanogens called hydrogenotrophic methanogens, which produce methane as the end product and 70% methane is produced through acetic acid pathway[40].
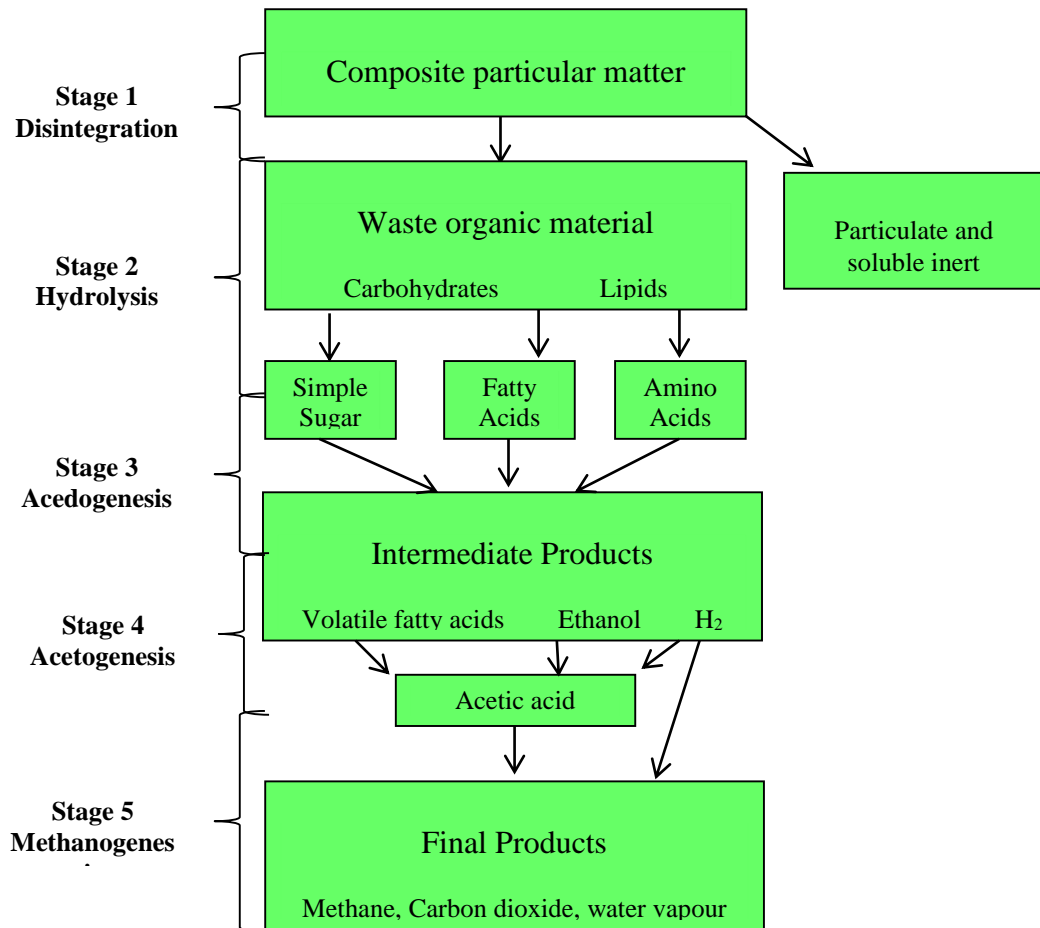
Figure 2.1: Conversion processes of anaerobic digestion

2.2.2 Anaerobic digestion of oxidized sulfur compounds

In biological sulfur cycle, sulfate is converted to sulfide through dissimilatory sulfate reduction. This conversion process takes place at strict anaerobic condition by sulfate reducing bacteria (SRB). Sulfate is the electron acceptor, whereas the organic compounds or the hydrogen acts as the electron donor.

SRB are unable to consume complex organic materials, but they use several intermediate products of anaerobic mineralization process. SRB consume substrates like molecular hydrogen(H_2), acetate, methanol, formate, propionate, butyrate, higher and branched fatty acids, lactate, ethanol and higher alcohols, fumarate, succinate, malate, alkanes and aromatic compounds[17]. It is been recorded that SRB also consume sucrose.

2.2.2.1   Sulfur reducing bacteria (SRB)

Sulfur reducing bacteria (SRB) activities were first discovered in 1895 by Beijerinck [46]. It was found that sulfate could be converted to sulfide through anaerobic respiration in sediments. This process is known as dissimilatory sulfate reduction and SRB owes to strict anaerobic micro-organisms.

Out of 40 genera of SRBs, 16 generas are incomplete oxidisers, 22 generas are complete oxidisers and the remaining 2 genera, Desulfotomaculum and Desulfomonile, do not exactly align with the characteristics of either groups, but express to have both complete and incomplete oxidizing species [47]. Usually the name of the SRB begins with "Desulfo". Desulfovibrio, Desulfomicrobium, Desulfohalobium and Desulfonatronum are some of the incomplete oxidizers. On the other hand, Desulfothermus, Desulfobacter Desulfobacula and Desulfofrigus are complete oxidizers. Most of these species are vibro, rod and curved in shape.

SRB are capable of surviving in wide spectrum of environmental conditions such as temperature range 0-100˚C, salinity from freshwater to sea water, pH range from 3-9.8 [48] and even in aerobic habitants[49], despite from their obligatory anaerobic metabolism.

2.2.2.2   Microbial pathways for sulfate reduction

In biological degradation process, two major microbial pathways can be identified. In the presence of sulfate, some SRBs produce CO_2, HCO_3^- and sulfide through complete oxidization. On the other hand, there are some other SRBs who produce intermediate products such as lactate, acetate and sulfide from partial oxidation. It has been reported that SRB has the capability of reducing sulfite and thiosulfate too. Nevertheless some SRBs, desufovibrio stains converts di, tri, tetra and thionate sulfur compounds to sulfide[9], [50]. Sulfate acts as an electron acceptor of this bacterial respiration. Electron donors are usually hydrogen and organic compounds with higher and branched fatty acids, ethanol and higher alcohols, other organic acids, alkanes and aromatic compounds [6]. The order of SRB affinity for substrate reduction is $H_2>$ propionate> other electron donors [51].

SRBs have the special ability of surviving through fermentative or acetogenic reactions by the time where even electron acceptors are not present [9]. SRBs easily

ferment pyruvate, lactate and ethanol. For example; desulfovibrio ferment lactate and ethanol, whereas desulfobulbus use propionate. But in the presence of sulfate, these microorganisms behave as true SRBs follow normal metabolic pathway, accepting propionate as electron acceptor, while reducing sulfate [6].

2.2.2.3 Competition between Sulfur reducing bacteria and Methane producing bacteria
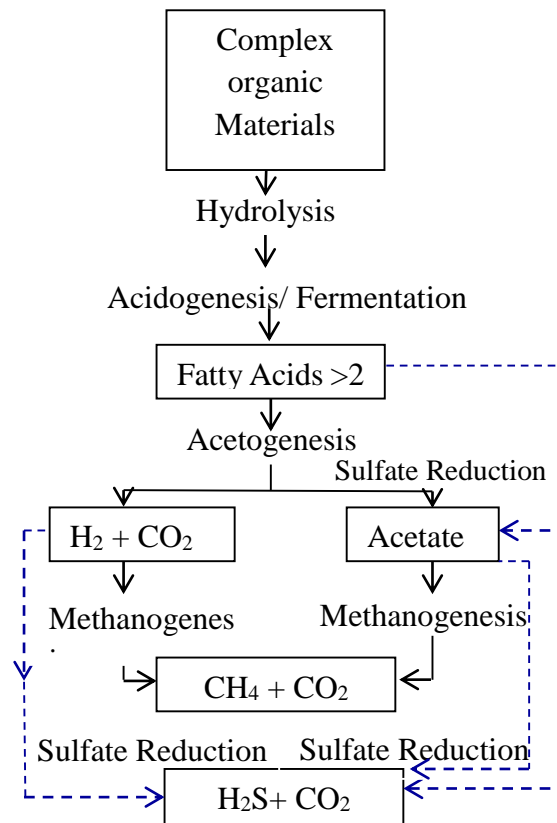
Figure 2.2:Microbial path ways of competition between MB and SRB for available substrate (Data extracted from Lens P.N.L.)

SRBs are not capable of degrading complex compounds and amino acids [52]. SRBs compete for intermediate substrates generated with acetogenesis and methanogenesis. When sulfate present in the wastewater in excess, SRB has to compete with acetogenic and methanogenic bacteria for the available substrate. This competition determines the final proportion of methane and sulfide produced. Main intermediate products identified in this anaerobic mineralization process are acetate, propionate, butyrate and hydrogen. When considering the competition between SRBs and methane producing

bacteria (MB), thermodynamically and reaction kinetically, SRBs are dominant in sulfate rich wastewater [9].The metabolic pathways of SRB and MB are summarized in Figure 2.2.

According to the standard Gibbs free energies (Table 2.2), at a condition where there is no sulfate limitation, SRB completely consume hydrogen whereas propionate and butyrate degrade faster by SRB than MB. In contrast, for acetate, either MB or SRB can be dominant as reported.

Apart from the Gibbs free energy and the kinetic reactions, there are some other factors which affect the dominancy of SRBs against MBs such as COD/SO_4^{-2} ratio, the type of substrate, the relative population and the characteristics of specific kinds of SRBs and other microorganisms , sensitivity for sulfide inhibition of each species, temperature and pH [42]. The COD/SO_4^{-2} ratio is a dominant factor in sulfate reduction. Thus, it will be discussed in next section.

Table 2.2: Stoichiometry of the anaerobic degradation by SRB and Gibb's free energy values at 37°C[9].

Reaction	$\Delta G°$ (kJ/mol)
Propionate $CH_3CH_2COO - +3H_2O \rightarrow CH_3COO^- +HCO_3^- + H^+ +3H_2$	+76.0
$CH_3CH_2COO^- +0.75SO_4^{2-} \rightarrow CH_3COO^- +HCO_3^- +0.75HS^- +0.25H^+$	-37.7
$CH_3CH_2COO- +1.75SO_4^{2-} \rightarrow 3HCO_3^- +1.75HS^- +0.5H^+ +0.25OH^-$	-88.9
Acetate $CH_3COO^- + H_2O \rightarrow CH_4 +HCO_3^-$	-31.0
$CH_3COO^- +SO_4^{2-} \rightarrow 2HCO_3^- +HS^-$	-47.6
Hydrogen $4H_2 +HCO_3^- + H^+ \rightarrow CH_4 +3H_2O$	-32.7
$4 H_2 +SO_4^{2-} + H^+ \rightarrow HS^- +4H_2O$	-38.1

2.2.2.4 The effect of COD/SO$_4^{-2}$ ratio

A. Sarti and M. Zaiai [13] explained that the division ratio of electron flow via sulfidogenesis and methanogens is mainly decided by the ratio of COD/SO$_4^{-2}$, but not the influent SO$_4^{-2}$ concentration. This hypothesis is demonstrated for hybrid reactors by Finnegan [12]. Further he suggested that increase in the COD/SO$_4^{-2}$ ratio can make a drastic change in the pattern of electron flow in a retained biomass in the hybrid reactor.

A. Rinzema and G. Lettinga has suggested that the optimum operating ratio of COD/SO$_4^{-2}$ for complete sulfate reduction is greater than ten times [14]. At this ratio, the minimum value of H$_2$S in the system would not ever exceed the critical threshold value of 150mg/l, in which sulfide inhibition occurs. Although it is regarded that the optimum COD/SO$_4^{-2}$ ratio is 10, there are some other researches carried out successively at lower ratios such as 8,5 and 3 respectively by Hilton and Archer [15], Mendez et al. [16], Derycke and Pypin [2].

C. Chen et. al. [7] has found that the sulfate reduction rate is increased from 54% when the COD/SO$_4^{-2}$ ratio is increased from 1:1 to 3:1 for 19 days HRT. Although there are several stoichiometric ratios of COD/SO$_4^{-2}$ found out for sulfate reduction, still the researchers are unable to find exact pathway or a clear explanation for the optimum stoichiometric COD/SO$_4^{-2}$ ratio for complete sulfate reduction [15]. Sulfide Inhibition. Sulfate is converted to sulfide by SRB at its anaerobic microbial degradation cycle. It has been discovered that un-dissociated H$_2$S causes the drastic inhibition. But the inhibition mechanism has still not been clearly explained in the literature. Some of the main reasons are that un-dissociated H$_2$S ions have the capability of diffusing through the cell membranes of micro-organisms and change the internal linkages, it interferes the assimilatory metabolic pathways or change the internal cell pH. Generally the methane production is known to be inhibited by 100-800mg/l of dissolved sulphides and 50-400mg/l of unionised H$_2$S [53]. Kroiss and Plahl-Wabnegg [54] reported that acetolactic methanogens is 50% inhibited by 50mg/l unionized H$_2$S and completely inhibited by 200 mg/l.

The quantity of H$_2$S present in the anaerobic reactor decides on the chemical and physical equilibrium as shown in Eq. (5) & Eq. (6).

$$H_2S(l) \leftrightarrow H^+ + HS^- \leftrightarrow 2H^+ + S^{-2} \qquad \dots\dots (5)$$

$$H_2S(aq) \leftrightarrow H_2S(g) \qquad \dots\dots (6)$$
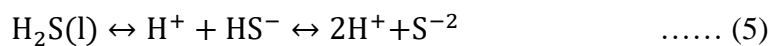
The reaction rates towards H2S ion formation are high in acidic pH levels whereas in high pH levels resultant HS- ion formation is high. Hence resultant products are affected by the pH of the wastewater and the temperature.

2.2.3 Anaerobic digestion of nitrogenous substances

Ammonia plays a vital role in the performance and stability of anaerobic digestion of nitrogenous organic matter rich wastewater. In anaerobic reactors, proteins are first hydrolysed to peptides and amino acids. Subsequently the amino acids are fermented and produce ammonia. Effluents from latex processing, fish canning and wastewater from poultry are some of the protein rich wastewater sources. Ammonia is an essential nutrient for bacterial growth. But it inhibits micro-organisms including methanogens, if it is available in high concentrations. Ammonia is regarded as a potential inhibitor during anaerobic digestion of protein rich wastewater.

2.2.3.1 Ammonia inhibition in Anaerobic Digestion

The two-principal form of ammonia in aqueous phase is Ammonium (NH_4^+) and Free Ammonia (NH_3). Both forms can directly and indirectly cause inhibition in an anaerobic digestion system, but free Ammonia (FAN) inhibit the micro-organisms the most above threshold concentration[55]. FAN concentration primarily depends on the TAN, pH and temperature. However, ionic strength is also considered as a significant parameter concentrated solutions[56].
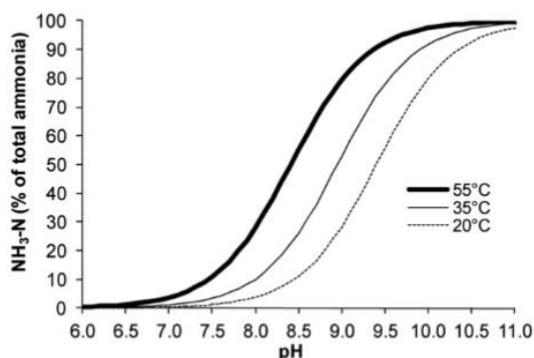
Figure 2.3:FAN as a percentage of TAN at temperatures 20, 35 and 55 °C Vs pH[23]

As shown in the Figure 2.3, FAN is less than 1% of total TAN at pH 7, whereas at pH 8 it has risen to 10% and at pH 9 it has increased to 48%. Thus the inhibition due to FAN also increased proportionately[22].

Nevertheless, FAN concentration at thermophilic temperatures is expected to be six times higher than under mesophilic condition with the same pH. Because the dissociation constant of ammonia nitrogen depends on the temperature. Free ammoniacal Nitrogen (FAN) of the system can be calculated following equation (7) given by Siles J.A. [57] and Rajagopalan R. et al.[23], using the TAN, pH and the operated temperature (35℃).

$$\text{FAN} = \text{TAN}\left(1 + \frac{10^{-pH}}{10^{-\left(0.09018 + \frac{2729.92}{T(K)}\right)}}\right)^{-1} \qquad\qquad \text{………(7)}$$

There are many pathways suggested for the ammonia inhibition, such as a change in intracellular pH of methanogens, increase energy requirement for cell maintenance and specific enzyme reaction. Existing knowledge contribution by Gallert C. and his team describes the ammonia inhibition to MB can be taken place in two pathways, (i) Ammonium ion may inhibit the methane producing enzymes directly and/or (ii) hydrophobic ammonia molecule may diffuse passively into bacterial cells, causing proton imbalance or potassium deficiency[23]. The literature on Ammonia inhibition to SRB are lacking, but it could be some similar mechanism. Further, Shanmugam P. and Horan N.J. proposed that acetate in the reactor are converted to Ammonium acetate or Ammonium bicarbonate. This phenomenon depletes the acetate which is the substrate for micro-organisms including SRB and MB. Thus, inhibits the biological activities in the AD reactor [58].

2.2.4 Controlling techniques for ammonia inhibition

There are several strategies for controlling the ammonia inhibition in an anaerobic digestion. Adequate choice of temperature, control of pH and influent C/N ratio and utilization of acclimatized microflora to higher ammonia concentrations are some of the ammonia inhibition controlling techniques utilized for stable and undisturbed anaerobic digestion.

2.3 Techniques to remove aqueous sulfide and gaseous hydrogen sulfide in biogas

The treatment methods available to treat high concentrations of sulfate wastewater is to anaerobically degrade the sulfate and let sulfate reduced to sulfide in the first step and removed formed sulfide using different methods. During the sulfate reduction in anaerobic reactors, gaseous hydrogen sulfide released. Thus, when effluent sulfate concentrations are decreased in AD reactors, toxic H_2S is released. Sulfide removal mechanisms are of two categories. Concentration of aqueous sulfide ions are controlled by direct liquid phase treatments or use of a separate gas phase treatment unit to remove gaseous hydrogen sulfide.

Table 2.3: Chemical compounds used in aqueous sulfide precipitation

Type of treatment	Description
Physio-chemical	Chemical precipitation using digester slurry (Addition of metal ions such as Zinc, Iron and copper)
	Adsorption using Activated carbon, Iron oxide (Iron sponge, Sulfa-rite, Sulfa-treat), Molecular sieve, Zinc oxide, Alkaline solids
	Absorption using Water, physical solvents without water, alkaline solutions, Zinc oxide slurries, Iron oxide slurries, Iron salt chelated or not chelated, Quinone and Vanadium salts, Chemical oxidants (H_2O_2, $KMnO_4$, Hypochlorite), Amines
	Membrane purification
	Clause process
	Incineration
Biological	Bio filter, Bio trickling filter
	Bio scrubbers
	Air/Oxygen dosing to the digester

The physical, chemical and biological processes for gaseous H_2S or liquid phase sulfide removal are summarized in Table 2.3. Considerable energy requirement, high operation, labour and maintenance cost, high chemical and disposal cost, disposing the spent chemicals are the other major drawbacks of these methods. Therefore, biological techniques are more preferred via chemical and physical methods.

There are systems developed for integrated simultaneous desulfurization and denitrification also but the principle and technique behind such units are different. Y. Yuan et al. [59] as well as C. Chen[60] et al. presented, in such systems, one compartment sulfate was reduced to sulfide while in another compartment of the same reactor the ammonium was converted to nitrate at higher DO levels. Then the nitrate and sulfide let to react in another third reactor to form elemental sulfur as per Eq (8).

$$S^{2-} + NO_3^- + H_2O \rightarrow S^0 + NO_2^- + 2OH^-$$…………………. (8)
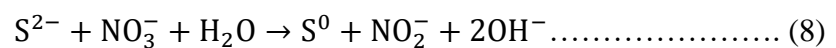
2.4 Micro-aerating Anaerobic digester for simultaneous sulfate and Hydrogen sulfide removal

Currently, supplying air or oxygen in micro level to Anaerobic digester is becoming more popular. As explained earlier in Chapter 1, Conversion of sulfate to elemental sulfur is a two-step process. Sulfate is broken down to sulfide by Sulfur Reducing Bacteria (SRB) in the first step and generated sulfide are converted to elemental sulfur by Sulfur Oxidizing Bacteria (SOB).

There are two different process configurations followed when reducing sulfate to sulfide and remove formed sulfide transforming into elemental sulfur via micro-aeration. However, Sulfate reduction under anaerobic condition and sulfide oxidation in micro aerophilic condition can be achieved using two separate reactors or using single reactor which integrate both the sulfate reduction and sulfide oxidation phenomena. However, in single stage micro aerated anaerobic reactors, micro-aeration condition or the DO concentration have to be always at controlled level to not to inhibit SRB. Both process configurations are shown in Figure 2.4. The sulfate reduction and sulfide removal in single reactor is becoming more popular nowadays as it is more economical. More detail information is presented in section 2.7 in this regard.
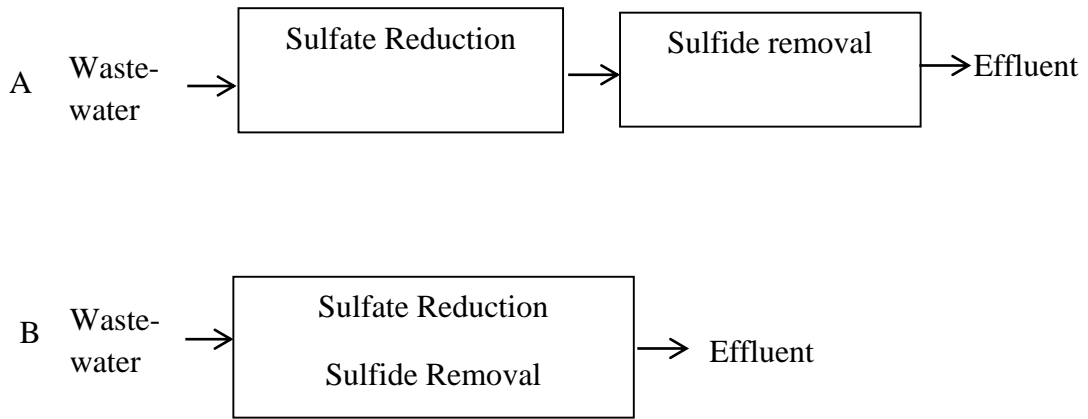
Figure 2.4: Different process configurations for influent sulfate reduction and sulfide removal

2.4.1 Biological Sulfide oxidation process

Biological sulfide oxidation in wastewater treatment is generally conducted by colourless sulfur oxidizing bacteria. These bacteria generate energy from the reactions given below (Equation (3) and Equation (4)) for their survival. With the oxygen supply under control level, sulfide is converted to elemental sulfur. On the other hand, if the level of oxygen is high, it will further oxidize to sulfate [61], [5].

SOB recorded to be existed in head space of the reactors as well as in the gas-bulk liquid inter phase of the micro-aerobic reactors[29], [19],[62]. Chemolithotroph SOBs are the mostly found microorganisms in the micro-aerobic reactors which utilise oxygen or nitrate or nitrite as electron acceptors. As reported by Tang et al. SOB have the ability to survive in the pH range of 1-9 and temperature of 4 to 90°C[63].

2.4.2 Simultaneous Sulfate reduction, Sulfide oxidization and micro aeration in single reactor

The process technique of supplying control amount of oxygen is defined as the introduction of small amount of oxygen which is less than the oxygen requirement for complete aerobic degradation[64]. It enables both anaerobic and micro-aerophilic biological activities to occur within a single bio reactor. In micro-aeration, all the

oxygen supplied are almost all consumed. Thus, the DO level of the medium always becomes nearly zero under continuous oxygen or air supply.
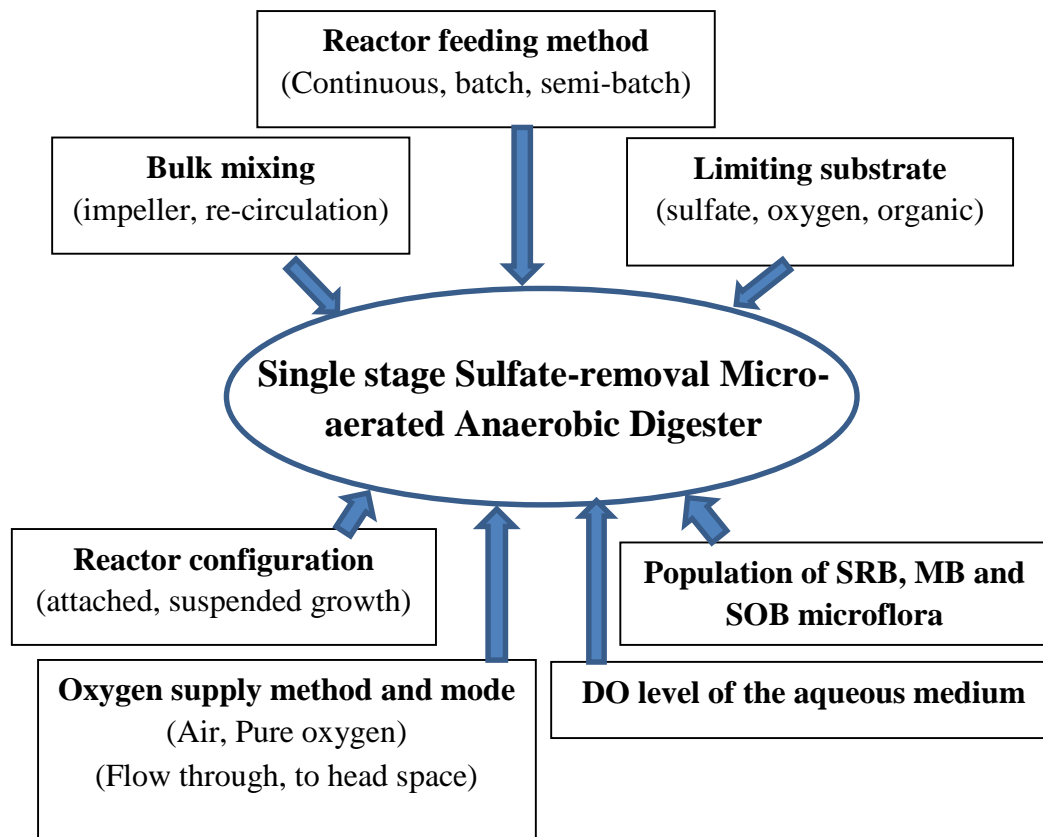
Figure 2.5:Main parameters affect SRB and SOB integrated reactor

Although SRBs and MBs are considered as strict anaerobes they are also aero tolerant up to some extent [61]. Therefore, they could survive in micro-aerophilic environments. A.T Kato and his team[65] explained the aero tolerance of microorganisms depends on the amount of superoxide dismutase [65]. Whereas Song and Logan explained that the rapid oxygen consumption of facultative or micro aerophilic organisms shield the other anaerobic organisms by scavenging on dissolved oxygen. Other hypothesis narrated by Shen C.T. et al. is that the steep oxygen gradient present in microbial aggregates such as flocks, granules and biofilms prevent the oxygen diffusion in to the aggregates [66]. Hence the anaerobes are shielded inside the granules, while facultative or aerobic organisms live closer to the surface[67].

There are several factors which affect the micro-aerobic reactor as shown in Figure 2.5, and researches have already carried out investigations. Some of the important findings are mentioned as below. The desired micro-aeration condition inside integrated SRB and SOB reactor for major end product of sulfide oxidation to be elemental sulfur generation is oxygen concentration below 0.1 mg/l[29]. Beyond 0.1 mg/l oxygenation level sulfate will be the major end product and obligate anaerobic bacteria will be inhibited with oxygen. In the past, micro aeration for sulfide removal has taken place in different oxygenation levels and in various types of reactor configurations. There are many advantages using this method in the industry. The capital cost and operation cost are minimum with investment on only one reactor than two, no chemical usage and no need of external biogas upgrading units.

Micro-aeration of anaerobic reactor, not only remove sulfide, but also reported that it enhance the hydrolysis process and increase the production of methane as well as significant reduction in COD[68]. D.H. Zitomer and J.D. Shrout [69] has discovered that COD removal increased from 25% to 87% for an aerated Fluidised Bed Reactor (FBR) than strictly anaerobic FBR for high sulfate and high COD wastewater. Polanco M. Fdz.[70] was able to achieve >99% [70] hydrogen sulfide in biogas by micro-aerating a anaerobic CSTR reactor with little or no effect on COD removal, biogas production or methane yield. He used sludge recirculation and biogas circulation as the mixing mechanism and observed that there is no effect on H_2S removal in biogas under micro-aerobic conditions.

2.4.3 Effect of O_2/S ratio on sulfate reduction and elemental sulfur formation

Although, initially the main focus of research and applications was to reduce H_2S in biogas using micro-aeration technique, recently it has been more concentrated to recover elemental sulfur for reuse. Theoretically 0.5 mol O_2 /mol S^{-2} is required for oxidation of sulfide to elemental sulfur as per Eq(1)[71] . In 1995 Janssen et al. were able to gain maximum sulfur recovery of 73±10% at O_2/S^{-2} ratio of 0.6 to 1.0 with 0.7 as the optimum. S. Alcantara et al.[72] found that elemental sulfur production at steady state was achieved at O_2/S^{-2} ratio ranging from 0.5 to 1.5, while maximum sulfur recovery of 85% was occurred at O_2/S^{-2} ratio of 0.5 while all the elemental sulfur was completely transformed to sulfate at O_2/S^{-2} ratio of 2. Munz et al. [29] observed

contradictory results to existing findings and it was observed that 91, 87 and 85% of sulfide conversion to elemental sulfur at O_2/S^{-2} ratios of 0.0015, 0.005 and 0.03.

2.4.4 Effect of Ammonia on Sulfate reduction and Sulfide oxidization

There are only very few research studies conducted for SRB and SOB in ammonia rich environment. Micro-aeration was carried out in by F. Straka and his team for both sulfate and nitrogenous compound rich wastewater, i.e. pig manure and H_2S in the biogas was decreased from 4000 mg/m^3 to 220 mg/m^3[24]. However, he has found that the ammonium nitrogen of the reactor increases steeply from 1.2 g N/l at 7.0-7.35 to 3.0 gN/l at 8.5-9.0 reducing the methane production from 15-20% down. Thus, he suggested that for micro-aerophilic system even ammonia inhibition can be minimized by initially increasing the C/N ratio higher than 10 by co-digestion with low ammonia waste and reducing the loading rate. W. Mulbry et al. also utilized miro-aeration successfully in plug flow reactors to reduce H_2S from 3500ppm to less than 100 ppm, for diary waste which is another protein rich waste, but analysis were not carried out on nitrogenous compounds[33]. Basically, from the previously reported literature it is evidenced that, sulfate reduction and sulfide oxidation are possible inside single reactor whereas protein in the wastewater breakdown to ammonia.

J.A. Siles and his team observed both sulfate reduction and protein breakdown to ammonia had taken place in a single complete anaerobic reactor whereas higher concentrations of FAN greater than 620 mg/l and influent sulfate concentrations greater than 1400 mg/l affected methanogenic bacteria indirectly inhibiting all the biological processes inside the reactor as both sulfate reduction and protein breakdown require partially degraded simple organic matter after acidogenic state for its biological conversion process. On the other hand the threshold values observed were C/N and C/SO_4^{-2} ratio of 4.4 and 1.6 [73].

Therefore, control of COD/SO_4^{-2} ratio and C/N ratio is an important factor for biological degradation. Kizilkaya and Bayrakli[73] found that the optimum C:N ratio was 25-30:1, but this ratio can be often lower or higher than this ideal value. It has been found that C:N ratio for sewage sludge is 9:1. However minimum COD/SO_4^{-2} ratio suggested was 10:1 by P. Hulshoff et al.[74] whereas at lower ratios, the biological AD are inhibited by H_2S.

SRB are strict anaerobic bacteria, thus inside microaerobic reactors, the desired DO concentration must be below 0.1mg/l for both SRB and SOB biological conversion process to take place. As there is ammonia inside the reactor, it is reasonable to suspect that ammonia might be nitrified to nitrate or nitrite, but nitrification of ammonia to nitrate would only take place in higher DO concentrations 1 mg/l than desired micro-aeration concentration for elemental sulfur formation which is less than 0.1 mg/l [75] or 0.10-0.12 mg/l [18] and at DO greater than 0.3 mg/l SRB were inhibited with completely failure of the integrated SRB and SOB reactor[18]. S. Luostarinen et al. [25] found out that at least 1-2 mg/l require for nitrification whereas B. Rusten et al. [26] explained for complete nitrification 2-3.5mg/l required in MBBR reactor. 2 ± 0.83 mg/l was maintained in the partial nitrification and anaerobic ammonia oxidation process in a single reactor[27]. However, Z. Zheng and his team[28] has found that at 3.50, 1.45 and 0.7 mg/l the inorganic nitrogen removal with nitrification were 93.4%, 87.5% and 92.7%. Therefore, it can be concluded that in the range of DO less than 0.1 mg/l, ammonia nitrification is impossible.

3 MATERIALS AND METHODS

3.1 Introduction to Experiments

Experimental methods conducted are presented at the beginning of this chapter followed by analytical methods. Developed experimental strategy to meet the expected objectives of the research is illustrated in Figure 3.1.

Experiments on Enhancement of SO_4^{-2} reduction

A. The effect of pH and external electron donor on mesophilic sulfate reduction during start-up period of Anaerobic reactor treating skim latex wastewater

B. Effect of pH and electron donor on sulfate reduction in Ammonia rich Anaerobic conversion

C. Effect of influent volumetric loading on sulfate reduction of anaerobic reactor treating skim latex wastewater

D. Effect of type of electron donor on sulfate reduction using synthetic wastewater

Enhancement of sulfide Oxidation to elemental sulfur

E. Effect of micro-aeration method on simultaneous sulfate reduction and elemental sulfur formation of synthetic wastewater

F. Investigate the effect of O_2/S ratio on elemental sulfur formation and sulfate reduction in Single-stage Sulfate-removal Micro-Aerobic Digester(SSMAD) feeding synthetic wastewater

G. Investigate the effect of O_2/S ratio on elemental sulfur formation and sulfate reduction in Single-stage Sulfate-removal Micro-Aerobic Digester(SSMAD) feeding skim latex wastewater

Figure 3.1: Experimental Strategy

3.2 Methodology

3.2.1 SLW sample collection and preservation

Although there are several centrifuged latex factories together with skim latex processing in Sri Lanka, latex processing factory located at Kalutara district was selected for sample collection.

The samples were collected after the final rubber trap as shown in wastewater generation flow diagram in Figure 1.1. which is the influent to wastewater treatment facility.

High concentrated wastewater discharged from the skim latex coagulation tanks and the wash water used in various other operations of the concentrated latex factory ultimately collected to this final rubber trap. Since the final rubber trap is large in size, it serves as equalization tank and the concentrated skim latex rich in both protein and sulfate get diluted to some extent. The samples were collected after this final rubber trap (Equalization tank). The collected samples were stored below 4℃, until it was used for experiments.

3.3 Effect of pH and external electron donor on mesophilic sulfate reduction during start-up period of Anaerobic Digester treating SLW (Experiment A)

Semi-continuously fed experiment was conducted to investigate the sulfate reduction and gaseous Hydrogen sulfide emission of natural SLW under complete anaerobic condition. Parameters such as TAN, COD, VFA and volumetric biogas production were closely monitored. Nevertheless, effect of sulfate reduction with increasing influent COD/SO_4^{-2} ratio was also investigated with this experiment.

3.3.1 Experimental setup

An airtight glass vessel was used as a completely mixed anaerobic reactor. The temperature of the anaerobic reactors was constantly maintained at mesophilic (35℃) temperature, circulating warm water inside stainless-steel coil which was installed inside the reactor. The warm water reservoir was an external water bath which is capable of maintaining its temperature at 42℃. The temperature controlling system

was designed to sense the temperature inside the reactor and automatically control the external submersible warm water circulation pump to maintain the temperature inside the reactor at 35 ±1 ℃. Two similar reactors were used to duplicate the experiment.
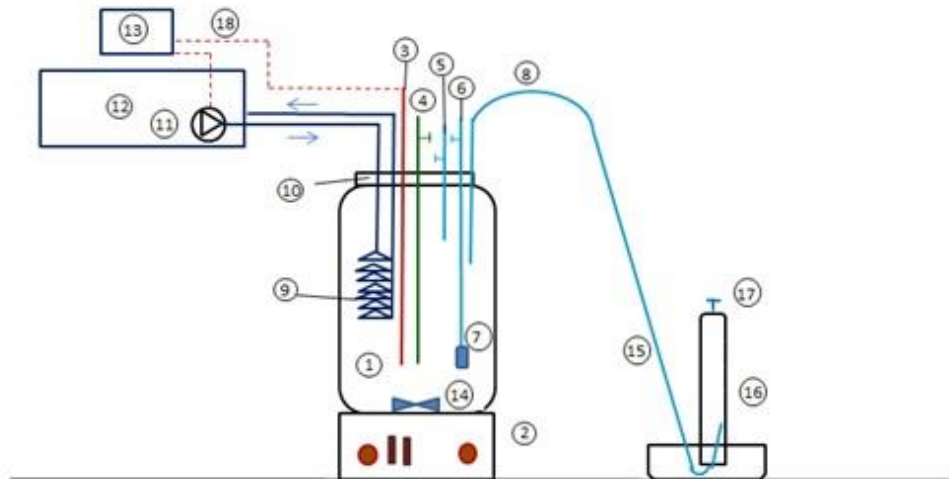
(1) Anaerobic reactor (2)Magnetic stirrer (3)Temperature probe (4)Sample inlet/outlet (5)Gas sampling outlet-1 (6)Gas flow inlet for micro-aeration (7)Diffuser (8)Clear horse for gas flow out (9)Stainless steel hot water circulation coil (10)Nylon top lid (11)Hot water bath (12)Submersible pump (13) Temperatures control unit (14)Magnetic stirrer (15)Clear horse for gas flow out (16)Gas collecting inverted measuring cylinder (17)Gas sampling outlet-2 (18) In/out signal from temperature control unit

Figure 3.2:Schematic diagram of the experimental setup

The working volume of the anaerobic reactor was 2.5L with 500ml head space. There were four outlets on the top of the reactor. One was to feed influent feedstock and remove effluent sample for analysis. The second outlet was to insert the temperatures probe. Third outlet was to collect gas sample for gas composition analysis when required. The fourth outlet was to connect the generated biogas to collect in a 1-litre inverted measuring cylinder with water displacement method. The pH of the water inside the inverted cylinder was maintained less than 2 using HCl, to minimize the gas dilution in the water including CO_2 in the biogas as J.A. Siles [73] et al. applied in their experiments. There was an additional gas sampling outlet at the top end of the measuring cylinder. The schematic diagram of the experimental setup is shown in

Figure 3.2. The anaerobic reactor was placed on top of the magnetic stirrer. Hence the mixing was achieved by magnetic rod placed at the bottom of the reactor.

Figure 3.3: Experimental set up before starting-up Experiment

3.3.2 Acclimation of the reactor

The inoculum used for this reactor was sludge taken from a well operating anaerobic reactor in wastewater treatment facility of skim latex processing industry. Inoculum was filtered through a 0.5mm size filter in order to remove any bulky particulates. Then, 800ml of inoculum and 800ml of raw SLW transferred into the reactor, Nitrogen gas was purged for about 20 minutes until the aqueous dissolved oxygen and head space oxygen depleted. Then 100 ml of natural SLW sample was fed daily during initial acclimation period until the reactor volume reaches 2.5 L. After the initial acclimatization period, the reactor was semi-continuously operated by removing 83 ml sample from the reactor and feeding the same volume of sample once in two days during initial start-up period.

Experiments were conducted using natural SLW. The characteristic of the wastewater fed to the reactor is given in Table 3.1. The tCOD value and the pH of the influent were adjusted according to the requirement of each phase of the experiment. The collected natural SLW was stored at 4°C until used, in order to minimize self-biodegradation[76].

Table 3.1:Characteristics of Influent Natural Skim Latex wastewater

Parameter	Value
pH	5.9
tCOD /(mg/l)	2662
sCOD /(mg/l)	2622
Sulfate /(mg/l)	950
Sulfide /(mg/l)	0
Total Ammoniacal Nitrogen /(mg/l)	210
Total Solid /(mg/l)	1220
Total Suspended Solid /(mg/l)	40
Total Dissolved Solid /(mg/l)	1120

3.3.3 Experimental Procedure

Laboratory experiment A was conducted using SLW. This experiment was conducted in 3 phases as described in Table 3.2. During the phase 01, the influent COD/SO_4^{-2} ratio was only 2.8(~3.0) which was the natural COD/SO_4^{-2} ratio existed in natural SLW, without any adjustment. However, in phase 02 and 03 experiments, the COD/SO_4^{-2} ratio was increased to 10 using one of the external electron donors, 3M acetic acid solution. The influent pH was adjusted using 3M Hydrochloric acid solution.

The anaerobic reactors were semi continuously operated, feeding 83ml of natural SLW once in two days. After six weeks of initial acclimation period, the reactors are used for this experiment. This experiment was used to observe the sulfate reduction during initial stage of acclimation.

Table 3.2:Parameters maintained in influent in each phase

Phase	Influent COD/SO_4^{-2} ratio (g/g)	Influent pH
I	2.8	7
II	10.0	7
III	10.0	3

3.3.4 Parameters measured

Influent and Effluent of the reactors were analysed for pH, Oxidation Reduction Potential (ORP), sCOD, aqueous SO_4^{-2}, Total Dissolved sulfide [S^{-2}(aq), HS^-(aq), H_2S(aq)] concentration, Gaseous Hydrogen sulfide composition, Total Ammoniacal Nitrogen (TAN), total VFA, component wise VFA and Volumetric biogas quantity. Gas volume was measured from the water displacement through an inverted 1litre measuring cylinder. All other parameters were measured according to the standard method given by American Public Health Association (APHA) and adapted from Standard methods for the examination of water and wastewater. All analytical methods used in experiment are in-detail explained in section 3.10.

3.4 Effect of pH and electron donor on sulfate reduction in ammonia rich Anaerobic conversion (Experiment B)

This research study investigates the quantitative sulfate reduction and hydrogen sulfide emission of SLW under anaerobic condition and influence of pH and addition of external electron donor on improvement of sulfate reduction under high ammonia concentrations. Under this experiment, effect of Ammoniacal nitrogen formation under anaerobic condition and effect of inhibitory compounds like Total Dissolved Sulfide and FAN concentrations on sulfate reduction, anaerobic degradation of other major constituents such as COD, VFA and volumetric biogas production were carried out.

Further it was observed that the inhibition was high when acclimation of the reactors carried out using SLW from the results of experiment A. Therefore, during this experiment, two reactors were acclimated using synthetic wastewater, which provided additional nutrient to accelerate microbial growth.

3.4.1 Experimental Setup

Completely mixed, identical 3-litres reactors were used in the experiment and temperature was controlled at mesophilic temperature, 35 ±1 ℃. The working volume of the reactors were 2.5 litres. The reactors were placed inside a water bath in which temperature was automatically controlled with a temperature control system. Temperature distribution was carried out evenly with a submersible wave making devise. The mixing was achieved by placing a magnetic stirrer under the water bath, directly coincident with the glass reactors on top.

There were three outlets on the top of the reactor. One was to feed influent feedstock and remove effluent sample for analysis. The second outlet was to connect the generated biogas to collect into a 1 litre inverted measuring cylinder with water displacement method. The schematic diagram of the experimental setup is as in Figure 3.4.
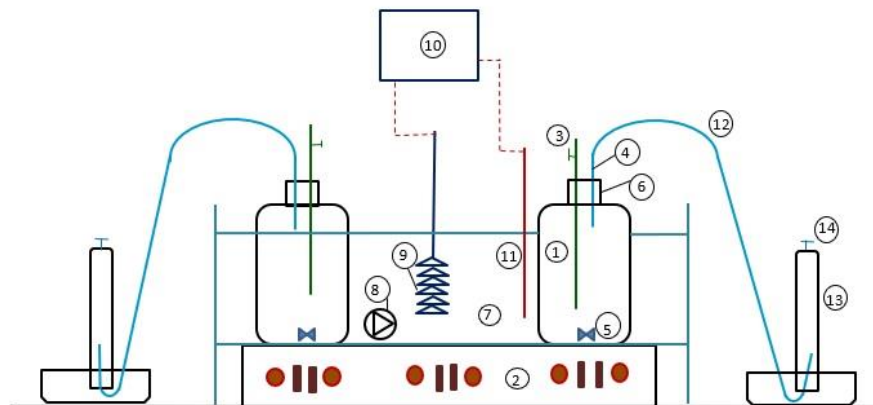
(1) Anaerobic reactor (2) Magnetic stirrer (4) Sample inlet/outlet -1 (5) Magnetic stirrer rod (6) Rubber cork (7) Hot water bath (8) Submersible wave maker (9) Water heater (10) Temperatures control unit (11) In/out signal from temperature (12) Clear horse for gas flow out (13) Gas collecting inverted measuring cylinder (14) Gas sampling outlet-2

Figure 3.4: Schematic diagram of the AD reactor setup

Figure 3.5: Experimental Setup

3.4.2 Acclimation of the reactors

Identical two reactors R_1 and R_2 described above were fed with 800ml of sludge as inoculum obtained from anaerobic reactors of SLW treatment plant located at Kalutara district, Sri Lanka. Nitrogen gas was purged for 20 minutes to remove dissolved oxygen and undesirable dissolved gasses. At the beginning, the reactors were fed with 100ml synthetic medium daily until the bulk volume reached 2500ml. After that the reactors were fed semi continuously, and 83 ml volume of mixed liquor was removed, and equivalent volume of synthetic wastewater was fed for three months' period for better microbial growth. Then 83 ml of natural SLW was fed with once in two days for about another three months to adapt the reactors for natural SLW before using for this semi-batch experiment. This procedure was carried out to provide sufficient nutrients for the microbes to grow well and to prevent the sludge washed-out before the reactors are well acclimated. Feeding pattern of initial acclimation period and the start-up period are as shown in the Figure 3.6(a) and Figure 3.6(b).
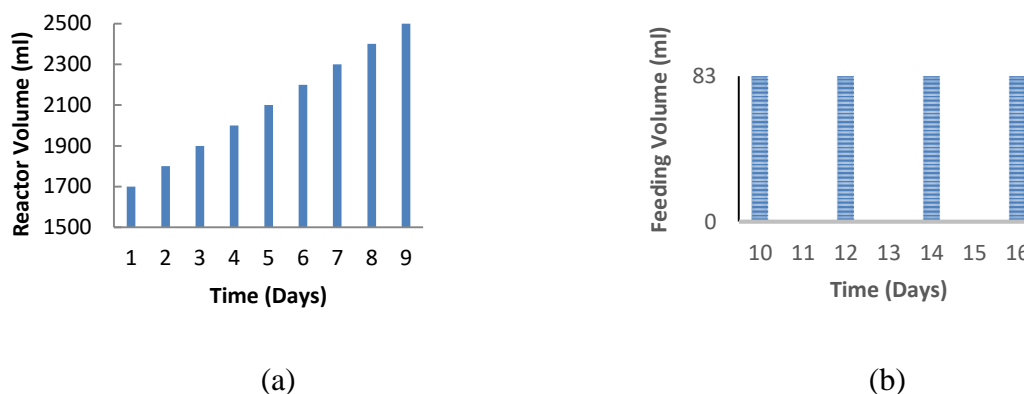
Figure 3.6: (a) Reactor volume during initial acclimation period (b) Feeding volume vs time during startup period

3.4.3   Substrate and nutrient medium for acclimation

When reactors were acclimated using natural skim latex wastewater, reactor failure occurred as per the experience of experiment A and B. This was due to unacclimated microorganisms unable to withstand high ammonia concentrations. Thus, synthetic media made of Acetic Acid and Sodium sulfate was used for acclimation of inoculum. Because microorganism easily grow on synthetic media composed of simple organic compound and sufficient nutrients. Initial COD/SO_4^{-2} ratio also kept at 10. According to A. Visser [77] 60ml of Basal nutrients and 10 ml of Trace Nutrients were mixed with 1 litre of base solution prepared using acetic and Sodium sulfate. The composition of basal nutrient solution consists NH_4CL (174 g/l), KH_2PO_4 (28g/l), $(NH_4)_2SO4$ (28 g/l) and KCL (45 g/l). Whereas the composition of the trace nutrient solution is $FeCl_2$ (2000mg/l), $MnCl_2$(500 mg/l), EDTA (500mg/l) H_3BO_3 (50 mg/l), $AlCl_3$ (50mg/l), $CuCl_2$ (50mg/l), $(NH_4)_6Mo_7O_{24}.4H_2O$ and HCL 36% (1ml/l)[77].

After the basal and trace nutrient solutions were prepared, it was autoclaved for 2 hours separately for sterilization before mixing with the base media made of acetic and Sodium sulfate. After synthetic wastewater solution was prepared, it was stored less than 4°C temperature until fed to the reactor.

3.4.4   Substrate for the experiments

Experiments were conducted using Natural Skim Latex wastewater. The characteristics of the wastewater fed to the reactor is shown in Table 3.3. The collected

natural SLW was stored at 4° C until used, in order to minimize self-biodegradation[76].

Table 3.3:Characteristics of the natural Skim Latex wastewater

Parameter	Value
pH	5.71
BOD/ (mg/l)	1590
tCOD/ (mg/l)	7965
sCOD /(mg/l)	7372
Sulfate/(mg/l)	2950
Sulfide/(mg/l)	4.4
TAN/(mg/l)	725
TKN/(mg/l)	820
Total suspended solid/(mg/l)	310
Total Dissolved Solid/(mg/l)	3220

3.4.5   Experimental Procedure

This experiment was performed in four phases using semi-batch fed AD reactor with feeding cycle time of 6 days. However, for all four phases, 125 ml of SLW was used with influent sulfate concentration of 2.95 kg-SO_4^{-2}/m^3. The natural SLW contains COD/SO_4^{-2} ratio of 2.7 (~ 3). During phase I, natural SLW with COD/SO_4^{-2} ratio of 2.7 was anaerobically digested without controlling the reactor pH, whereas in phase II, III and IV the pH of the reactors was controlled at range of 7.5-8.0. The influent COD of the natural SLW was increased to COD/SO_4^{-2} ratio of 5 and 10 in phase III and phase IV using acetate as electron donor. Operating conditions of each phase is summarized in Table 3.4. 3M Acetic acid solution was used to adjust the influent COD/SO_4^{-2} ratio, while 3M HCl was used to adjust the pH of the rector.

Table 3.4:Operating condition of the experiment

Phase	Influent COD/SO$_4^{-2}$ ratio	pH control status
I	2.7	not controlled
II	2.7	7.5-8.0
III	5	7.5-8.0
IV	10	7.5-8.0

3.4.6 Parameters measured

Measured parameters are same as parameters measured and in-detail explained in section 3.3.

3.5 Effect of influent volumetric loading on the sulfate reduction of anaerobic reactor treating SLW (Experiment C)

The objectives of this experiment were to investigate the effect of influent volumetric loading on the sulfate reduction and on the Ammonia inhibition.

3.5.1 Experimental Setup

Completely mixed, identical 3-litres reactors were used in the experiment and temperature was controlled at mesophilic temperature, 35 ±1 ℃. The working volume of the reactors were 2.5 litres. Experimental setup was similar to the setup described and used in Section 3.4, but these two reactors were acclimated and operated for 6 months.

3.5.2 Substrate for semi-batch experiments

Laboratory experiments were conducted using skim latex wastewater. The characteristics of this wastewater are shown in Table 3.5. The tCOD and the sulfate concentration of the wastewater collected from the skim latex processing factory was 8228 mg/l and 3009 mg/l respectively. Therefore, the influent COD/SO$_4^{-2}$ ratio was

2.7 (~3), whereas the COD/TKN ratio was 10.1. Then the influent COD/SO$_4^{-2}$ ratio was adjusted to 5 using 3M acetic acid which automatically change the COD/TKN ratio to 18.5. pH of the sample was reduced to 3.00 at the beginning of the experiment. Then pH of the reactors was not controlled.

Table 3.5:Characteristics of Influent skim latex water

Parameter	Concentration
pH	5.12
tCOD/(mg/l)	8228
SO$_4^{-2}$/(mg/l)	3009
TAN/(mg/l)	459
Kjeldahl Nitrogen/(mg/l)	815

3.5.3 Experimental Procedure

Experiment was conducted in three phases and the feed volumes were varied to increase the influent volumetric loading to the AD reactor. The feed volumes of respective phases were 83 ml, 125 ml and 250 ml. Thus, the influent sulfate loading of the reactor vary as 0.1 kgSO$_4^{-2}$/m^3.d, 1.5 kgSO$_4^{-2}$/m^3.d and 3.0 kgSO$_4^{-2}$/m^{3}d respectively. In order to maintain constant reactor bulk and head space volumes, equal volume of mixed liquid was first removed by a syringe and replaced with same volume of substrate into reactor. From the starting day of the experiment, samples were taken out for analysis and the semi-batch fed reactor was operated until the sulfate concentration and the gas production were zero. Then reactor was fed using respective sample as mentioned earlier. Similar procedure was carried-out for all the feed volumes. From the daily sample collection,10ml was taken out for analysis. The summary of the influent sulfate loadings of three phases are shown in Table 3.6.

Table 3.6:Conditions of three phases of the experiment

Sample	Sample size (ml)	Influent volumetric loading (l/m^3)	Sulfate loading (kgSO$_4^{-2}$/m^3.d)
VL 01	83	33.2	0.10
VL 02	125	50.0	0.15
VL 03	250	100.0	0.30

3.5.4 Parameters measured

Daily sulfate, total dissolved sulfide, gaseous H$_2$S concentration, volumetric gas production, total dissolved Ammonia concentrations, pH and ORP were monitored and recorded. The analytical methods used for the experiment are explained under section 3.10.

3.6 Effect of type of electron donor on sulfate reduction using synthetic wastewater (Experiment D)

The main objective of this experiment was to find out the effect of type of electron donor, complete oxidizer and partial oxidizer on the sulfate reduction. As explained in-detail in section 2.4.2, complete oxidizers convert into CO_2, HCO_3^- while sulfate reduced to sulfide. Whereas partial oxidizers turn in to intermediate products such as lactate and acetate while sulfate turn in to sulfide. It was found that for SLW the optimum COD/SO$_4^{-2}$ ratio was 5. Thus, the prepared synthetic wastewater with COD/SO$_4^{-2}$ ratio of 3 was further increased to 5 using two types of electron donors, Acetate as the complete oxidizer and Ethanol as a partial oxidizer. Ethanol is known to be a partial oxidizer because at the sulfate reduction process, sulfate converted to sulfide, while ethanol transforms to acetate, not for carbon dioxide.

3.6.1 Experimental Setup

Completely mixed, identical 3-litres reactors were used in the experiment and temperature was controlled at mesophilic temperature, 35 ±1℃. The working volume of the reactors were 2.0 litres whereas the head space was 1 litre used in this study. Experimental setup was same as the setup described and used in Section 3.3. These two reactors were acclimated and initially operated for about 8 months before using for this experiment to allow the micro-organisms to grow well. Feeding and removing 100 ml of sample of synthetic wastewater was conducted once in two days.

3.6.2 Substrate for the experiment

Experiments were conducted using synthetic media made of Acetic Acid. Sulfate concentrations were adjusted using Sodium sulfate. According to A. Visser[78] 60ml of Basal nutrients and 10 ml of Trace Nutrients were mixed with 1 litre of base solution prepared using acetic and Sodium sulfate. The composition, preparation method and the storage of basal nutrient solution and trace nutrient solutions were same as mention in section 3.4.

Synthetic wastewater was developed to contain same COD/SO_4^{-2} ratio compatible with skim latex wastewater. An assay of 3M acetic acid was diluted with varying volumes of distilled water and corresponding volume ratio required to achieve the COD of 9950 mg/l. Then the sulfate concentration of the prepared synthetic wastewater mixture was adjusted to 3315 mg/l. COD of the prepared synthetic wastewater was increased to achieve COD/SO_4^{-2} ratio of 5 using acetic and Ethanol and two synthetic wastewater mixtures were developed. Finally, the influent pH was adjusted to 3.

3.6.3 Experimental procedure

Each anaerobic reactor was fed with 100 ml of above mentioned pre-prepared synthetic wastewater mixture and the COD/SO_4^{-2} ratio was adjusted by Acetic acid and main parameters as, sulfate, TDS, Gaseous H_2S concentration, volumes of biogas and CH_4 and CO_2 concentrations in biogas were observed for 5 days including sulfate. Then same procedure was carried-out for synthetic wastewater mixture with the COD/SO_4^{-2} ratio of 5 which had already been adjusted by ethanol. From the reactor bulk liquid, 10ml sample was taken out daily for analysis.

3.6.4 Parameters measured

Measured parameters were same as parameters measured and explained in experiment 3.3, except VFA concentration and sCOD were not measured during this experiment.

3.7 Effect of micro-aeration method on simultaneous sulfate reduction and elemental sulfur formation of synthetic wastewater (Experiment E)

The most suitable air feeding mechanism for sulfate fed semi batch operated micro-aerobic reactors were studied during this experiment. The air was supplied using several air feeding techniques and sulfurous compounds in the micro-aerobic reactors were analysed. The experiment was conducted in four phases, completely anaerobic phase and another three phases with three different air feeding mechanism utilizing synthetic wastewater. Under this study, the most suitable air feeding mechanism for semi-batch fed reactor for optimal sulfate to elemental sulfur conversion was investigated. The variation of sulfurous compounds inside the micro-aerobic reactors with different air feeding mechanisms were investigated in-detail.

3.7.1 Experimental Setup

Completely mixed, identical two 3-litres reactors were used in this experiment. The reactors were operated semi-batch with 2.0 L working volume and 1.0 L head space in this study. An airtight glass vessel was used as completely mixed reactor. The temperature of the anaerobic reactors was constantly maintained at mesophilic 35 ±1℃. The experimental setup similar to section 3.3 was further modified with air feeding mechanism.
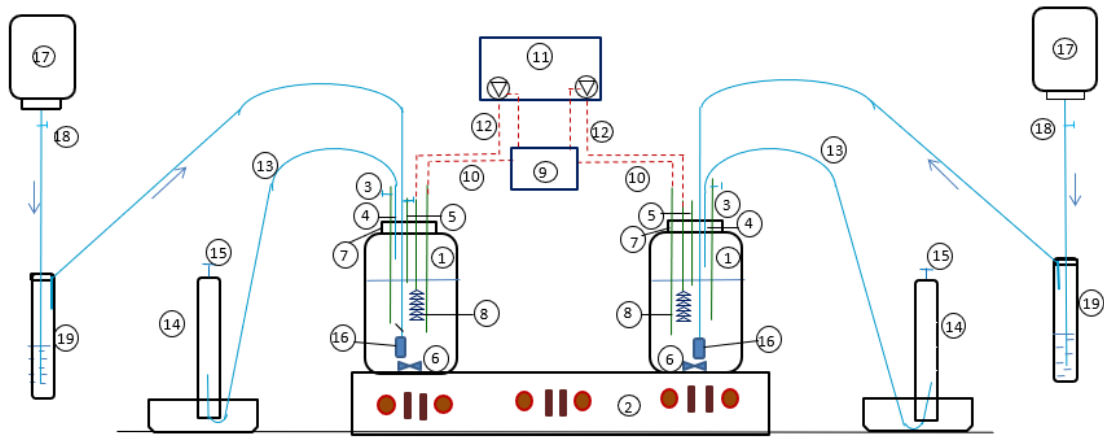
(1) Anaerobic reactor (2) Magnetic stirrer (3) Sample inlet/outlet (4) Gas sampling outlet (5) Sludge sample out (6) Magnetic stirrer rod (7) Nylon top lid (8) Hot water coil (9) Hot water bath (10) Temperature probe (11) Temperatures control unit (12) In/out signal from temperature control unit (13) Clear horse for gas flow out (14)Gas collecting inverted measuring cylinder (15)Gas sampling outlet (16)Air diffuser (17) Liquid filled bottle (18) Controlling valve (19) End capped measuring cylinder

Figure 3.7:Experimental setup for microaeration

There were seven outlets on the top of the reactor. One was to feed influent feedstock and remove effluent sample for analysis. The second outlet was to insert the temperature probe and there were another two outlets to fit the hot water inlet/outlet lines to the hot water circulating coil inside the reactor. Fifth outlet was to remove samples from bulk liquid near the air-bulk liquid interphase for analysis of elemental sulfur Sixth outlet was to feed air sample in micro-aerobic experiments. The end of the clear horse tubing of sixth sampling point was fixed to a diffuser to distribute the air uniformly inside the reactor and the other end was connected to properly sealed end capped measuring cylinder. The inverted acidic water filled bottle was connected to the other end of the end-capped measuring cylinder. The flow from the acidic liquid bottle fill the above said measuring cylinder, thus from air displacement method air in the head space of the measuring cylinder was let to flow into the reactor through diffuser. Reverse flow from the reactor to this measuring cylinder was prevented using non-return valve. The control valve was adjusted to have the required air flow rate to the reactor.

The seventh outlet was to connect the generated biogas to collect in a 1-litre inverted plastic measuring cylinder with water displacement method. The pH of the water

inside the inverted cylinder was maintained less than 2 using HCl, to minimize the gas dilution in the water including CO_2 in the biogas as J.A. Siles et al. [57] applied in their experiments. The gas sampling outlet was at the top end of the measuring cylinder. The schematic diagram of the experimental setup is shown in Figure 3.7. The anaerobic reactors were placed on top of the magnetic plate Hence the mixing was achieved by magnetic rod placed inside at the bottom of the reactor.

The setups were previously acclimated, operated and used for AD experiments for one year. However, before utilizing these micro-aerobic reactors for this experiment, they were fed with synthetic wastewater for another one month daily removing 200 ml of gas sample from the head space and supplying air sample of 200ml to the liquid phase at 100ml/hr half an hour after the feed. This type of adaptation system was carried out to introduce the oxygen to the AD reactor and to increase the activities of the SOB (Sulfur Oxidizing Bacteria) in the reactor.

3.7.2 Experimental Procedure

Experiments were conducted using the same synthetic media made of Acetic Acid, sodium sulfate, basal and trace nutrient solutions of influent COD/SO_4^{-2} ratio of 3 which compatible for influent COD/SO_4^{-2} ratio of SLW and increase its COD/SO_4^{-2} ratio to 5 using alcohol as the optimum influent COD/SO_4^{-2} ratio was found to be 5 which was already in detail explained in section 3.6.

Semi-batch wise fed reactors were operated with 100 ml of feed sample. Before feeding synthetic wastewater sample, N_2 gas was purged for 10min to remove any dissolved oxygen comes with the liquid feed. The head spaces of the reactors were also flushed using N_2 gas.

Air was used to feed the required amount of oxygen to the reactor. The sulfate concentration in the feed was 3000mg/l whereas the sample size fed to the reactor was only 100ml. Thus, the required amount of oxygen moles was calculated for stoichiometric $O_2/S\text{-}SO_4^{-2}$ ratio of 0.5 and influent Sulfur concentration via SO_4^{-2}. Then the corresponding air volume was calculated considering that the air pressure was at atmospheric condition and the room temperature at 33°C using universal gas equation (PV=nRT). Calculated air sample volume was 188 ml. Then as per Table 3.7, this air volume was fed into the reactor with three different methods. Synthetic

wastewater sample of 100ml was fed in for each phase with feeding cycle time of two days. As a reference phase (phase 1), the sample was anaerobically digested without feeding air. Air sample of 188ml fed into the reactor following several feeding methods shown in Table 3.7 in each phase. Gas volume equivalent to the air sample of 188 ml was fed to the reactor through the bulk liquid. Before an air sample fed in, equivalent volume of 188 ml was taken out from the headspace with aid of a syringe to facilitate air feed into the reactor.

In each phase, the degradation and generation behaviour of the main sulfurous compounds in the micro-aerated reactor such as sulfate, TDS, gaseous H_2S were measured. Before feeding each sample into reactor, the cloudy layer of generated sulfur in between the gaseous and liquid surface was removed via outlet tube which was located near the surface of the reactor. The four phases carried out during the experiment were as follows:

Table 3.7: Summary of reactor operation in each phase

Phase	Condition of the reactor	Description
I	Anaerobic	No air fed
II	Micro-aerobic	Air sample fed in 2 mins soon after feeding at a rate of 94ml/min
III	Micro-aerobic	Air sample fed as continuous micro aeration at rate of 0.065 ml/min
IV	Micro-aerobic	Air sample fed in 2 hours after half an hour following feeding at a rate of 1.6 ml/min

During phase I, synthetic wastewater sample of 100ml was anaerobically degraded. Then in phase II to IV, corresponding air volume (188 ml) calculated for atmospheric pressure and room temperature 33°C for O_2/S ratio 0.5 was fed to the reactor by means of 3 different methods. During phase II, the air was fed just after feeding to the reactor. Total air volume was fed within 2 minutes. In phase III the air was fed to the reactor

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at a slow rate (0.065 ml/min) continuously for 48 hours, started soon after the reactors were fed with synthetic wastewater. Then in phase IV, the air was fed to the reactors in half an hour after the synthetic wastewater sample was fed to the reactors and corresponding air volume was fed within 2 hours. The air was decided to feed in lag of half an hour to provide sufficient time for sulfate reduction to sulfide providing strict anaerobic condition for most sulfate to be reduced to sulfide. In all three phases, the air sample was fed into the liquid phase of the reactor through diffusers. It was conducted with the objective of supplying O_2 directly for SOB for sulfide conversion process as well as to dissolve some amount of O_2 in the liquid media, whereas the remaining O_2 would transfer in to head space and SOB present in the liquid media and the headspace both able to consume them for further sulfide to elemental sulfur conversion process.

3.7.2.1 Parameters measured

Measured parameters were same as parameters measured and in-detail explained in experiment 3.5.

3.8 Effect of O_2/S ratio on elemental sulfur formation and sulfate reduction in Single Stage Sulfate-removal Micro-aerated Anaerobic Digester (SSMAD) feeding synthetic wastewater (Experiment F)

All Initial experiments were carried out to study the sulfate reduction and investigated the most suitable conditions for sulfate reduction of both synthetic wastewater and Skim latex wastewater. From this experiment onwards the second stage of sulfate conversion to elemental sulfur process, the sulfide oxidation step was studied in detail. Many literatures are found for direct sulfide(S^{-2}) oxidation to elemental sulfur(S^0), but very few previous studies were found for influent sulfate conversion to elemental sulfur. Although high O_2/S ratios are used for direct sulfide oxidation, limited O_2/S ratios are used when influent sulfurous compound is sulfate. Because in return sulfate reduction which was a strictly anaerobic reaction is inhibited by high O_2/S ratios. With respect to the past experiments, the O_2/S ratio of planned Sulfide oxidizing this

experiment was set at 0.25, 0.5, 1.0 and 1.5. The oxygen was fed to the reactor via supplying air.

The effect of O_2/S ratio on the gaseous H_2S in biogas, O_2/S ratio on elemental sulfur formation as well as the effect of O_2/S ratio on biological sulfate reduction were studied. Nevertheless, the variation of elemental sulfur formation with the time was investigated under this experiment.

3.8.1 Experimental Setup

Two Suspended grown anaerobic semi- batch reactors with 2.0 L working volume and 1.0l head space were used in this study. Experimental setup was same as the setup described and used in Section 3.7 which was maintained at temperature 35 ±1℃.

3.8. 2 Experimental Procedure

Experiments were conducted using the same synthetic media made of Acetic Acid, sodium sulfate, basal and trace nutrient solutions of COD/SO_4^{-2} ratio of 3 and increase its influent COD/SO_4^{-2} ratio to 5 using alcohol, which was already explained in section 3.7.

The setup was then operated for another 2 months, daily removing 200 ml of gas sample from the head space and supplying air sample of 200ml to the liquid phase at 100ml/hr after half an hour following feeding. Then the setup was operated for another one month, daily removing 350 ml of gas sample from the head space and supplying air sample of 350ml to the liquid phase at 175ml/hr after half an hour after feeding. The micro-aeration levels are smoothly increased to adapt the micro-organisms to survive in the micro-aerobic condition and increase the activities of the Sulfur oxidizing bacteria.

Semi-batch fed reactors were carried out with 100 ml of feed sample. Before feeding the synthetic wastewater sample to the reactor, N_2 gas was purged for 10min to remove any dissolved oxygen comes with the liquid feed. Then oxygen was fed to the reactor via air, after half an hour the feed sample was fed to the reactor. The head space of the reactors was flushed using N_2 gas before each sample fed into the reactor. Before air sample was fed into the reactor, head space gas volume nearly equivalent to the air sample to be fed was taken out to facilitate air feed into the reactor. The oxygen was fed to the reactor with half an hour lag to optimize the sulfate reduction which was a

strict anaerobic degradation. Each of the corresponding air sample was fed to the reactor for two hours at a slow rate to prevent flushing off the fed air samples out of the reactors. Then observed the degradation and generation pattern of the main sulfurous compounds in the micro-aerated reactor. The same procedure was carried out increasing the air quantity in steps of O_2/S ratio 0.25, 0.5, 1.0 and 1.5. O_2/S ratio of each phase is as shown in Figure 3.8. Before feeding each sample to the reactor the cloudy surface in between the gaseous and liquid surface was removed via outlet tube which was located near the surface of the reactor.
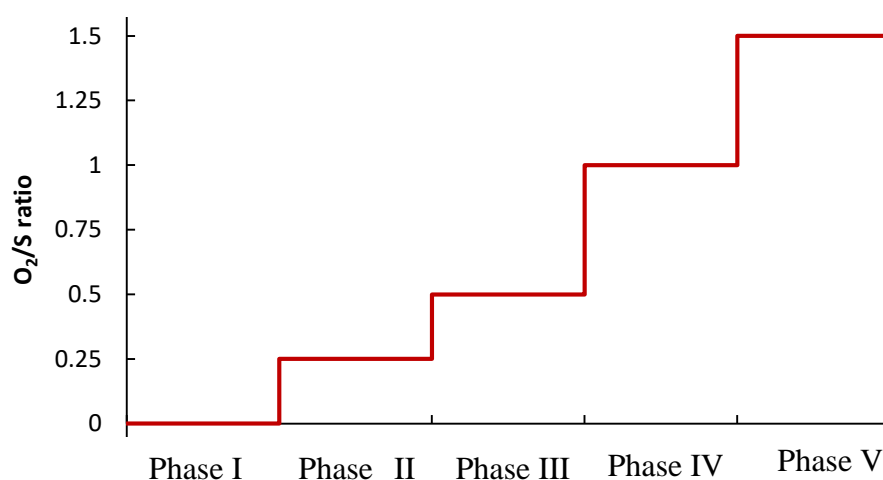
Figure 3.8: O_2/S ratio in each phase

3.8.2   Parameters measured

Measured parameters were same as parameters measured and in-detail explained in experiment 3.5, additionally elemental sulfur amount was measured.

3.9 Effect of O_2/S ratio on mesophilic sulfate reduction and elemental sulfur formation in SSMAD using SLW (Experiment G)

Series of experiments were conducted to understand the anaerobic digestion behaviour of SLW which has low COD/SO_4^{-2} as well as low COD/TKN ratio for biological treatment process and enhance first stage sulfate reduction and identify the most suitable condition for second stage elemental sulfur formation. In this final experiment, adaptation of all the findings of all experiments to enhance sulfate reduction and elemental sulfur formation was utilized to maximize the influent sulfate treatment together with elemental sulfur formation.

Average COD/SO_4^{-2} ratio of natural SLW which was used in the study was 2.7-3.3 and COD/TKN ratio was 10.1 which is in the range of causing severe inhibition under biological anaerobic digestion as found in Experiment A, B and C. Therefore, addition of external electron donor, increased not only the COD/SO_4^{-2}. ratio but also the COD/TKN ratio of SLW required to improve stability of the reactor. Maintaining pH of the micro-aerobic reactor at 7.5-8.0 minimized both the ammonia inhibition in the reactor and enhance efficient sulfate reduction. Ethanol which is a partial oxidizer was found to be the most efficient electron donor than complete oxidizing agent acetic. From the series of experiments, most suitable COD/SO_4^{-2} ratio was 5, which simultaneously increased the COD/TKN ratio to 18.5. The suitable hydraulic loading to the reactor was found to be 50.0 l/m^3.d.

From the experiment conducted to maximize the efficiency of conversion of reduced sulfide to elemental sulfur for synthetic wastewater in experiment F, it was found that feeding air of O_2/S ratio of 0.8 - 1 with air fed direct through the bulk liquid, half an hour after feeding at a rate of 1.6 ml/min for two hours was given the maximum elemental sulfur yield. Hydraulic loading used in the experiment was 50.0 l/m^3.d whereas the Sulfate loading was 0.15 $kgSO_4^{-2}/m^3$.d. During this experiment O_2/S ratio was varied in steps of 0.5, 1.0 and 1.5. However, the DO concentration of the natural SLW was about 3 mg/l. Although the oxygen concentration feed into the reactor as dissolved oxygen was very small with respect to the oxygen fed with air supply, it was considered in calculating the required O_2/S ratios of 0.5, 1.0 and 1.5. The most effective method of supplying air was the supply through the bulk liquid.

During the phase I, II, III and IV, of this experiment, best suitable O_2/S ratio for simultaneous sulfate reduction, hydrogen sulfide reduction and elemental sulfur formation at COD/SO_4^{-2} ratio of 5 were investigated, whereas, in phase IV, COD/SO_4^{-2} ratio was increased to 10 while maintaining the O_2/S ratio at 1.5 to find out the optimized condition for Skim latex wastewater. The effect of micro-aeration process on the COD reduction and methane formation was also investigated.

From this experiment, investigations were carried out to study the effect of O_2/S ratio on the gaseous H_2S in biogas, elemental sulfur formation, biological sulfate reduction, biological COD reduction and methane formation using skim latex wastewater.

3.9.1 Experimental Setup

Two Suspended growth semi-batch reactors with 2.0 L working volume and 1L head space were used in this study. Experimental setup was same as the setup described and used in Section 3.4 which was maintained at temperature 35 ±1℃ and modified with air feeding mechanism for the micro-aeration (Figure 3.9).
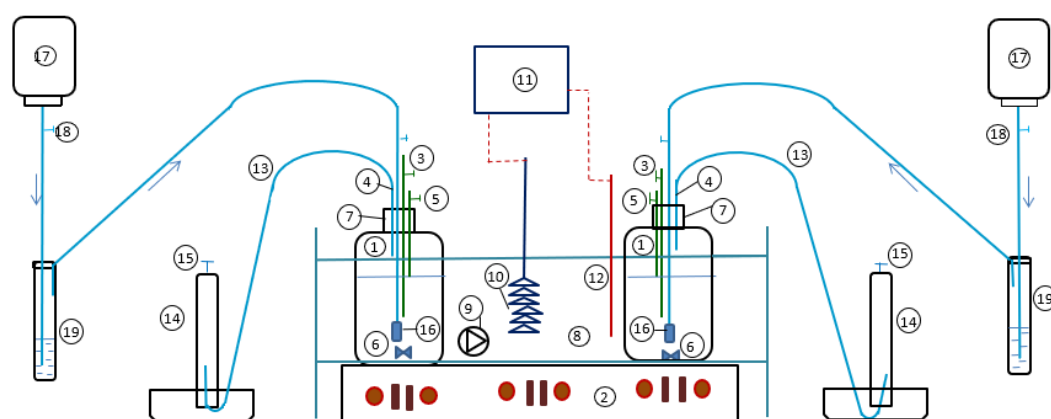
(1) Anaerobic reactor (2) Magnetic stirrer (3) Sample inlet/outlet (4) Gas sampling outlet (5) Sludge sample out (6) Magnetic stirrer rod (7) Nylon top lid (8) Hot water coil (9) Hot water bath (10) Temperature probe (11) Temperatures control unit (12) In/out signal from temperature control unit (13) Clear horse for gas flow out (14)Gas collecting inverted measuring cylinder (15)Gas sampling outlet (16)Air diffuser (17) Liquid filled bottle (18) Controlling valve (19) End capped measuring cylinder

Figure 3.9: Schematic diagram of the Micro-aeration setup

3.9.2 Start-up procedure

SRB and SOB are slow growing bacteria and it took about one year for the reactor to achieve high treatment efficiency. Thus, for this experiment two identical reactors operated for one and half years were used. before starting up of this micro-aerobic experiment the reactors were adapted for micro-aerobic condition while SLW was fed for 3 months semi-continuously with feeding cycle of 2 days. SLW sample of 100ml was removed from the AD digester before feeding 100 ml feed sample, while 200 ml of gas sample from the head space of the AD digester was removed and 200ml of air was supplied through the bulk liquid at 100ml/hr after half an hour of feeding the wastewater sample for two hours once in two days. Thereafter the setup was operated for another one month, daily removing 350 ml of gas sample from the head space and supplying air sample of 350 ml to the liquid phase at 175 ml/hr after half an hour after the feed for two hours. During this period of time even the feed wastewater sample was 100 ml. The micro-aeration levels are smoothly increased to adapt the micro-organisms to survive in the micro-aerobic condition and increase the activities of the Sulfur Oxidizing Bacteria.

3.9.3 Experimental method

Experiments were conducted using the SLW possessing the characteristics as shown in Table 3.5. Then the COD/SO_4^{-2} ratio of the wastewater was increased to 5 using ethanol.

Semi batch fed micro-aerobic reactors were carried out with 100 ml of SLW sample. The experiment was conducted in duplicate and average results were considered in the evaluation. Then oxygen was fed to the reactor via air, and after half an hour sample fed into reactor. The head space of the reactors was flushed using N_2 gas before feeding into the reactor. Before air sample was fed into the reactor, head space gas volume equivalent to the air sample to be fed was removed to facilitate air feed into the reactor. The oxygen was fed to the reactor with half an hour lag to optimize the sulfate reduction which was a strict anaerobic degradation. Each corresponding air sample was fed to the reactor within two hours at a slow rate to prevent flushing off the fed air samples out of the reactors. Then observed the degradation and formation of the main sulfurous compounds in the micro-aerated reactor; sulfate, gaseous H_2S, TDS

and generated elemental sulfur. Samples for elemental sulfur analysis were collected from the cloudy interface of bulk liquid and headspace via two outlet tubes which were located near the surface of the reactor. The same procedure was carried out increasing the air quantity in steps of O_2/S ratio 0.5, 1.0 and 1.5. The feeding cycle time of each phase is 48 hours.

Table 3.8: Summary of influent and reactor condition of each phase

Phase	Condition of the reactor	Description	COD/SO_4^{-2} ratio
I	Anaerobic	No air fed	5
II	Micro-aerobic	$O_2/S = 0.5$	5
III	Micro-aerobic	$O_2/S = 1.0$	5
IV	Micro-aerobic	$O_2/S = 1.5$	5
V	Micro-aerobic	$O_2/S = 1.5$	10

3.9.4 Parameters measured

Measured parameters were same as parameters measured and explained in detail under experiment 3.5, additionally elemental sulfur amount was measured. Initial and final tCOD after 48 hours, following the complete sulfate reduction were measured. TAN was monitored intermittently throughout the experiment to check the ammonia inhibition condition. The analytical methods used for the experiment is explained under section 3.10.

3.10 Analytical Methods for all experiments

3.10.1 Sulfate concentration

Aqueous SO_4^{-2} concentration was measured using HATCH DR/890 colorimeter according to the Sulfa Ver 4 Method adapted from standard methods for the Examination of Water and Wastewater and USEPA method 375.4 for wastewater.

Sample was prepared by filtering through 0.45 μm syringe filter and diluted correspondingly. Both the 10 ml sample cell and blank sample cell were filled with filtered and diluted wastewater sample. Then, Sulfa Ver 4 Sulfate reagent powder pillow was added to the sample cells. The cell was capped and inverted several times to mix. Thereafter the cell was allowed to stand still for reaction time of 5 minutes. Then the sulfate measurement of the sample was measured with respect to the blank sample placing the cell in the cell holder and tightly covering the instrument cap in mg/l.

3.10.2 Total Dissolved Sulfide (TDS) Concentration

Aqueous TDS concentration was measured using HATCH DR/890 colorimeter according to the Methylene Blue Method adapted from standard methods for the Examination of Water and Wastewater and USEPA method 376.2 for wastewater or Standard Method 4500-S^{2-} D for wastewater.

Sample was prepared by filter through 0.45 μm syringe filter and dilute correspondingly. One 25 ml sample cell was filled with the sample and the other one with 25 ml deionized water(blank). 1.0 ml of sulfide 1 reagent was added to each cell and swirled to mix. Then 1.0 ml of sulfide 2 reagent to each cell and swirled to mix. After 5 minutes of reaction time the sulfide measurement in mg/l was obtained with respect to the blank sample placing the cell in the cell holder and tightly covering the instrument cap

3.10.3 Elemental Sulfur concentration

Analytical method for elemental sulfur analysis was used and described by G.C. Stefess et al. [79]. Samples for the analysis of elemental sulfur were centrifuged at "centrifuge Eppendorf – 5804R" for 15,000 rpm for 15 minutes by careful decantation and drying overnight at 30C. The residuals were extracted with acetone for 3 days, and

subsequently extract samples were prepared and analysed by UV spectrophotometer (UV 1800 of Shimadzu) at 465 nm as the method described by J.K. Bartlett and D.A. Skoog Standard solutions of S_8 (orthorhombic sulfur) in acetone were used for calibration[19].

3.10.4 Total Chemical Oxygen Demand (tCOD)

tCOD of samples were analysed according to standard methods 5220 C (Closed Reflux Titrimetric method). Samples were diluted using dilution factor 10 -50. For each composition, two samples were analysed.

3.10.5 Soluble Chemiacal Oxygen Demand (sCOD)

Liquid samples with suspended solids were centrifuged at 7,000 rpm for 10 min and supernatant was filtered through syringe filter (25 mm Agilent syringe filter- Nylon with 0.45 μm pore size). Samples were diluted using dilution factor 10 -50.

Following preparation of samples, sCOD of samples were determined according to standard methods 5220 C (Closed Reflux Titrimetric method). For each composition, two samples were analysed.

3.10.6 Total Ammoniacal Nitrogen (TAN)

TAN concentration was measured using HATCH DR/890 colorimeter according to the Salicylate Method adapted from Clin. Chim. Acta., 14 403 (1966) standard methods. Sample was prepared by filtering through 0.45 μm syringe filter and dilute correspondingly. One 10 ml sample cell was filled with sample and the other one with 10 ml deionized water(blank). Ammonia Salicylate reagent powder pillow was added to each sample cell. Both cells were capped and shake well to dissolve. After 3 minutes' reaction time, one Ammonia Cyanurate reagent powder pillow was added to each above-mentioned cell. The cells were capped and shake well to dissolve the reagent. After 15 minutes' reaction time, the sample cells NH_3-N was measured with respect to blank cell, placing the cell in the cell holder and tightly covering the instrument cap in mg/l.

3.10.7 Biogas volume

Biogas volume was measured using an inverted 1L plastic measuring cylinder with water displacement method which connect one outlet of the reactor to the measuring

cylinder. The pH of the water inside the inverted cylinder was maintained less than 2 using HCl, to minimize the gas dilution in the water including CO_2 in the biogas as Siles J.A. et al. applied in their experiments[57].

3.10.8 Gaseous Hydrogen sulfide (H_2S) concentration

Gaseous hydrogen sulfide was measured using Sensorcon hydrogen sulfide Detector and pump kit. The resolution was 1ppm.

3.10.9 Gas Chromatography (GC) analysis

Biogas composition (CH_4, CO_2, O_2, N_2) was determined using Shimadzu GC-2014 series gas chromatography. Thermal Conductivity Detector (TCD) was used with Argon as the carrier gas. The column details are as follows, Column (MC – 2) – MS-13X packed column, 1.0mm ID*1.5M.

Gas bags with samples were connected to the GC and automatically 10 ml sample were taken into the GC. For each composition two samples were analysed, and average composition was calculated.

3.10.10 Volatile fatty acid (VFA) analysis

Individual concentrations of VFA were measured using Agilent Technologies 7890 N series gas chromatography with flame ionization detector (FID) and carrier gas Helium. Filtered diluted sample of 1.35 ml was first acidified by adding 150µl of 0.65M formic acid and enclosed in a vial.

3.10.11 pH measurements

pH measurements were taken using Ohauns – Starter 2100 pH meter. Two-point calibrations were performed using solutions pH = 7 and pH = 4.

3.10.12 Oxidation Reduction Potential (ORP) measurements

ORP measurements were taken using YSI 1200 meter.

3.10.13 Dissolved Oxygen measurements

Dissolved oxygen concentration was measured using YSI model 55 meter. Before each experiment, single point calibration was performed placing the probe under 100% humidity conditions. Accuracy of the probe is ± 0.3 mg/l with 0.01 mg/l resolution at an ambient temperature of -10 to 50°C.

3.10.14 Total Solid (TS), Total Suspended Solid (TSS), Total Dissolved Solid (TDS) and Total Volatile Solid (TVS) analysis

TS, TSS, TDS and TVS were analysed according to standard methods, 2540 B, 2540 D, 2540 C and 2540 E respectively.

4 RESULTS AND DISCUSSION

4.1 Effect of pH and external electron donor on mesophilic sulfate reduction during start-up period of Anaerobic Digester treating SLW (Experiment A)

Semi-continuous feeding experiments were carried out to investigate the sulfate reduction during start-up period, soon after the initial acclimation of Anaerobic Digester which treat SLW under complete anaerobic condition and the effect of complete electron donor on sulfate reduction under mesophilic condition was also investigated. The influent COD/SO_4^{-2} (g/g) ratio, COD/TAN (g/g) ratio and COD/TKN (g/g) are 2.8, 12.7and 8.9 respectively. As discussed earlier, SLW is high in protein. Thus, the COD/TKN ratio is lower than COD/TAN. According to P. Kongjan et al. [64] the protein content in the skim latex serum coagulated by formic acid was 6.25 times the organic nitrogen content and it was estimated to be 7.56 ± 0.5 g/l. N. Pake [80] and his team also has reported that the skim latex serum is low in COD/N ratio and it was approximately one third of that in food waste. Although there are many literatures found for skim latex coagulation through formic acid only very few literatures available for skim latex coagulation by sulfuric acid. Natural SLW coagulated by sulfuric acid is rich in high concentrations of sulfate, protein and organic matter[5]. Sulfate is broken down into Total Dissolved sulfide (S^{-2}, HS^-, H_2S(aq)) and gaseous Hydrogen sulfide under anaerobic degradation. Under this experiment, the sulfate reduction was examined at initial stage acclimation period. It had been recorded by P.H. Tessa et al. [81] that sulfate reduction was efficiently carried out with reactors which operated for long time as SRB are slow growing micro-organisms.
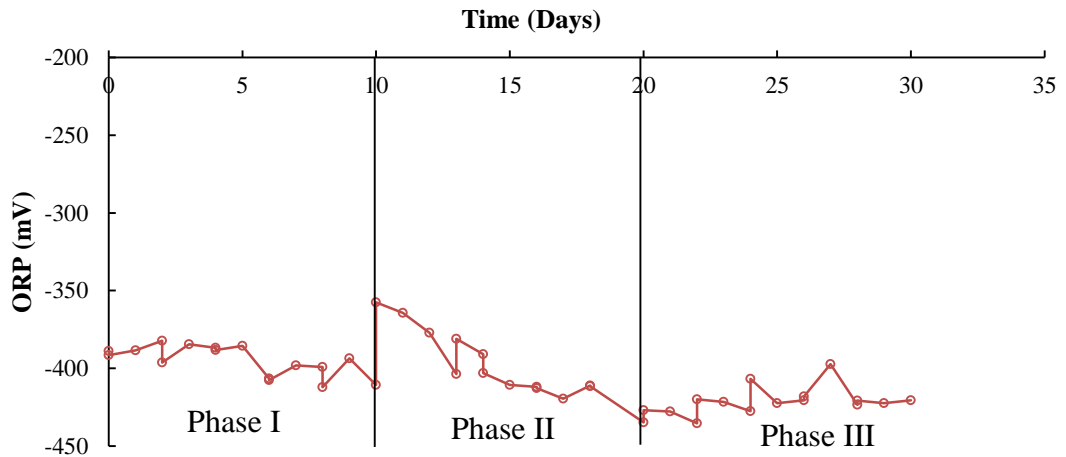
Figure 4.1:ORP Vs Time

Oxidation Reduction Potential (ORP) values which indicates the degree of anaerobic reactions were in the range of -360 mV to -435 mV. The ORP values are only shown in Figure 4.1. However, it represents the net ORP value corresponding to the net major break down reactions related to the major constituents in the anaerobic reactor, the sulfate, organic matter and the protein degradation.

4.1.1 Sulfate reduction and Sulfide formation in the anaerobic reactor

Under anaerobic condition, sulfate is broken down to sulfide. Some of the broken-down sulfide leave the reactor with biogas as gaseous hydrogen sulfide and remaining sulfide present in the reactor as a Total Dissolved Sulfide (TDS) which consists of sulfide (S^{-2}), bisulfide (HS^-) and dissolved Hydrogen sulfide ($H_2S(aq)$). The sulfate and the Total Dissolved Sulfide (TDS) profiles of the anaerobic reactor in all three phases are shown in Figure 4.2.
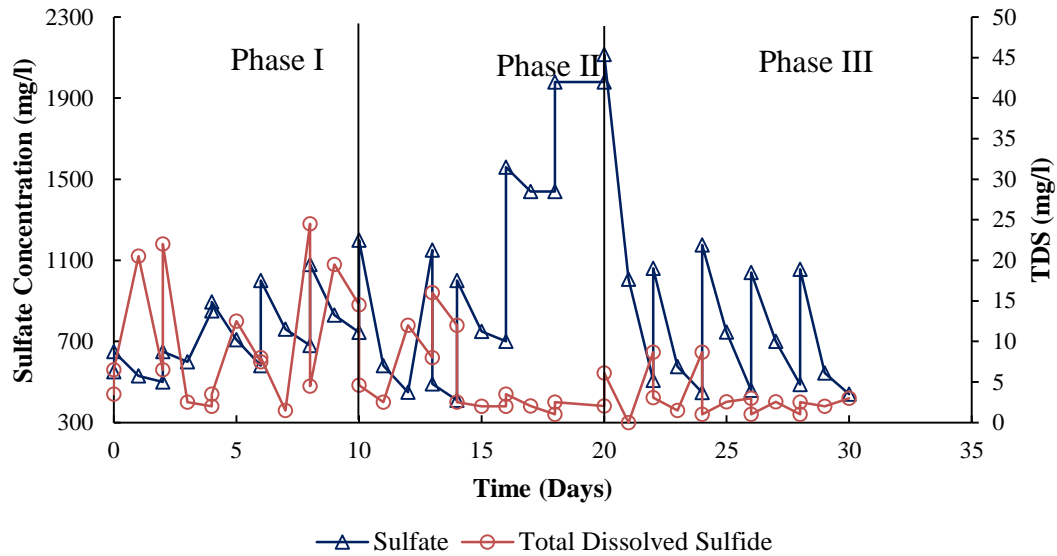
Figure 4.2: Sulfate and TDS Vs Time

Under Phase I in which the influent COD/SO_4^{-2} ratio was 2.8 with influent pH 7, the maximum sulfate reduction rate occurred on the first day of the cycle which had cycle time of 2 days. As the reactant concentration was high, soon after feeding, it is obvious to have high rate of reaction. It could be observed that the high sulfate reduction after feeding (Figure 4.2), as well having high gradient in the first day of the cycle. The first day and the second day average sulfate reduction percentages were 23±2% and 33±2% respectively. It seemed to be comparatively low degradation. For quantitative sulfate degradation, several factors affect such as influent COD/SO_4^{-2} ratio, Gibbs free energy, kinetic reaction, sensitivity of microbes for inhibition, type of substrate, relative Microbial population, pH and temperature. P.H. Tessa et al. [82] reported that SRB are slow-growing microbes and they require more time for adaptation to new condition of semi-continuous feeding. It was observed that when this reactor was tested for sulfate degradation experiment after about 6 months following the acclimation, the first day and second day treatment efficiency improved to 57.1% and 65.7%. (Data not shown). Therefore, it is reasonable to assume that SRB are slow adapted micro-organism which take reasonable time for efficient treatment. SRB consume partially degraded organic compounds only for sulfate reduction. Therefore, hydrolysis of organic matter and the Acidogenesis are limiting factors for sulfate reduction. Latex wastewater contains

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complex organic matter such as protein, [5]. Therefore, those compounds have to be converted to simple products such as VFA for SRB to consume. It was observed that when COD/SO$_4^{-2}$ ratio of the influent SLW was increased from 3 to 10 using acetic, the percentage sulfate reduction per cycle has improved to 63.5%. Not only the percentage sulfate reduction but also the rate of sulfate reduction improved with the influent COD/SO$_4^{-2}$ ratio (Figure 4.3). Addition of external electron donors provided simple organic substrate for SRB. Nevertheless, its improved COD/N ratio of the influent which is another essential parameter affects inhibition of sulfate and protein rich wastewater.
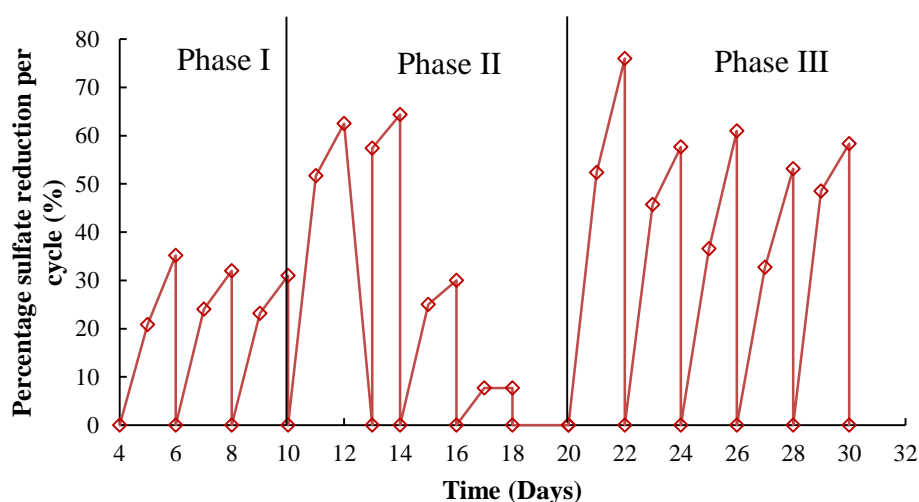
Figure 4.3: Percentage cumulative Sulfate Reduction Vs Time after feeding

Although sulfate reduction improved within the first two cycles of phase II, in the second two cycles, it decreased significantly. It was due to increase of pH in the anaerobic reactor. The pH profile is shown in Figure 4.4.

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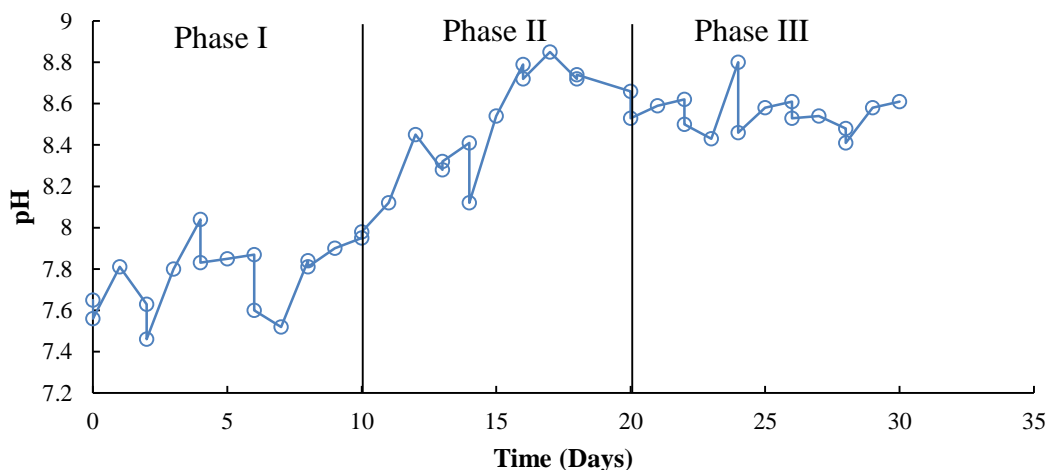
Figure 4.4: pH Vs Time after the feed

It was evidenced that the sulfate reduction, adversely affected by high pH when excess simple organic substrates were available. When the influent pH of the feed sample was adjusted to 7 in phase I and II, the pH inside the reactor was rising to 8.85 (Figure 4.4), whereas the percentage sulfate reduction per cycle gradually reduced to 30%, 8% and 0%. Because of less buffering capacity, the anaerobic reactor showed high pH variation.

From the beginning of the anaerobic reactor acclimation, pH of the reactor showed more biased towards alkalinity. T.V. Nguyen [83] has observed similar trends of high concentration of NH_4^+ in the seed sludge, the initial TAN concentration in the effluent was high up to 1100 mg/l and following acclimation of reactor about 6 months, it reduced into concentration of 450mg/l. High concentration of ammonia released from seed sludge inhibited few anaerobic reactors at the initial stage of acclimation and failed at the time of acclimation (Data are not shown here).

Further, the SLW contains high amount of sulfate, ammonia and protein. The pH of the anaerobic reactor depends on by products formation due to dominant reactions such as VFA formation in acidifying, HCO_3^{-2} formation and VFA consumption in sulfate reduction, and ammonia generation in protein degradation as explained detail in section. 2.5.

In phase III, when influent pH of the feed sample was reduced to 3 using 3M HCl maintaining the influent COD/SO_4^{-2} ratio at 10, fully inhibited anaerobic reactor

recovered again. The original pH of the feed sample before adjusting to pH 3 is 5.5. Addition of acidity with the feed sample reduced the significant increase of pH inside the reactor. Thus, sulfate reduction again increased in phase III. In the first cycle soon after the low pH influent fed to the reactor, percentage sulfate reduction per cycle increased to 76%, decreasing the accumulated sulfate in the reactor. But within other three cycles thereafter average sulfate reduction gained was 58 ± 3%. In all three phases there were some unexpected increase of sulfate noticed in the reactor. It was suspected that this is due to release from seed sludge.

Methane production is inhibited by 100-800mg/l of dissolved sulfide and 50-400mg/l of un-ionized H_2S [84]. The measured TDS concentration of phase from I to III were 9.7 mg/l, 4.8 mg/l and 2.6 mg/l respectively, these values were lower than the inhibition value. TDS concentration decreases with increase of pH as observed by Omil F. [61]. But in phase III gaseous H_2S concentration increased as per the Figure 4.5. Both volumetric gas production and gaseous H_2S concentration decreased at the end of the phase II, inhibition period. The measured H_2S concentrations were lower in phase I in which sulfate reduction is less and high in phase II and phase III. The generated H_2S concentrations have shown random increments at the acclimation. It is because of the uneven biogas production. L. Krayzelova et al. also observed similar results during initial stage acclimation period of a UASB reactor[15].
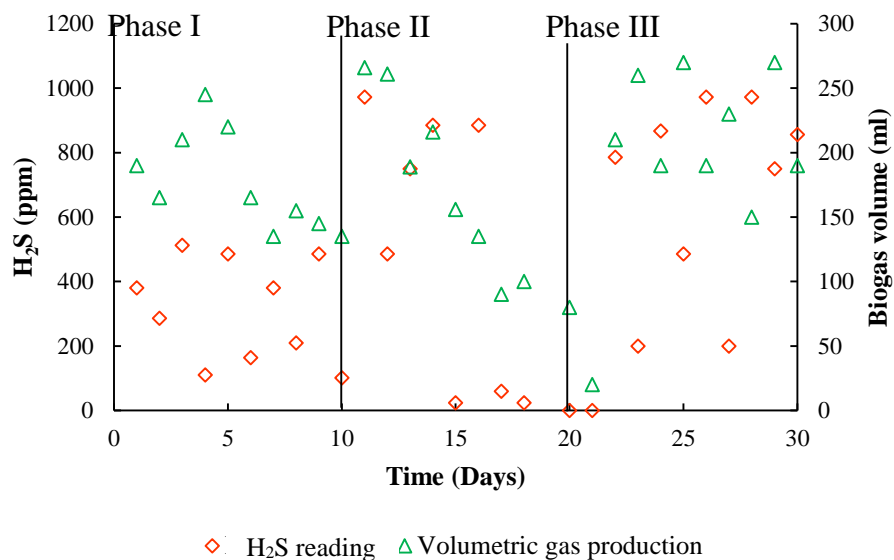
Figure 4.5:H_2S concentration and volumetric gas production Vs Time after feeding

4.1.2 Organic matter degradation and VFA formation

The sCOD concentration of each phase were 6.6 ± 1.9 g/l, 6.6 ± 3.3 g/l and 6.8 ± 3.1 g/l. Only in phase II, sCOD was high. But comparatively there weren't much significant variation in the sCOD concentration.
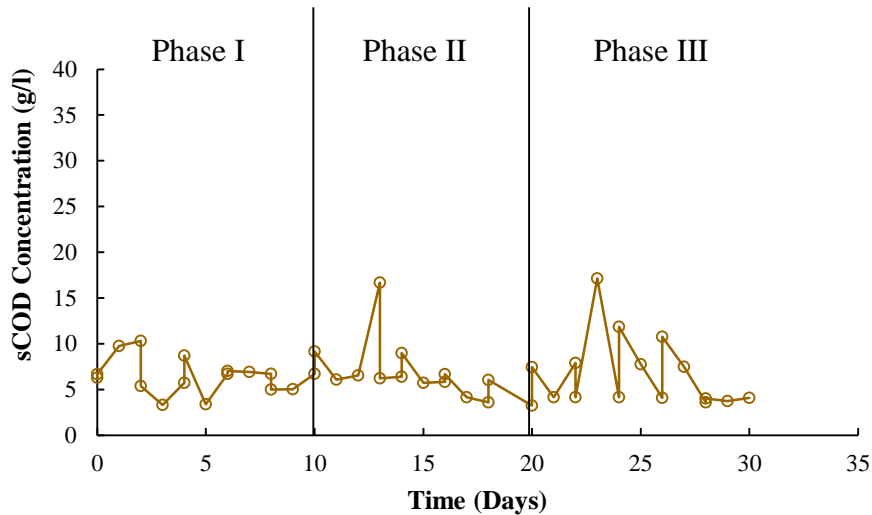
Figure 4.6: sCOD Vs Time

Component wise VFA profiles for 25 days are shown in Figure 4.7. But due to some practical difficulties at the final 5 days, component-wise VFA could not be measured. Following feeding at 10th day, only Acetic, Butyric and Iso-butyric could be observed in the reactor. Though tVFA was high during the phase I, it was lower in other two phases, II and III. tVFA of the phase I was less due to accumulation of VFA inside the reactor as MB and SRB were inhibited by the high FAN as in detail explained in section 4.1.3. Acetic and Butyric were the major observed VFA constituents while propionic, Iso-butyric, Iso-valeric and N-Valeric could also be observed in minor concentrations. phase I, in which the sulfate reduction is less, propionic and Iso-butyric were detected. Presence of these acids in different phases can be well explained from thermodynamic and kinetic theorems as well.

Propionic acid was only found in phase I in which sulfate reduction was less. But propionic acid was not found in observable concentrations in phase II and III in which sulfate reduction was high. It might be propionic acid generated in the reactor, but it was consumed fast by SRB. Thus, net propionic acid was not observed in measurable

concentrations. V. O'Flaherty and his team[85] also noticed that propionic acid was only observed at the initial period of operation of anaerobic reactors only in absence of sulfate, but with presence of sulfate, propionic acid was not observed. It is because SRB carried out an incomplete oxidation of propionic to acetate or complete oxidation to bicarbonate more easily than use of acetate as the electron donor. This phenomenon can be explained theoretically with Gibb's free energy. As expressed in Table 2.2, Gibb's free energy for sulfate reduction, converting propionic acid to acetate and bicarbonate is -33 and -88.9 kJ/kmol, whereas the Gibb's free energy(ΔG) for sulfate reduction using acetate as the electron donor is only -47.6 kJ/kmol. The importance of SRB in the degradation of propionate agrees with previous reported results using various anaerobic reactors and sulfate adapted sludge, which an indirect oxidation of propionate to acetate was found to be the key degradation pathway of that substrate ([10], [86],[87]).

Absence of Propionate and presence of high percentage of acetate and butyrate inside the reactor agrees with the explanation above. The affinity of SRB for substrate decreases from hydrogen to propionate and then other electron donors. This provides an explanation for the failure of SRB to outcompete butyrate and acetate.
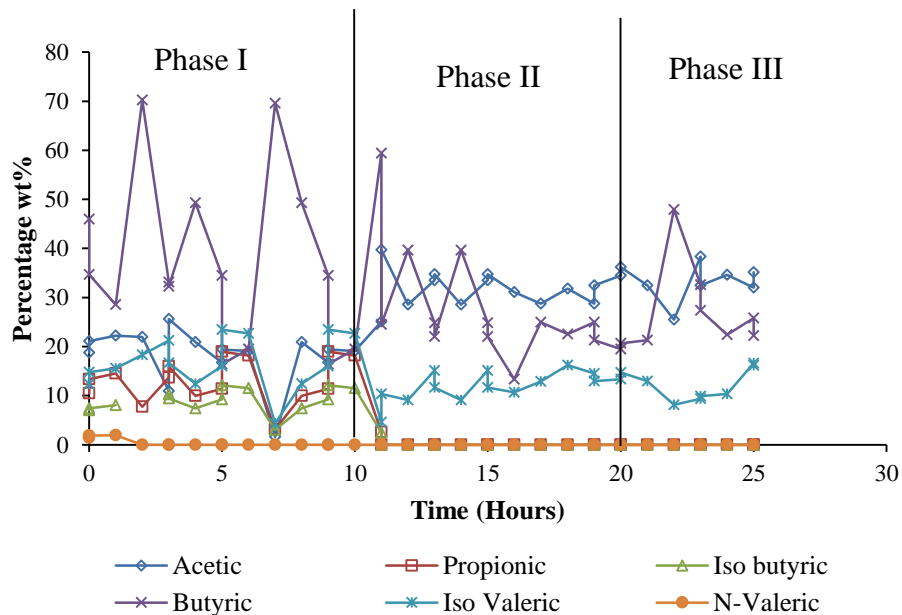
Figure 4.7: Component of VFA Vs Time

As per the results, N-valeric was also observed during initial period. Thereafter it was not detected.

Average methane production obtained in phase I, was only 11.18 ± 8.0 v/v%. which is comparatively less value. This may be due to consumption of VFA for sulfate reduction as well as methanogenic inhibition in high pH values and due to NH_3 inhibition. Gaseous CO_2 detected was 1.26 ± 1.6 v/v% and H_2S was only 0.6 v/v% after correction was made for oxygen and nitrogen. Measured components in gas phase were oxygen, nitrogen, methane, carbon dioxide and gaseous Hydrogen sulfide only. But the major component of other gasses was 87.17 ± 9.5 % and the major component of this unknown gas is assumed to be gaseous ammonia. because of ammonia dominance seed sludge in the liquid phase. After about six months of acclimation, it was observed that the methane composition in the biogas has improved to 30% (Data not shown here). Therefore, although the methane composition during the acclimation period was less, with the well growth of micro-organisms it can be increased to higher value.

4.1.3 Effect of Evolution of Ammonia under sulfate reduction

During the initial stage of acclimation, the profile of TAN, FAN plotted together with sulfate is shown in Figure 4.8. During the acclimation period, the TAN concentration was high because of the high NH_4^+ concentration in the seed sludge and the feed. As a result, the pH of the reactor increased. FAN depends on the pH and the temperature. As reported, FAN is the most inhibitory substance than the TAN for any micro-organism. SRB withstand high pH values like 8.8, thus even though the anaerobic reactor inhibited, it did not completely fail. But when the FAN of the system reached 679.5 mg/l, the system completely inhibited.
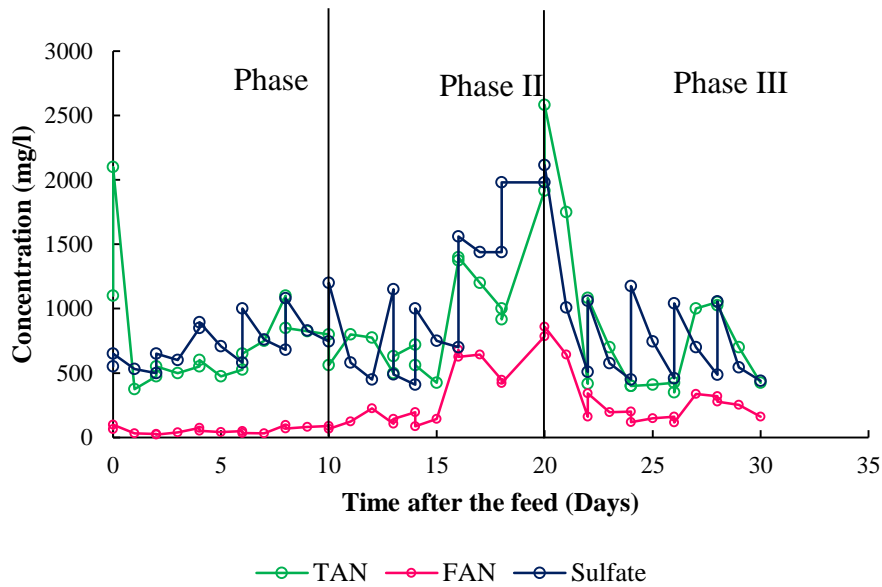
Figure 4.8:TAN, FAN Vs Time

Sulfate concentration Vs FAN graph is shown in Figure 4.9. From the graph, It is further evidenced that the sulfate reduction lower or the sulfate accumulation in the system is high when FAN is high and sulfate redcution is efficient when the FAN is lower. This may be due to direct inhibition of FAN on SRB or indirect inhibition on MB.

S. Chaiprapat et at. [88] and Jariyaboon R. [89] found that in skim latex serum experiments the resultant pH reduced in the reactors due to excess formation of VFA in an Up flow Anaerobic sludge blanket (UASB) and Anaerobic sequencing batch reactor (ASBR). Further Chaiprapat S. et al was able to achieve the desired pH values inside the reactor pre-treating the original influent pH of 2.43 ± 0.5 with para-wood ash by adjusting the influent pH to $7 - 8$. But, in contradictory T.V. Nguyen [5] has observed pH rise during the anaerobic digestion of SLW due to formation of ammonia which is similar to the results of this study.
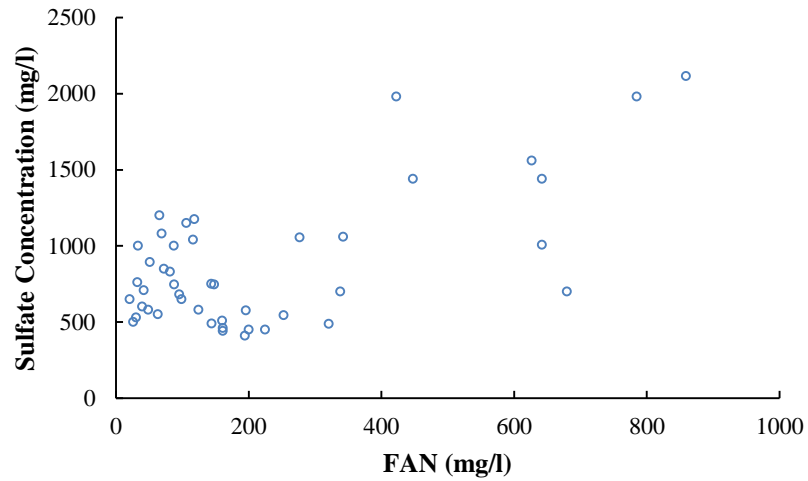
Figure 4.9: Sulfate Concentration Vs FAN

If the pH was controlled at 7.5, the FAN concentration would have maintained 92% lower. Thus, the system inhibition would have been avoided and efficient sulfate reduction might have achieved as shown in Figure 4.10.
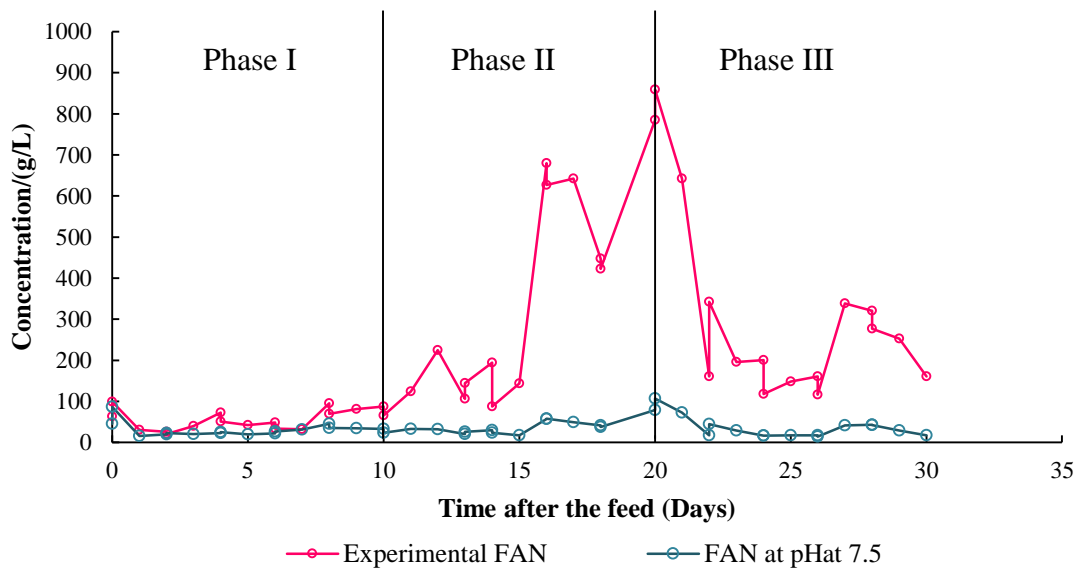
Figure 4.10: FAN Concentration Vs Time after feeding

4.1.3 Conclusions Derived from the Experiment A

When summarizing all the results obtained from experiment A, following conclusions can be drawn. For skim latex wastewater, controlling of pH around 7.5 is essential for

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both MB and SRB in order to have high sulfate reduction efficiency and minimize inhibition caused by free ammonia and free hydrogen sulfide which are continuously produced under anaerobic condition. For skim latex wastewater, the sulfate treatment efficiency can be increased by increasing the influent COD/SO_4^{-2} ratio using external electron donors. Additionally, adding external electron donors or organic matter to the anaerobic reactor system, would automatically control the ammonia inhibition which may occur due to lack of COD/TKN ratio in the feed stock. Adding external electron donor alone doesn't increase the sulfate reduction, but it is essential to control the pH around 7.5 while addition of external electron donor to get the optimum sulfate reduction efficiencies.

Major VFA components observed in sulfate reducing anaerobic digestion system are Acetic and Butyric. Whereas, some of the VFA components can only be seen during the initial acclimation period in which the sulfate reduction is less. But with high sulfate reduction, propionic acid was not observed. It is because SRB partially oxidizes the propionate to acetate and bicarbonate easily as the Gibb's free energy is less during sulfate reduction.

4.2 Effect of pH and electron donor in Ammonia rich Anaerobic conversion (Experiment B)

4.2.1 Effect of Ammonia on sulfate reduction in Anaerobic digestion without controlling pH (Phase I)

Phase I of experiments were carried out to identify the characteristics of Natural SLW under anaerobic conditions without controlling pH. The COD/SO_4^{-2} and the COD/TKN ratios of the influent were 2.7(~3) and 9.8 respectively. As a feed medium, Natural SLW is rich in high concentrations of sulfate, protein and organic matter. Under the anaerobic digestion, sulfate reduced to dissolved sulfide (S^{-2}, HS^-, $H_2S(aq)$) and gaseous hydrogen sulfide. Ammonia production was due to hydrolysis of protein and presence of initial ammonia due to ammonia added in preservation process. Results of major constituents are presented and discussed under each subtopic: Sulfate reduction, Ammonia evolution and Organic matter degradation.

The major difference of experiment A and Experiment B is that the operated digesters were used for experiment A, have been acclimated for six weeks only whereas experiment B was performed after 6 months of start-up period. However, in experiment A, the digesters were acclimated using natural skim latex wastewater, but in experiment B, initially during the acclimation period synthetic media added to enhance microbial growth providing more external nutrients. Nevertheless, during the experiment A, the digesters were operated semi-continuously whereas in experiment B, the digesters were operated semi-batch wise with cycle time of 6 days.

Oxidation Reduction Potential (ORP) values which indicates the degree of anaerobic reactions were in the range of -415 mV to -430 mV. Experiment was done in duplicate and both reactors showed similar pattern of variation of parameters. Hence, the average values are only presented and discussed. High negative ORP value depicts that the two reactors were maintained well under strict anaerobic condition.
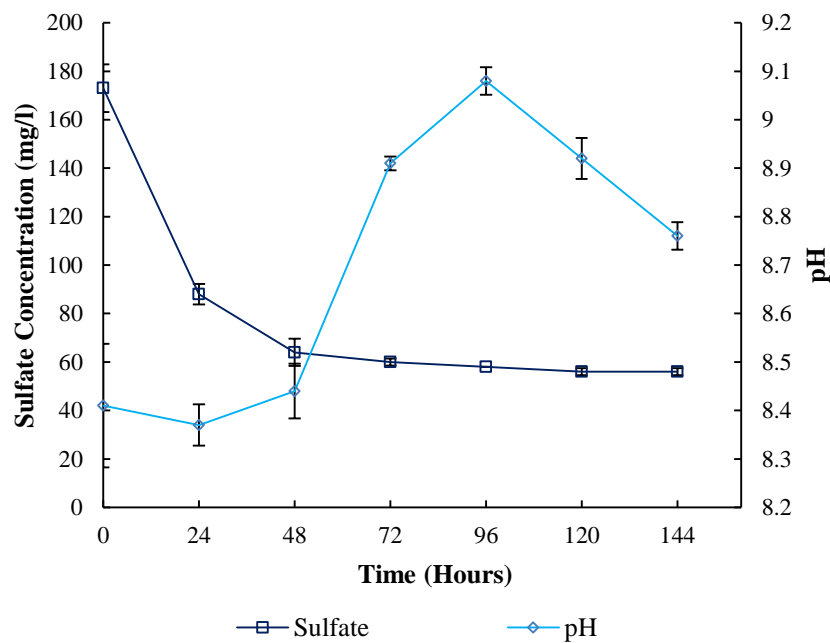
Figure 4.11: Sulfate concentration and pH Vs time

The most preferable pH for SRB is 7.5 to 8.5[90]. Therefore, the initial reactor pH was adjusted to 7.5 expecting pH reduction in the reactor with degradation of influent.

However, the experimental pH value showed a slightly decrease in the initial two days, but in latter days, the pH increased steeply and reached a maximum on 5th day after feeding. As recorded by R. Jariyaboon and S. Chaiprapat, pH reduction was recorded for Skim Latex Serum with continuous high rate anaerobic reactors with low HRT value. In anaerobic reactors where Sulfate Reducing Bacterial (SRB) is in progress with reducing the sulfate, alkalinity automatically increases due to formation of HCO_3^- [3] as described earlier, pH in the reactor is determined from the resultant effect of dominant reactions such as HCO_3^- formation in sulfate reduction. TAN formation from protein hydrolysis and total Volatile Fatty Acid formation by organic matter in the stage of Acidogenesis. The highest Sulfate reduction was recorded on initial two days (Figure 4.11), Thus the HCO_3^- generation is highest. But after about two 48 hours, pH increased steeply due to TAN generation through protein hydrolysis. However as per the experimental results reported by T.V. Nguyen[83] similar pH variation was observed for introduction of rubber latex processing wastewater with granular sludge in batch reactor; the batch reactor pH was decreased 5.2 - 5.6 in intial 2-3 days and then increased steeply to 7.2. The percentage pH reduction depend on the the accumulation of VFA. But, pH gradually increased with formation of ammonia and consumption of VFA by MB and SRB.

4.2.1.1 Sulfate Reduction

Sulfate concentration and Total dissolved sulfide concentration (S^{-2}, HS^-, $H_2S(aq)$) variation inside the reactor is shown in Figure 4.12. Sulfate steeply diminished initially, and subsequently no significant variation could be seen.
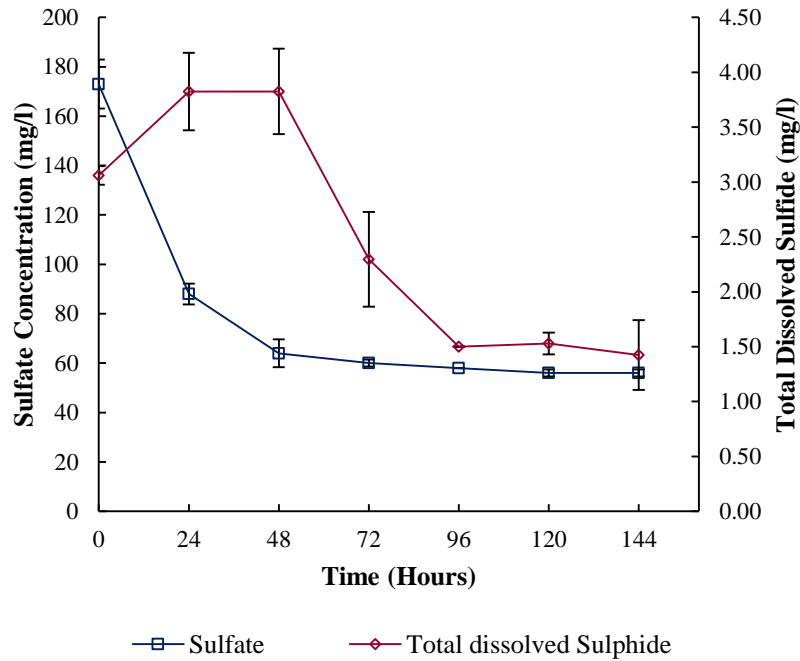
Figure 4.12:Sulfate and total dissolved sulfide concentration Vs Time
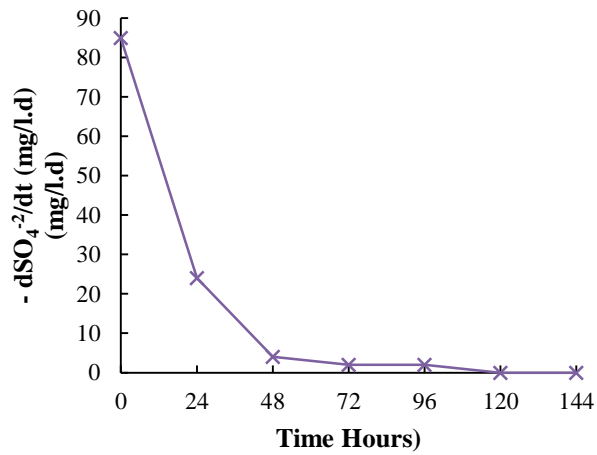
Figure 4.13:dSO$_4^{-2}$/dt Vs Time

According to Figure 4.13, it is evident that the rate of Sulfate reduction as well as the percentage sulfate reduction was high in first two days (Figure 4.14). However, both

the maximum rate of sulfate reduction per day, i.e. 85mg/day and the maximum Sulfate percentage reduction per day, i.e. 49.4% were during the first day after. feeding.
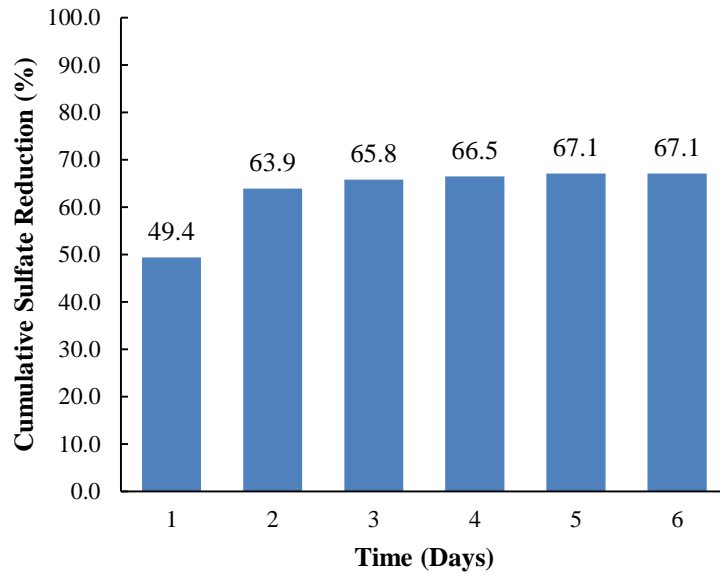
Figure 4.14:Cumulative percentage sulfate reduction Vs Time

The daily percentage sulfate reduction on second and third days were 14.5% and 1.9% respectively. Then on fifth day, the maximum cumulative percentge sulfate reduction of 67.1% was attained and thereafter the sulfate concentration was stagnant. Either the reactor has subjected into sulfide or ammonia inhibition or the available substrate for sulfate reduction may be not sufficient. D.M. McCartney and J.A. Oleszkiewicz [91] has also observed same pattern of sulfate reducing trends , but heir reactors were under sulfide inhibition.

The level of free H_2S concentration (un-dissociated H_2S) causes the severe inhibition on micro-organisms, penetrating through the cell membranes of micro-organisms and change the internal linkages, it interferes the assimilatory metabolic pathways or change the internal cell pH. Generally the methane production is known to be inhibited by 100-800mg/l of dissolved sulfide and 50-400mg/l of unionized H_2S[84]. In this batch reactor, Total dissolved sulfide concentration was below 8mg/l. Therefore, total dissolved sulfide concentration observed was always below the inhibitory sulfide

content. Thus, the Free H_2S content in the reactor was obviously below the inhibitory level. Therefore, slow sulfate reduction or incomplete sulfate reduction could not be due to free sulfide inhibition. According to the Figure 4.12, the total dissolved sulfide concentration was high at initial three days after feeding, as the rate of sulfate reduction was high.

The following composition analysis shown in Figure 4.15 emphasize that the phases exists with respect to Sulfur in the sulfurous compounds such as $S-SO_4^{-2}$, S-TDS and $S-H_2S$ in the reactor. On the first day after feeding, more than 50% of the sulfur in the reactor, emitted as gaseous $S-H_2S$ of 279 ppm (45.3%) and as total dissolved sulfide (6.9%). Gaseous H_2S emitted decreased by 44.7% gradually while reduction in sulfate break down diminish. However, the variation of TDS ($S-S^{-2}$, S^-SH^-, $S-H_2S$) has changed by 8.3% ± 2.3%.
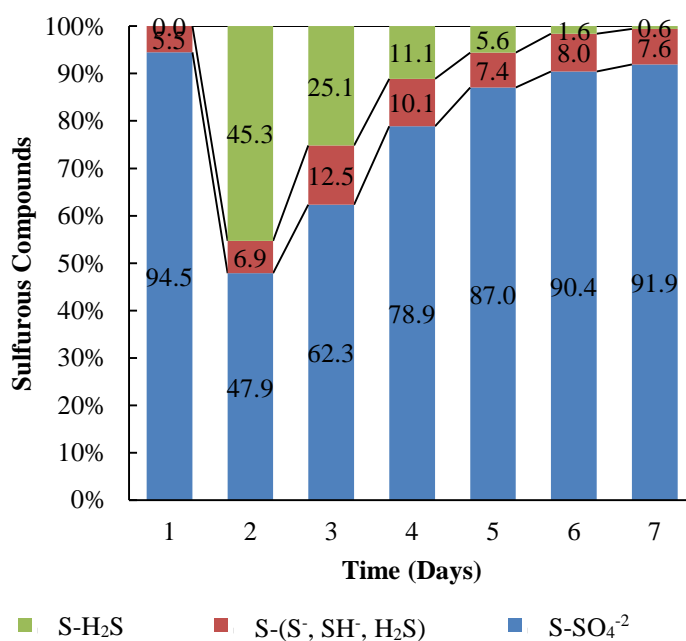
Figure 4.15: Variation of sulfurous compounds in the reactor Vs Time

The COD/SO_4^{-2} ratio represents the available organic matter for sulfate reduction which was only 2.7 g/g. SRB competes with MB for same available substrates for sulfate reduction. Therefore, sufficient substrate has to be available for completing sulfate reduction. Complete sulfate reduction was reported at COD/SO_4^{-2} ratio of 10

and 5, but strongly deteriorated at a COD/SO_4^{-2} ratio of 0.5 in a methanol fed UASB reactor [92] Sulfate removal efficiency decreased when the substrate to sulfate ratio became less than 6, because SRB faced strong competition for substrate with MB in an ethanol fed expanded granular sludge blanket (EGSB) reactor[93]. The COD/SO_4^{-2} ratio at 0.67 on molar basis is the minimum requirement recorded for sulfate reduction [17]. The stoichiometric amount of organic matter requirement for complete sulfate reduction is still under experiments with real wastewater using different configurations of reactors. However, lower COD/SO_4^{-2} ratio available in the influent SLW might have been a reason behind low sulfate reduction percentage observed.

4.2.1.1 Evolution of Ammonia

SLW is rich in ammonia and protein compounds as well. Ammonia was readily inclusive in the influent as Total Ammoniacal Nitrogen (TAN) 725 mg/l Skim Latex wastewater. because Ammonia is used heavily in the latex industry for preservation. Nevertheless, influent contains proteins with COD/TKN ratio of 9.8. Under anaerobic condition, organic nitrogen within the protein chains converted into ammonia. Release of Ammonia from hydrolysis of amino acids increases both alkalinity and pH of the digester. Acetate in the reactor are converted to ammonium acetate or ammonium bicarbonate acetate into ammonium acetate by generated ammonium ions. This phenomenon depletes the acetate which is the substrate for the Sulfur Reducing Bacteria (SRB) and Methanogenic Bacteria (MB). Thus, this inhibits the biological activities in the reactor[58].

T.V. Nguyen has found that the rubber latex processing serum contains 810 – 1,565 mgN-or g/l as proteins which consists of 14 types of amino acids such as glycine, alanine, leucine, glutamic, etc. This is equivalent to about 5,000-9,800 mg/l proteins[5]. Under anaerobic condition, the proteins are first hydrolysed to peptides and amino acids and subsequently the amino acids are fermented to short chain or branched chain fatty acids, ammonia and CO_2. As per the Figure 4.16, the protein hydrolysis rate was high at the beginning of the experiment, which is represented by the increase in net aqueous Total Ammoniacal nitrogen concentration (TAN). Highest Ammoniacal nitrogen generation rate or protein hydrolysis rate recorded on the first day after feeding, was 361.5 mg/l.d. Then TAN gradually decreased. Then again on

the 7th day, there was an increment in the TAN. It might be due to the dominance of breakdown of balanced protein in the reactor. The TAN , Free Ammonia Nitrogen (FAN) and Total Dissolved Sulfide (TDS) of the reactor are shown in the Figure 4.16, Free Ammoniacal Nitrogen (FAN) of the system was calculated using equation 7 in section 2.4.4 given by J.A. Siles [57] and R. Rajagopalan et al.[23], using the TAN, pH and the operated temperature (35 °C).
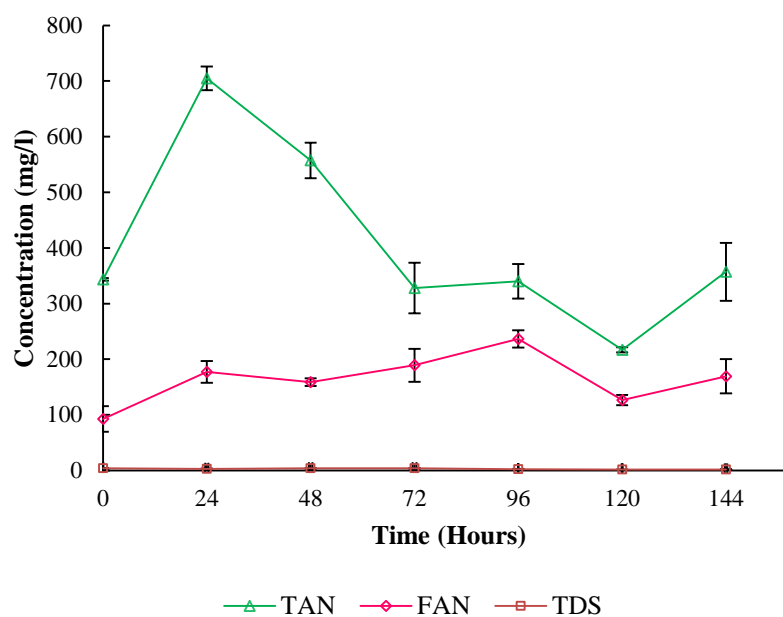
Figure 4.16:TAN, FAN nd TDS Vs Time

Throughout the experiment, the total dissolved sulfide concentration was below the inhibitory level. Thus the free H_2S level is also below the inhibition level of 110 mg/l[17] . But the aqueous free ammonia was 166.7± 50 mg/l, which exceeded the FAN level of inhibition reported, 150mg/l by McCarty and McKinney[17] and 80mg/l Koster and Lettinga[73]. FAN amount depends mainly on TAN concentration, pH and the temperature. Although the TAN was very high in initial days, free ammonia content did not increase up to higher concentration due to comparative low pH in the bulk liquid. In final days, because of the high. free ammonia concentration in the reactor, pH also increased. Rajagopal R. and his team also confirmed that at high pH values, above 8.5 the fraction presents as FAN steeply increased as in Figure 4.17

adapted from R. Rajagopal [23]. With the variation of pH value of the reactor, FAN concentration varies. FAN is less than 1% of total TAN at pH 7, whereas at pH 8 it has risen to 10% and at pH 9 it has increased to 48%. Thus the inhibition due to FAN also increased proportionately[22].

However, Free Ammonia causes the highest inhibition on micro-organisms than the TAN and the amount of free ammonia was dependent on the pH of the reactor medium[58]. Existing knowledge contribution by C. Gallert and his team [23]describes the ammonia inhibition to MB can be taken place in two pathways, i.e. (i) Ammonium ion may inhibit the methane producing enzymes directly and/or (ii) hydrophobic ammonia molecule may diffuse passively into bacterial cells, causing proton imbalance or potassium deficiency. The literature on Ammonia inhibition to SRB are lacking, but there could be some similar mechanism.
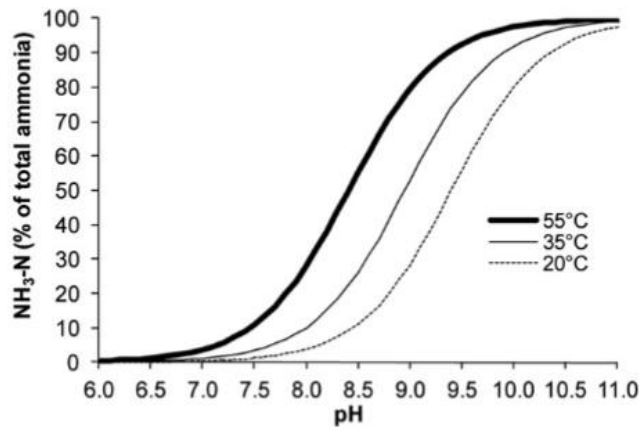
Figure 4.17:FAN percentage in solution at 20, 35 and 55 °C Vs pH
(Adapted from Rajagopalan R. et al.[23])

F. Omil et al has found that for anaerobic reactors which treat wastewaters rich in both protein and sulfate face the challenge of increase high FAN and free H_2S concentration. Because of high free ammonia and free H_2S inhibit micro-organisms, specially MB. Specially COD removal efficiency was affected as shown in Figure 4.18. As per the Figure 4.18, with increasing the pH starting from 7.2, Free H_2S diminished while the Free NH_3 (FAN) increased. In order to achieve successful anaerobic treatment of these kinds of wastewaters which both sulfate and ammonia,

minimization of both free hydrogen sulfide and free ammonia are essential and it was easily achieved maintaining the pH around 7.25- 7.6. At this pH range both free H_2S and free NH_3 gets minimum as shown in Figure 4.18.
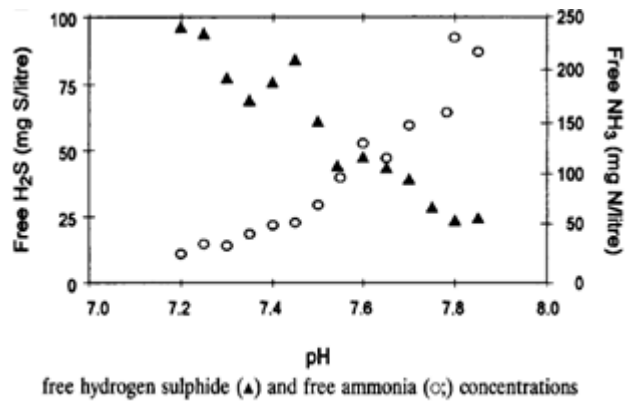
Figure 4.18: Free H_2S and Free NH_3 concentration vs pH in the digester
(Figure Adapted from Omil F.[10])

Although F. Omil et al. suggested that dilution of the protein rich wastewater before entering the anaerobic reactor, in most of other studies acid solution was added in adequate amount to maintain the pH at the desired value. T. Imai. and his team also were able to control the inhibition of both Ammonia and dissolved Sulfide by controlling the pH at 7.0-7.5[94]. But with pH adjustment, Ammonia inhibition can be controlled and operation of the reactor under stable condition is possible, but methane yield is less.

The free ammonia concentration at pH 7.5 was calculated using the TAN of the anaerobic reactor by both N. Krakat et al. [95] and R. Rajagopal et al. As illustrated in Figure 4.19, the free ammonia concentration reduced by 90% by maintaining free ammonia concentration at 17.2±7.7 mg/l. Therefore, anaerobic reactor pH is suggested to be maintain pH at 7.5 to reduce the free ammonia inhibition and increase the treatment efficiency of the system.
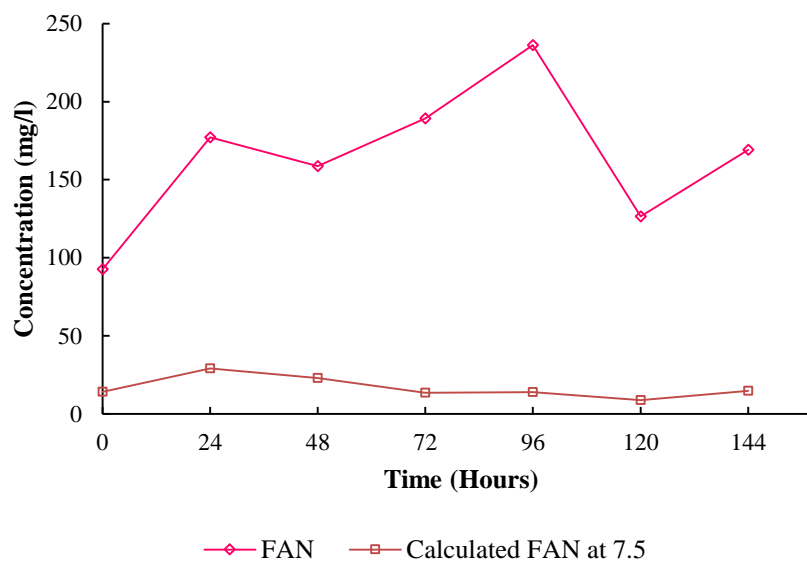
Figure 4.19:Experimental FAN and FAN at 7.5 Vs time

Strategies to overcome the ammonia inhibition are acclimation of microflora, pH control, adjusting COD/TKN ratio of the feed stock, temperature control, dilution of reactor content. But among all, pH control and adjusting COD/TKN ratio of the feed stock are the most commonly used[55], [23], N. Krakat et al. also confirmed it through their review on ammonia inhibition prevention methods[95]. Therefore, in further studies on skim latex wastewater, pH control and adjusting COD/TKN ratio of the feed stock will be performed under this research.

4.2.1.2 Effect of sulfate removal on Organic matter degradation

, The soluble Chemical Oxygen Demad (sCOD) and Total Volatile Fatty Acid (VFA) concentration value in the two reactors are shown in Figure 4.20. There is a steep reduction in the sCOD in the firstday after feeding the substrate and 74% sCOD drop was observed. But only 22.5% of total Volatile Fatty Acid (VFA) formed. Thus it was evidenced that the reduced sCOD is mainly consumed by SRB for sulfate reduction as SRB reactions are dominant than MB[96], [97]. Total sCOD reduction observed was 80%.
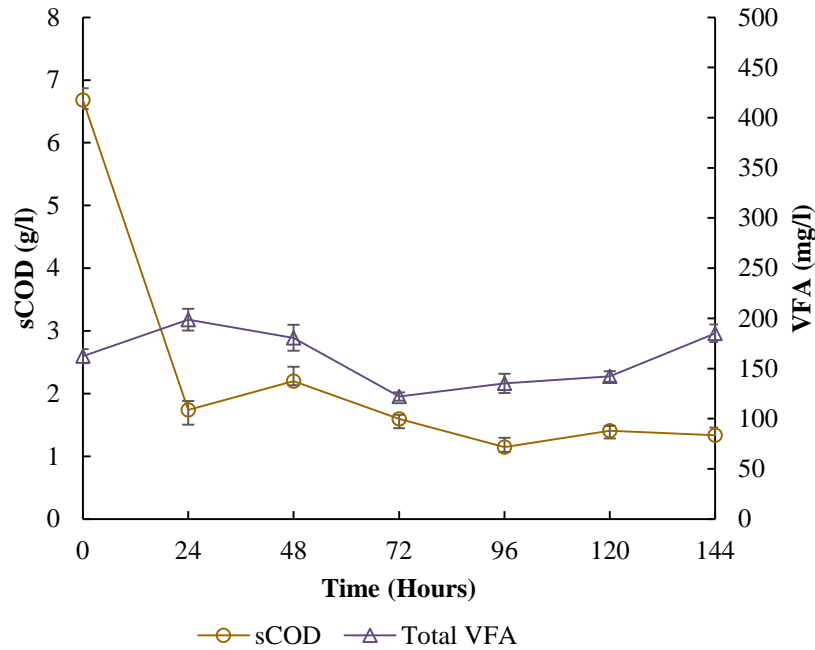
Figure 4.20:sCOD and Total VFA in the reactor Vs Time

SRB consume only simple organic compounds which produce at stages after the acedogenesis stage of anaerobic digestion of organic matter, such as fatty acids, ethanol and Hydrogen [71]. With the feed, SRB sufficiently had received simple organic substrate via sCOD of 7,372 mg/l with the feed as well as simple organic substances generated through organic matter degradation on the firstday and SRB dominate over MB in competition for the availavle substrate [98] . This must have been the reason for the extensively high rate of sulfate reduction in the first day and less there after. Complex organic matter must be degraded in to simple organic matter for SRB to be utilized in the sulfate degradation process. Hence rate of acedogenesis is a rate limiting factor for SRB.

Further, as illustrated in the Figure 4.20, although there is a steep reduction in the sCOD, the increment in the VFA is very less. Therefore, it can be concluded that the sCOD manily comprises of VFA and balance VFA might have consumed by SRB for sulfate reduction. It was further observed that the percentage sulfate reduction is correlated to the sCOD removal according to the Figure 4.21. The correlation between

sulfate reduction and lactate removal was observed by D.M. McCartney and J.A.Oleszkiewicz as well[91].
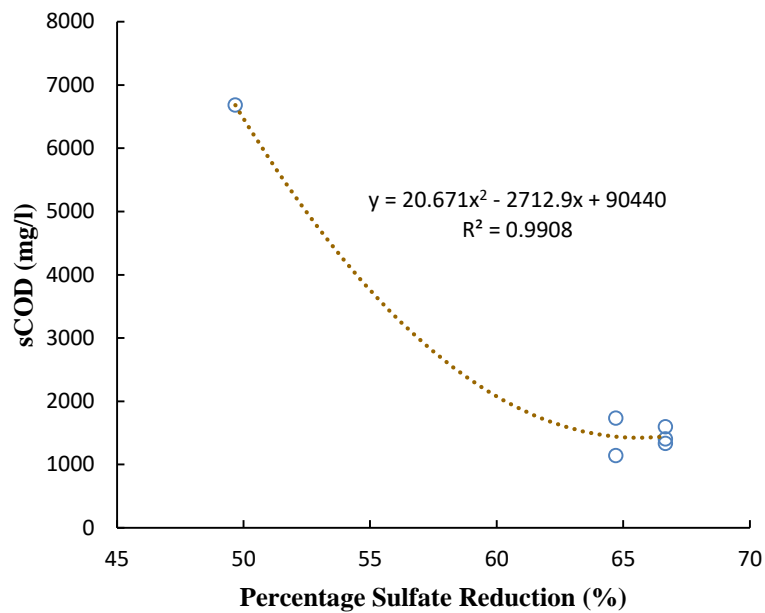
Figure 4.21:sCOD Vs Sulfate Concentration

As per the Figure 4.11, even after the 7th day following the feeding, 32.9% sulfate concentration remained in the reactor without breaking down, where as the final Total VFA was 185.0 mg/l. S. Chaiprapat [1] also recorded that in his experiment 55.5% and 72.6% sulfate remained respectively in the effluent of Up flow Anaerobic sludge blanket and anaerobic sequencing batch reactor while the effluent Total VFA (asCH$_3$COOH) was 5,885±493 mg/l and 6,370±614 mg/l in each anaerobic digester. This was due to inhibition of micro-organisms. After the 4th day onwards, percentage sulfate reduction of the batch reactor was less than 1.85% and the pH of the reactor was also above 8.9.   D. Mara and N. Horan emphasized that not only MB but also SRB inhibited above pH 9 or below 5.5[99]. Therefore, it can be concluded that high pH value in the reactor affected SRB. Thus, sulfate reduction is very less or no change in subsequent days even acetic acid was available as a substrate. there are some more factors which affect the sulfate reduction such as the type of substrate, the relative population and the characteristics of specific kinds of SRBs and other microorganisms,

sensitivity for sulfide inhibition of each species, temperature and pH. Although the experiment was conducted for six days until sulfate reduced significantly, the experiment would have extended more, to get more clear understanding on the remaining sulfate concentration.

4.2.1.3 Effect of sulfate reduction on Volatile fatty acid
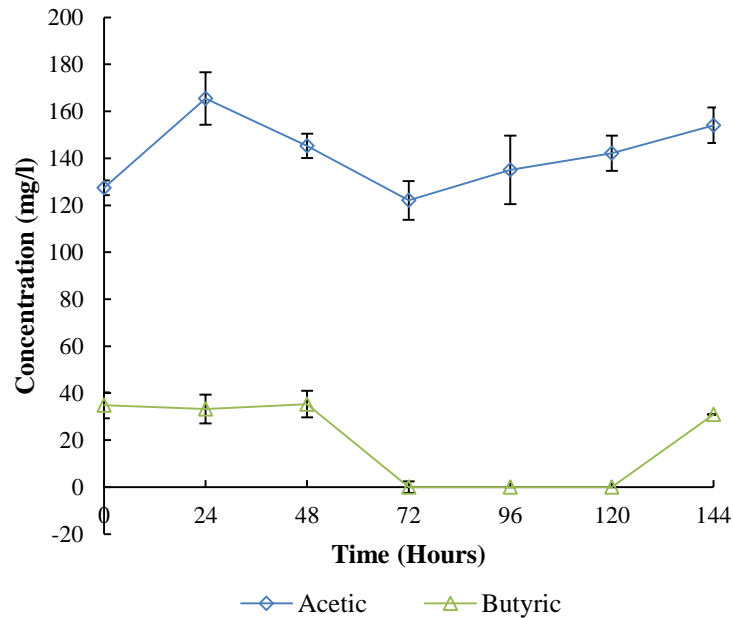
Figure 4.22:Acetic and Butyric Concentration Vs. Time

Volatile Fatty Acids found in the anaerobic batch reactors, were acetic and butyric and their concentrations were 141.6 ±15.1 and 19.2 ± 18.0 mg/l respectively (Figure 4.22). V. O'Flaherty et al.[100] has also observed acetate, butyric and propionic at the acclimation or initial period of anaerobic reactor which was treating sulfate rich wastewater, But after long period of operation, where sulfate reduction was high, acetate and butyric were found , but not propionic. As these reactors also have high sulfate reduction and the reactors were operated for some time before the experiment, propionic was not observed.

Acetic concentration was 88.1% higher than butyric. Sulfate acts as an electron acceptor of sulfate reducing bacterial respiration. Electron donors are usually hydrogen and organic compounds with higher and branched fatty acids, ethanol and higher alcohols, other organic acids, alkanes and aromatic compounds [84]. The order of SRB affinity for substrate reduction is H_2> propionate> other electron donors[42]. In

complete oxidation process SRBs produce CO_2. On the other hand, there are some other SRBs who produce intermediate products such as lactate, acetate and sulfide from partial oxidation. Most SRBs reduce sulfate to sulfide, partially oxidizing such products as ethanol and propionic to acetate[100] in the first step. This could be the reason that the fraction of acetate was high in the reactor and propionic was not observed. Further at high pH values above 8.9, butyric was not seen, but only acetic. As explained earlier, both MB and SRB were inhibited at high pH values[101].
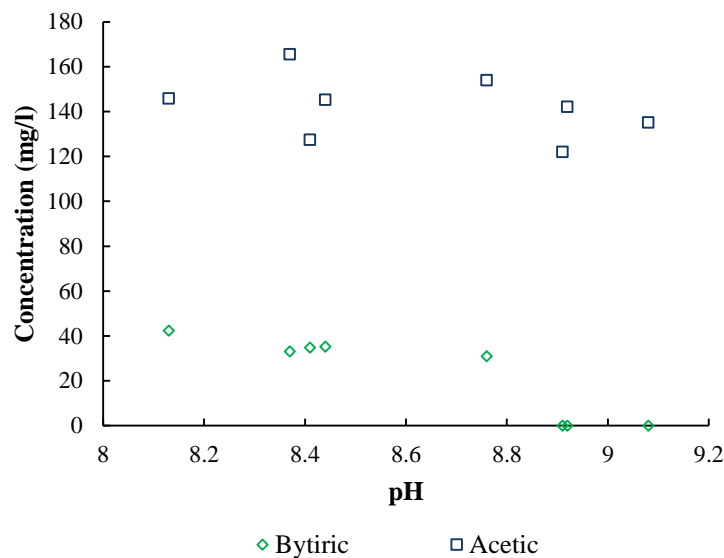
Figure 4.23:Acetic and Butyric Concentration Vs. pH

Biogas volumes produced is shown in Figure 2.24, illustrated. The largest biogas volume produced in the first day after feeding, and gradually decreased with time. Emmision of H_2S, NH_3 and CH_4 and CO_2 gasses in biogas are related with major reactions involved with sulfate, TAN and sCOD. But gas composition analysis was not able to carried out to confirm the fraction of gasses which indirectly explained liquid phase reactions, but only the volumetric bio gas quantities
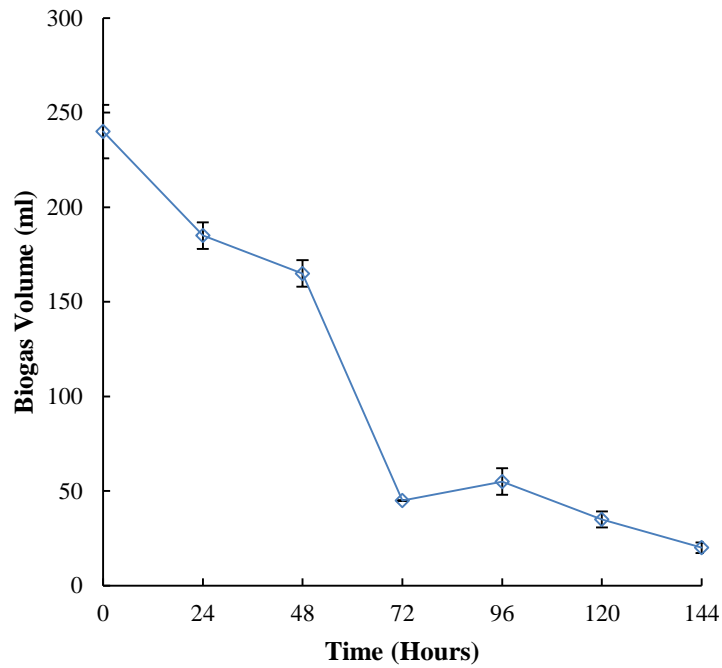
Figure 4.24:Biogas volumeVs. Time

4.2.2 Effect of pH control on sulfate reduction (Phase I and Phase II)

Under phase II, there was 83% reduction of inhibitory FAN in the reactor, in which the pH was controlled at 7.5 to 8.0. The FAN concentrations of the uncontrolled pH condition was 164 ± 47.7 mg/l, whereas it was only 28.2 ± 10.2 mg/l when pH of the AD maintained at 7.5 to 8.0 in phase II (Figure 4.25). Therefore the cumulative percentage sulfate reduction as well as the rate of sulfate reduction has been improved by 10.1% and 15.7%. The cumulative sulfate reduction percentage variation of both experiments are illustrated in Figure 4.26. FAN concentrations of phases III and IV were also below the inhibitory level. Thus it is convinced that sulfate reduction enhanced with control

of pH of the reactor at 7.5 to 8.0, while inhibition caused by FAN was minimized and operation of the reactor was stable.
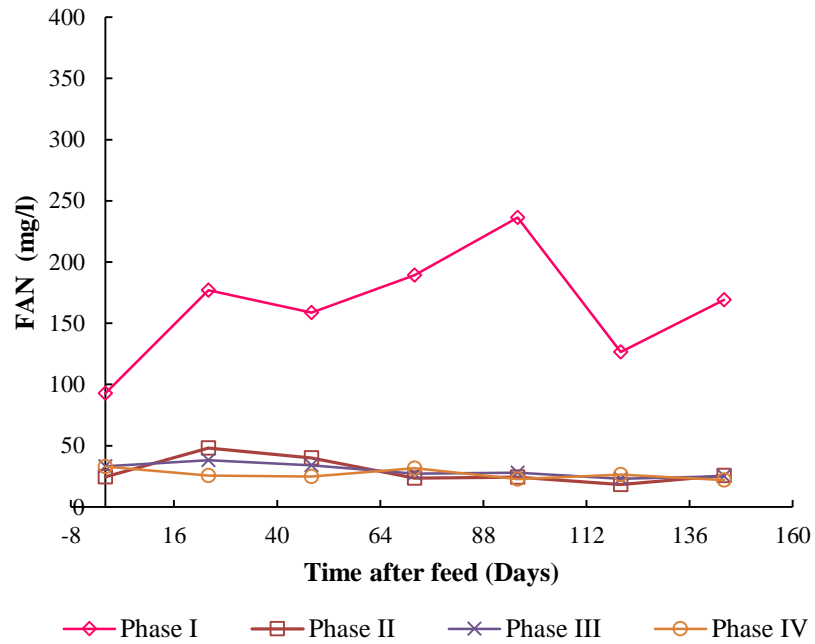
Figure 4.25: FAN Concentration with time
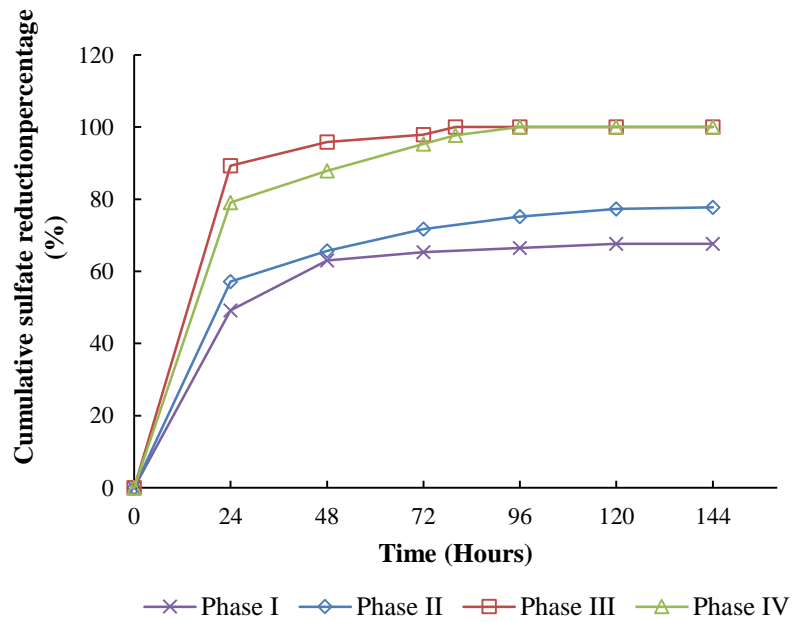
Figure 4.26: Cumulative sulfate reduction percentage Vs Time

4.2.3 Effect of influent COD/SO$_4^{-2}$ ratio on the sulfate reduction (Phase II, III and IV)

Natural SLW was fed to complete anaerobic reactor which was operated in semi-batch wise with 6 days' cycle time by varying the influent COD/SO$_4^{-2}$ ratio at 3, 5 and 10 whereas the pH of the reactor in three phases were maintained at 7.5 -8.0. Then sulfate reduction was closely monitored in the reactor by measuring the daily sulfate concentration. The pattern of Sulfate degradation was as shown in Figure 4.27.
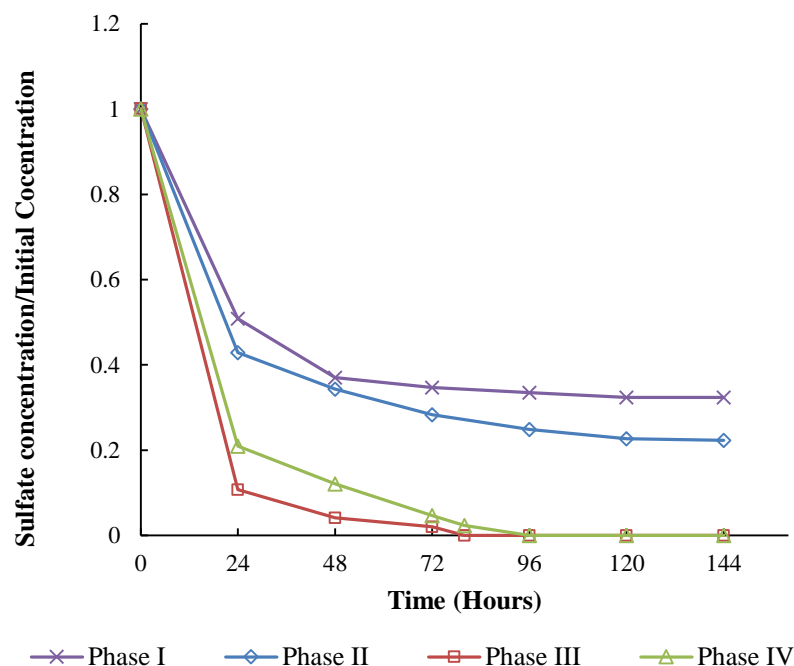
Figure 4.27:Sulfate concentration in the anaerobic reactor vs time

4.2.3.1 Effect of semi batch cycle time on Sulfate reduction

As shown in Figure 4.27, sulfate drastically decreased on the first day after feeding for all COD/SO$_4^{-2}$ ratios at 2.7, 5 and 10. However, during phase II, for all three COD/SO$_4^{-2}$ ratios, both maximum daily sulfate reduction percentage and the highest rate of sulfate reduction recorded on the first day after feeding. Then gradually decreased with time. The daily percentage sulfate reduction for SLW for COD/SO$_4^{-2}$ ratio at 2.7 decreased from 57.1% to 1.9% in the second day. Percentage sulfate reductions are presented in Figure 4.26. SRB require simple organic substrate as intermediate

products of acidogenesis and acetogenesis stages for sulfate reduction. Influent contained 1390mg/l carbonic matter via BOD which can be readily consumed by SRB as well as sCOD of 7372 mg/l which represents the easily biodegradable fraction of organic matter. Therefore, as soon as the anaerobic reactors are fed with the influent SLW sample, SRB who are dominant than MB according to past literature, have sufficient organic matter for sulfate reduction process. Therefore, the maximum average sulfate reduction rate was recorded during the first day after feeding which was 120mg/l.d. (Figure 4.28). But both daily sulfate reduction percentage as well as rate of sulfate reduction decreased with time due to steep reduction of available reactants in the digester, reaching accumulated sulfate reduction of 77.7% on the 6th day after the feed. Rate of organic matter hydrolysis and acidogenesis are rate limiting reactions for sulfate reduction. Following consumption of all simple organic matter, the remaining complex organic matter in the influent SLW has to be broken-down into simple compounds by acidogenesis which are subsequently degraded by SRB.
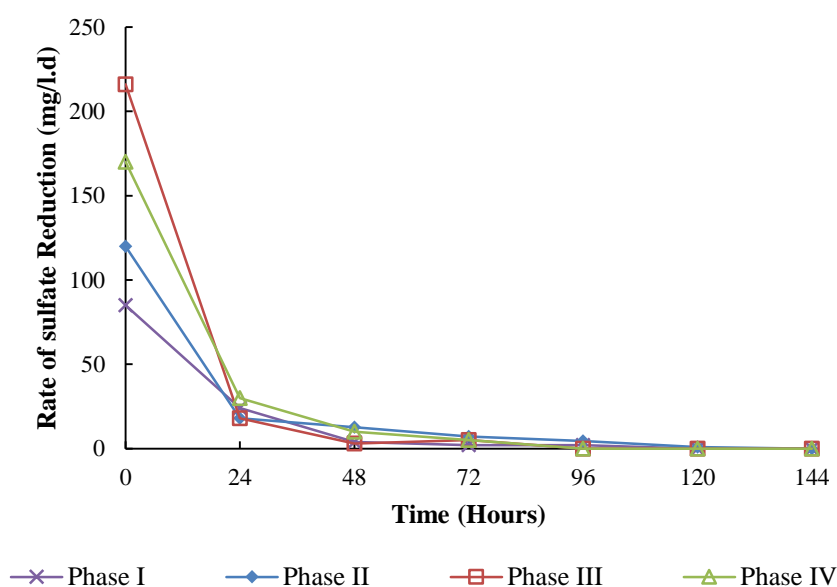
Figure 4.28: Rate of Sulfate reduction vs time

Next, the available substrate concentrations of the natural SLW were increased using external electron donor, acetate in phase III and phase IV. Similar Sulfate reduction

trends were seen in other levels of COD/SO_4^{-2} ratios at 5 and 10 as well. Their maximum daily sulfate reduction percentages and rates were 89.3%, 79.1% and 216.4 mg/l.d, 170.0 mg/l.d respectively. Following feeding, 100% sulfate removal reached for COD/SO_4^{-2} ratio at 5 and 10 with 80 hours and 96 hours respectively. According to above stated results, it is evidenced that both the percentage sulfate reduction and rate of sulfate reduction in phase III (COD/SO_4^{-2} ratio 5) were higher than the phase IV (COD/SO_4^{-2} ratio of 10). Nevertheless, complete sulfate reduction was reached faster under COD/SO_4^{-2} at 5 than 10.

The above explanation for sulfate reduction was also evidenced according to the variation of total dissolved sulfide (HS^-, S^{-2} and aqueous free H_2S) concentration as per Figure 4.29. Fraction of broken-down sulfate prevail as aqueous total dissolved sulfide and the other fraction emit as gaseous hydrogen sulfide. For all COD/SO_4^{-2} ratios, first day total sulfide concentrations were higher corresponding to the maximum sulfate reduction period and then it decreased with the time. But during total operation period, the total dissolved sulfide level was below the inhibition range of 100-800 mg/l dissolved sulfide and 50-400 mg/l un-dissociated H_2S[71]. H_2S produced during the experiment was as shown on Figure 4.30.
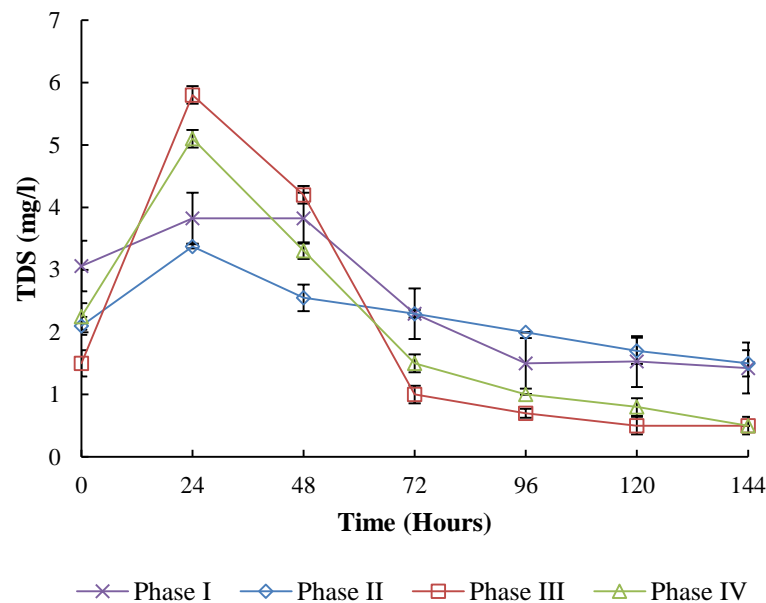
Figure 4.29:Total Dissolved Sulfide Concentration vs time
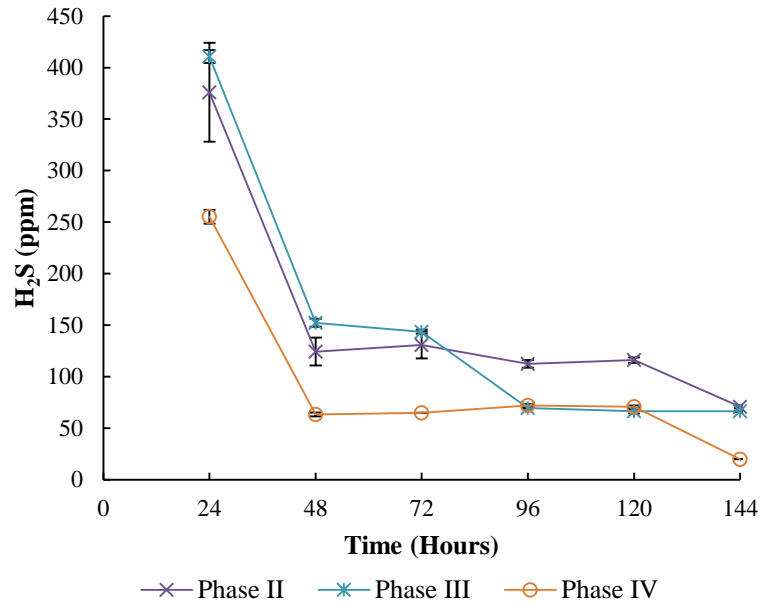
Figure 4.30: H_2S concentrations measured during the experiment

4.2.3.2 Effect of influent COD/SO₄⁻² ratio on sulfate reduction.

SRB converts sulfate into sulfide via dissimilatory sulfate reduction. This process of bacterial respiration occurs under strict anaerobic conditions and uses sulfate as the terminal electron acceptor. Electron donors are usually organic compounds and hydrogen.

In phase II at COD/SO₄⁻² ratio 2.7, the first day percentage sulfate reduction was only 57.7% and the cumulative sulfate reduction was 77.7% on the 6th day after feeding. The total dissolved sulfide concentration was also below the inhibition range of 100-800 mg/l [17] and Koster and Lettinga proposed a 80mg/l of Free Ammonia inhibition-N as minimum inhibitory level, while 150mg/l free ammonia was proposed by McCarty and McKinney [57]. After increasing the COD/SO₄⁻² ratio to 5, addition of external electron donor in phase III sulfate reduction increased considerably. From Figure 4.22 and 4.23, it can be clearly understood that the sulfate reduction percentage as well as the rate of sulfate breakdown has increased in SLW when the COD/SO₄⁻² was increased from 2.7 to 5 (phase II and III). First day percentage sulfate reduction improved from 57.7% to 89.3% and rate of sulfate reduction has enhanced from 120.0 mg/l.d to 216.4 mg/l.d which was 80.3% increment. Nevertheless, Sulfate was 100%

removed by adding external electron donor with adjusting the COD/SO_4^{-2} ratio to 5 with the minimum time period with less than 80 hours after feeding. It is hindered, that although the influent COD/SO_4^{-2} ratio expresses as 2.7, the simple fraction of organic matter available for consumption of SRB might be less in SLW due to presence of complex organic and inorganic substance. Thus, hydrolysis and acidogenesis are the rate limiting step for sulfate reduction.

tCOD is considered in calculating the COD/SO_4^{-2} ratio, it is hindered, that although the influent COD/SO_4^{-2} ratio expresses as 2.7, the simple fraction of organic matter available for consumption of SRB might be less in SLW due to presence of complex organic and inorganic substance. Thus, hydrolysis and acidogenesis are the rate limiting step for sulfate reduction.

Following addition of external electron donors, increasing the COD/SO_4^{-2} from 2.7 to 10(phase II and IV) also increased the percentage sulfate reduction and rate of sulfate reduction as well. But it is slightly lower compared with phase II. After providing the external electron donor, the daily percentage sulfate reduction for natural SLW at $COD/SO4^{-2}$ ratio of 2.7 was increased from 57.1% to 79.1%. On the other hand, it was convinced that the rate of sulfate removal also improved from 120.0 mg/l to 170mg/l at phase III. Therefore, not only the quantitative sulfate reduction, but also the rate of sulfate reduction was able to be improved as required by adjusting COD/SO_4^{-2} by addition of appropriate external electron donors.

According to the results, sulfate reduction enhanced from phase II to phase IV after external electron donors were added up to $COD/SO4^{-2}$ ratio of 5, but when organic substances were increased further up to $COD/SO4^{-2}$ ratio of 10 in phase IV, percentage sulfate reduction and average rate of sulfate reduction did not increase, on the other hand it slightly decreased (Figure 4.31 and 4.32). The average rate of reductions from phase II to phase IV is 1.13, 2.52 and 2.24 mg/l.h respectively as shown in Figure 4.32.
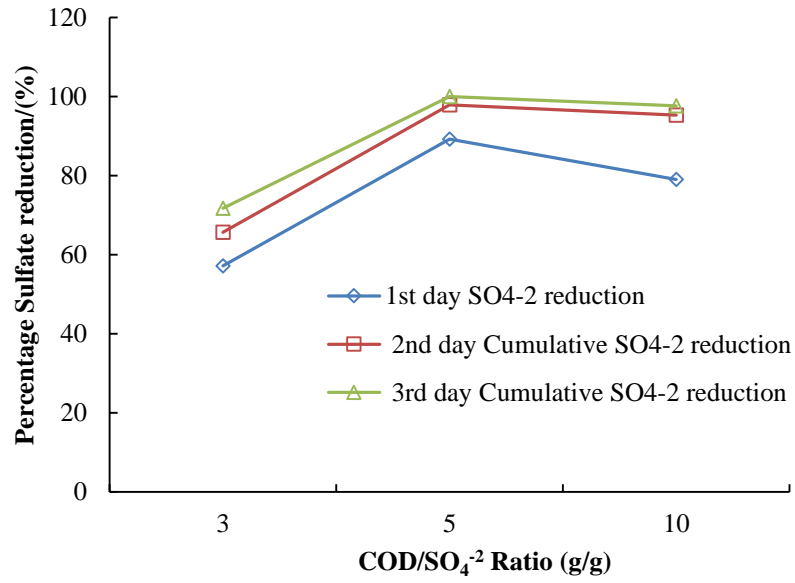
Figure 4.31: Percentage Sulfate reduction vs COD/SO_4^{-2}
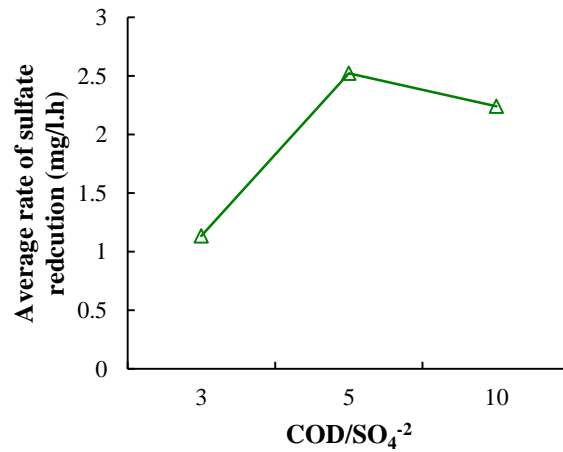
Figure 4.32: Average rate of sulfate reduction Vs time

4.2.3.3 Competition between SRB and methanogens

When excess sulfate present in the wastewater, SRB has to compete with acetogen and methanogen bacteria for the available substrate. This competition determines the final proportion of sulfate reduction and methane production. This is highly competitive at low COD/SO_4^{-2}. According to the standard Gibbs free energies (Table 2.2), at a

94

condition where there is no sulfate limitation, SRB completely consume hydrogen whereas acetate, propionate and butyrate degrade faster by SRB than MB[71].
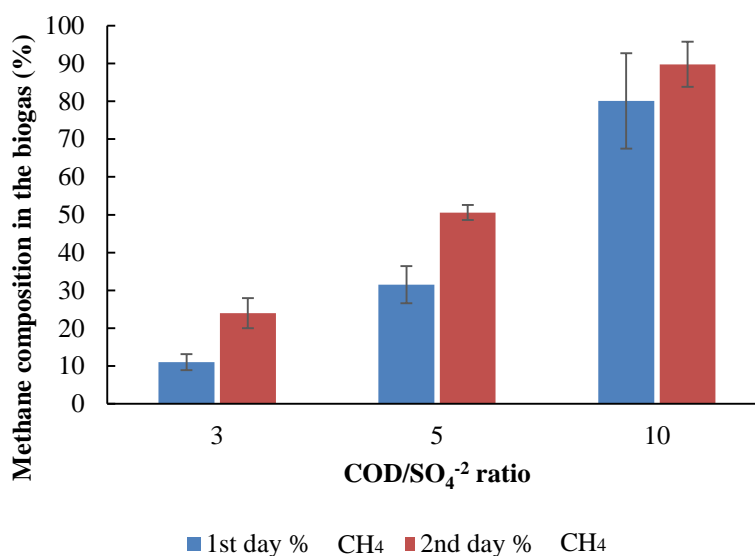
Figure 4.33: Percentage Sulfate reduction vs COD/SO$_4^{-2}$ Ratio

According to this experimental results, the methane formation of the SLW under complete anaerobic condition with COD/SO$_4^{-2}$ ratio of 2.7 (Phase II) was very less; first day methane production was only 11% and the second day methane composition was 24% (Figure 4.33) whereas the first day sulfate reduction was 57.1%. Therefore, with these observations, the sulfate reduction reaction seems to be dominant than methane formation.

In phase III, at influent COD/SO$_4^{-2}$ ratio of 5 when more carbonic substances added, the SRB dominate over the MB. Thus, the first day sulfate reduction further improved to 89.3% and methane formation was still 31.5%.

As per phase IV, after adding excess electron donors, until influent COD/SO$_4^{-2}$ of 10, percentage sulfate reduction was 79.1% and methane composition in the biogas increased to 80.1%. Although, Sufficient amount of organic substrates are essential for sulfate reduction, in excess COD, with increasing influent COD/SO$_4^{-2}$ ratio from 5 to 10 has created a more favourable condition for MB. However, the first day percentage sulfate reduction has reduced from 10.2%, and the first day rate of sulfate reduction also decreased by 46.4mg/l.d and 48.6% increase in methane formation. There was

another special result notified in the Figure 4.33. Methane formation was less in the first day, while the maximum sulfate reduction taken place on the first day in all three phases; i.e. 11.0%, 31.5% and 80.1%. respectively. The second day methane formation comparatively increased, when the remaining sulfate concentrations were relatively low; 24.0%, 50.6% and 89.8%.

Similar results were also observed by some researches. At low COD/SO_4^{-2} ratios SRB dominate and in high COD/SO_4^{-2} ratios especially with using acetic as the electron donor, dominance of MB was reported. Gupta et al., Middleton and Lawrence had observed the expected pre-dominance of acetate utilizing SRB (ASRB) over acetate utilizing MB (AMB) in continuously stirred tank reactors and in anaerobic contact process[102]. Although usually SRB are more dominant than MB in sulfate rich anaerobic environment, for acetate, either MB or SRB can be dominant according to the past literature with high COD/SO_4^{-2} ratio values or in modern high rate anaerobic reactors. Several studies have reported the dominance of MB or methane formation process even with excess sulfate, while some other researches like Omil et al. reported predominance of ASRB[10].
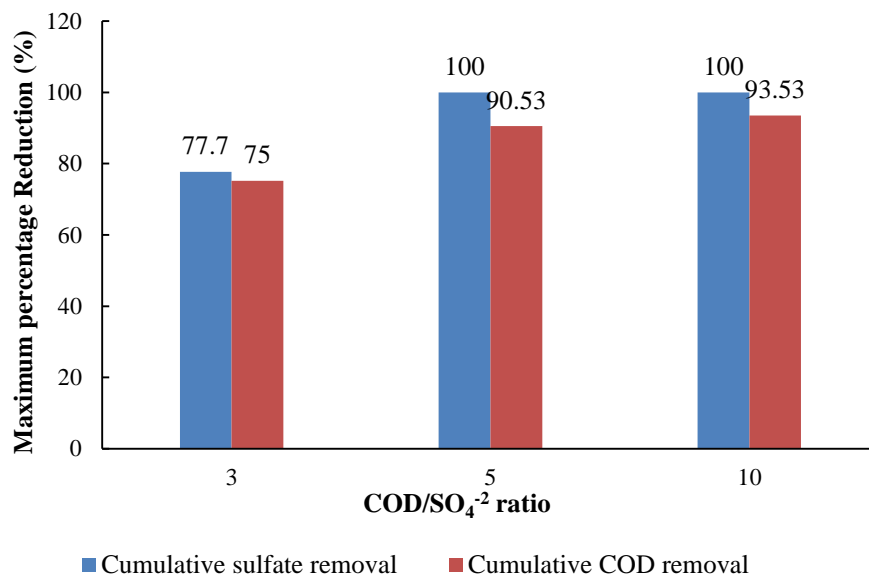
Figure 4.34: Maximum Percentage reduction of Sulfate and COD after 6 days of batch time

The maximum sulfate reduction and the maximum COD removal recorded were shown in Figure 4.34. Sulfate reduction or kinetic reactions of SRB was faster than

COD reduction. Biogas volumes produced in the experiment are shown in Figure 4.35. Daily collected biogas volumes were measured by water displacement method using inverted measuring cylinder. Collected biogas volumes were daily removed using valve on top of the inverted cylinder as shown in the schematic diagram of the experimental setup in Figure 3.40 for gaseous H_2S analysis.
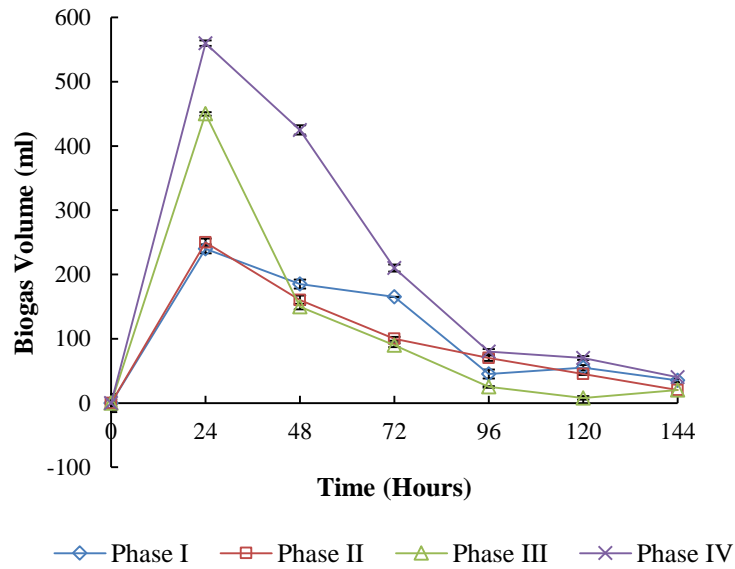
Figure 4.35: Generated Biogas Volumes Vs Time

Dominance of MB was difficult to be predicted from the ORP value of the anaerobic reactor as different variety of reactions taken place in the single reactor and the ORP measured is a result of all these reactions. Measured ORP values are shown in Figure 4.36. The ORP values of the three phases from phase II to phase IV were -426.6 $\pm$ 4.4mV, -418.11 $\pm$ 7.8 mV and -396.9$\pm$ 10.8 mV. respectively.
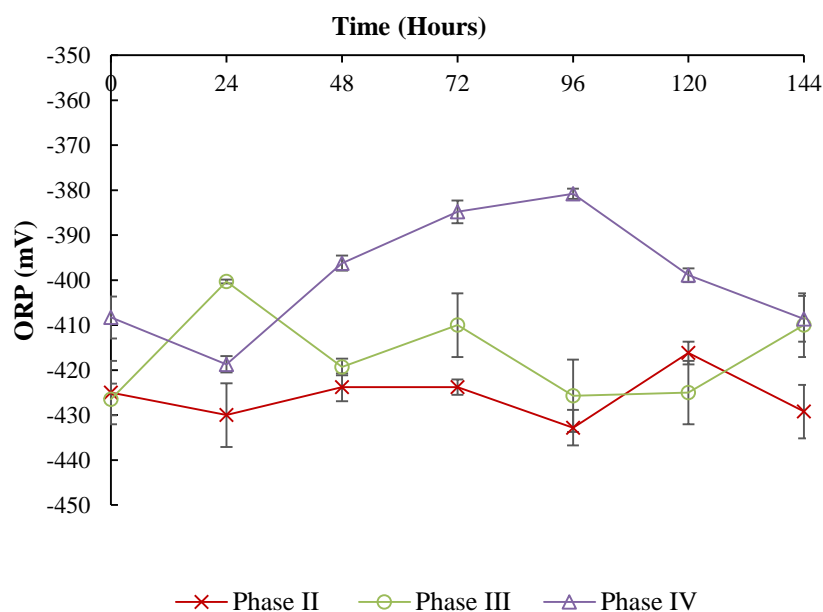
Figure 4.36:Oxidation Reduction Potential vs Time

During phase II and IV in which the sulfate reduction or SRB were dominant, because of high negative value reported i.e. below the range of -400 mV. However, in the phase IV the ORP value were higher in the range of -396.9 ± 10.8 mV, which corresponded to more methane production. Literature on correlation between biochemical reactions and corresponding ORP values are very less. However, M.H. Gerardi[103] has reported that the ORP value for sulfide formation is -50 to -250 mg/l and methane production is -175 to -400 mV and I. Diaz. et al.[104] reported sulfate reduction exist even at -506 to -518 mV. C.W. Leung reported that MB was inhibited at -285mV. As the reactor ORP was below -380mV, it is preferable for both the SRB and MB.

4.2.3.4 Competition between SRB and Acetogens

During this experiment volatile fatty acid components observed were only acetic and butyric. The Variation of these parameters inside the reactors are shown in Figure 4.37. During the acclimation period when the sulfate reduction is less and the population of the SRB on their growth stage, of the reactors it was observed that Acetic, Propionic, Butyric present as main components and Iso-Butyric, Iso-Valeric and N-Valeric as other compounds. (Data not shown here) whereas after about 6 months' acclimation

period, acetic was the dominant product and butyric varied in trace level. The Acetic acid concentration in the anaerobic reactor was 6.2 times higher than butyric acid concentration. Enhancement of the Acetate production may be due to exploiting the metabolism of incomplete oxidizing SRB, which use numerous substrates (butyrate, propionate, lactate and ethanol) potentially available after acidogenic stage as electron donor to perform sulfate reduction[71]. In incomplete sulfate reduction, the end product is acetate.
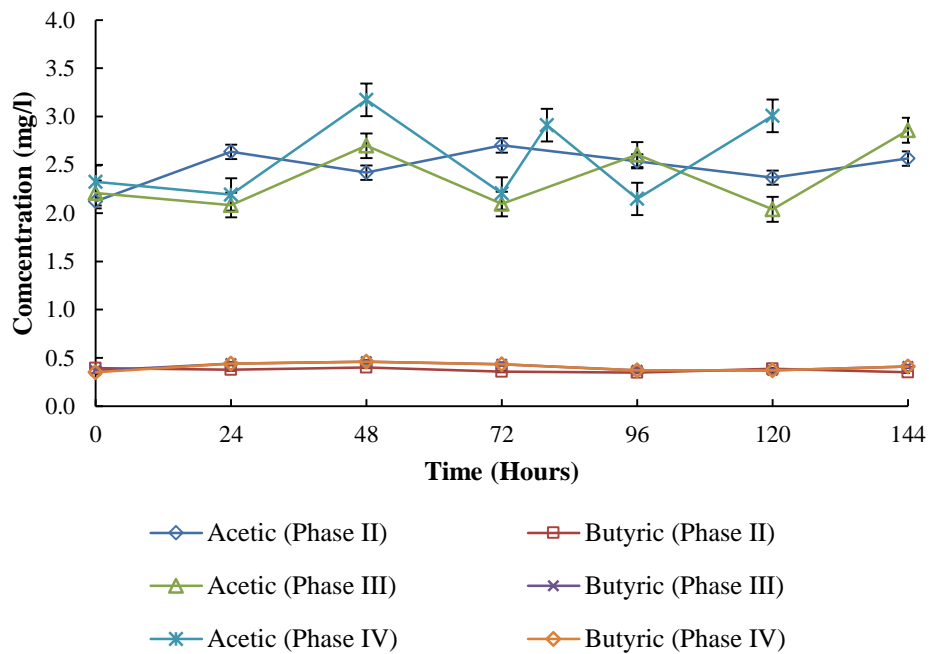
Figure 4.37:Volatile Fatty Acid Concentration vs Time

Absence of propionate could be due to rapid conversion of propionate to acetic by SRB in incomplete sulfate reduction. The electron affinity of SRB varies in the order of H_2>Propionate>other electron donors[87]. Nevertheless, the Gibbs free energy (Table 4.1) of the incomplete sulfate reduction using propionate has high negative value; comparatively with acetic or butyric. Thus, propionate easily converted to acetate. D.M. McCartney and J.A. Oleszliewicz [105] has also reported that propionic acid was only seen in un-acclimated biomass, but propionate was not produced as a product in acclimated biomass which sulfate reduction rates are high.

Table 4.1: Some Gibb's free energies of sulfate reduction

Compound	Reaction	G° (kJ mol^{-1})
Butyrate	SO_4^{-2} + 2Butyrate →HS^- + H^+ +4Acetate	-55.5
Propionate	SO_4^{-2} + 4Propionate →$3HS^-$ + $4HCO_3^-$ + H^+ +4Acetate	-150.6
Lactate	SO_4^{-2} + 2Lactate →HS^- + $2HCO_3^-$ + H^+ +2Acetate	-160.1
Ethanol	SO_4^{-2} + 2Ethanol →HS^- + $2H_2O$ + H^+ +2Acetate	-132.7
Acetate	SO_4^{-2} + 2Acetate → HCO_3^- + HS^-	-47.0
H_2	SO_4^{-2} + $4H_2$ + H^+ → HS^- + $2H_2O$	-152.2

Adapted from C.Gil -Garcia et al. [106]and A. Visser [87].

4.2.3.5 Ammonia inhibition in the reactor

The FAN concentrations were below the inhibitory levels recorded; 150mg/l by McCarty and McKinney [91] and 80mg/l Koster and Lettinga[73] when the reactor pH are maintained at 7.5. Therefore, pH control was a good strategy for preventing ammonia inhibition prevention as discussed by T. Imai et al.[94] , F. Omil [59], R. Rajagopalan et al. [23] and N. Krakat [95].
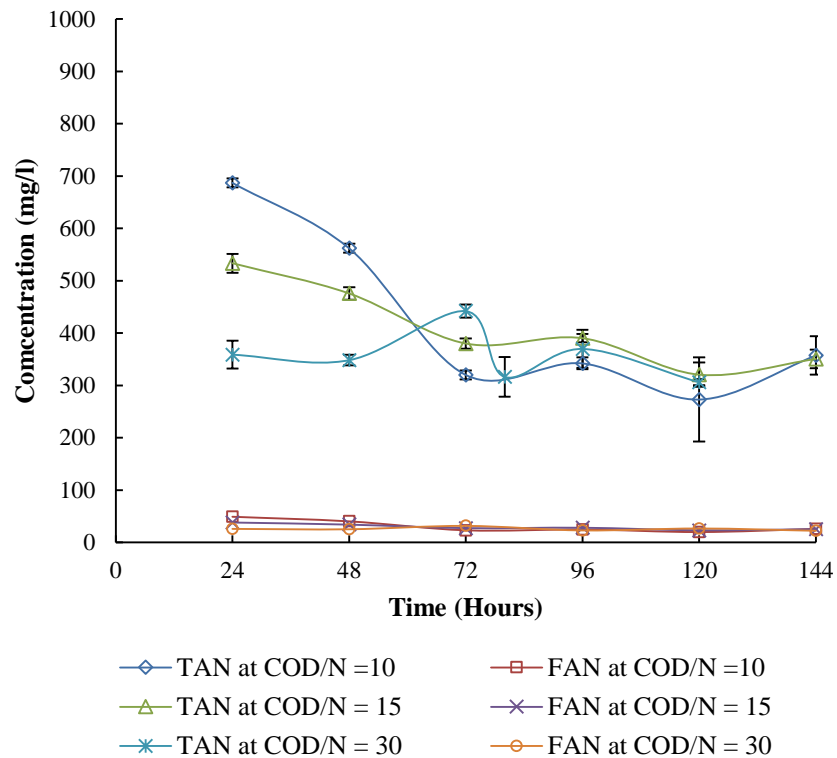
Figure 4.38; TAN, FAN Vs Time

When the COD/SO_4^{-2} ratio of the reactors was adjusted adding more organic substance, acetate automatically increased the influent COD/TKN ratio of the influent from 9.7, 18 and 36.0 respectively which will further avoid ammonia inhibition and further improve biological processes in the reactor. Researchers have found that the low COD/TKN ratios lead to accumulation of ammonia in the fermenter sludge that may consequently lead to an inhibition of the microbial consortia. Among many ammonia inhibition control strategies, the optimization of COD: TKN ratios is widely used.

P. Shanmugam and N.J. Horan[58] demonstrated the effect of C:N ratios between 3.2 and 30 on ammonia reduction in particularly for leather fleshing waste. They found that anaerobic digestion of leather fleshing waste with C: N ratio of 15 produced more cumulative biogas and less FAN at pH 6.5; which reduced the concentration of ammonia released during digestion by 80%, compared to unblended leather fleshing wastes with a pH rising to as high as 11.4. However, deterioration of performance and stability of the reactor could take place at higher C/N ration, due to lack of NH_4-N for microbial growth. As reported by Esposito G. et al. optimal C/N ratios for effective

anaerobic digestion were found to be indicated between 20 to 30. Therefore, different C/N ratios for various types of wastewaters have been investigated by researches.

. When organic compounds are found with less C/N ratios, these are co digested with other substrates by increasing C/N ratio. This is more cost effective as well. Starch from energy crops or glycerine (by product of biodiesel production) are some of the carbon rich substrates mixed with less C/N wastewaters to prevent inhibition. But during laboratory scale experiments, ethanol and acetates are often used as external carbon sources[7],[97].

When the digester pH was maintained at average 7.5, any ammonia inhibition could not be observed, and the average FAN was only 28.2 ± 7.1 mg/l (Figure 4.38). Thus, anaerobic reactor system was stable. Nevertheless, with addition of external electron donor, not only increase COD/SO_4^{-2}, but also the influent COD/TKN ratio automatically increased which would further increase the stability of the digester over ammonia overloads.

4.2.4   Conclusions Derived from the Experiment B

Summarizing the results obtained from experiment B which is a semi-batch experiment using well acclimated reactors with cycle time of 6 days, following conclusions can be drawn. Sulfate reduction efficiency can be increased, by increasing the influent COD/SO_4^{-2} ratio with acetate as external electron donor (carbon source), Influent COD/SO_4^{-2} ratio of 5 was found to be optimum for sulfate reduction than 3 and 10 for skim latex wastewater. Although addition of sufficient external electron donors, increases the sulfate reduction, excess electron donors with high influent COD/SO_4^{-2} ratio of 10 reduces the rate of sulfate reduction because of the dominance of MB become higher than SRB at high COD/SO_4^{-2}. However, adding external electron donors to the AD reactor would automatically improves the system stability over ammonia inhibition with increasing COD/TKN ratio in the feed stock. Nevertheless, it was found that by maintaining the pH 7.5-8.0 of AD reactor further enhances the sulfate reduction efficiency and minimize inhibition caused by free ammonia and free hydrogen sulfide which are continuously produced under anaerobic digestion of SLW. Enhancement of sulfate reduction was observed with pH adjustment in experiment A. Experiment A was carried out semi-continuously with cycle time of

2 days during acclimation period. But the daily sulfate reduction efficiency has increased in experiment B compared to experiment A, which may be due to proper microbial growth with the time than acclimation period.

4.3 Investigation of effect of influent volumetric loading on sulfate reduction of SLW (Experiment C)

The influent volumetric loading examined in this experiment are summarized in Table 3.6. During anaerobic digestion of SLW, breakdown of main influent compounds i.e. sulfates and generation of ammonia was studied in detail to understand the impact caused by variation in volumetric loading. The observed sulfate concentrations of the digesters are shown in Figure 4.39. The ratio of daily sulfate concentration to Initial sulfate concentration vs time was plotted to observe the variation of sulfate within the complete anaerobic digesters. According to the Figure 4.39, the highest sulfate degradation pattern with minimum total time period for complete sulfate reduction was observed at VL 02 when the sulfate loading rate was 0.10 $kgSO_4^{-2}/m^3$.d or the reactor was fed with 125ml of substrate. During VL03 in which the feed volume was 250 ml, anaerobic digester showed symptoms of overloading, unstable and most affected with the shock load been fed to the digester at once. Because VL03 has shown the least rate of sulfate reduction, at the beginning. However, curves corresponding to VL01 and VL 03 of feed volumes 250ml and 83ml reached zero at 72 hours and 94 hours after feeding while feed volume 125ml curve has reached the zero only at 53 hours after feeding. Therefore, the average rate of sulfate reduction during VL02 is higher than the VL01 and VL03.
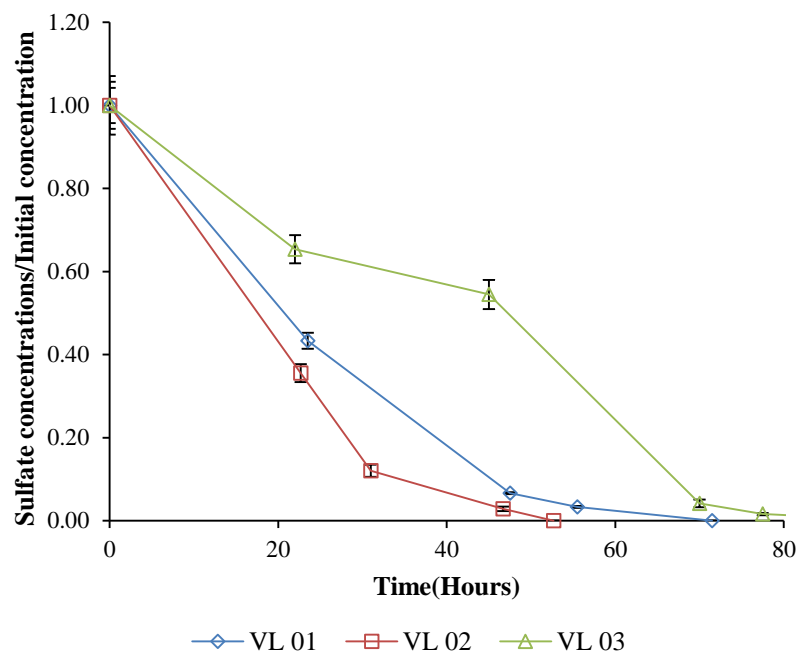
Figure 4.39: SO_4^{-2}/Initial SO_4^{-2} concentration Vs Time

The total aqueous sulfide (HS^-, S^{-2} and $H_2S(aq)$) content in the reactor varied as shown the Figure 4.40. The highest aqueous total sulfide concentration recorded first under VL 02, at 30 hrs following feeding with sulfate loading of 0.15 $kgSO_4^{-2}/m^3$.d. Then it decreased with the time. The second maxima observed for VL01 with sulfate loading of 0.10$kgSO_4^{-2}/m^3$.d was around 48 hours after feeding. This may be due to low influent sulfate loading. However, for VL 03 with the highest sulfate loading rate of 0.30 $kgSO_4^{-2}/m^3$.d, the total aqueous sulfide generation or the sulfate reduction was less, and it reached the highest value around 76 hours after feeding. Thus, it is evidenced that the sulfate reduction variation recorded in Figure 4.39 was correct. Nevertheless, for all the three phases the Total Dissolved Sulfide (TDS) was below the inhibition level.
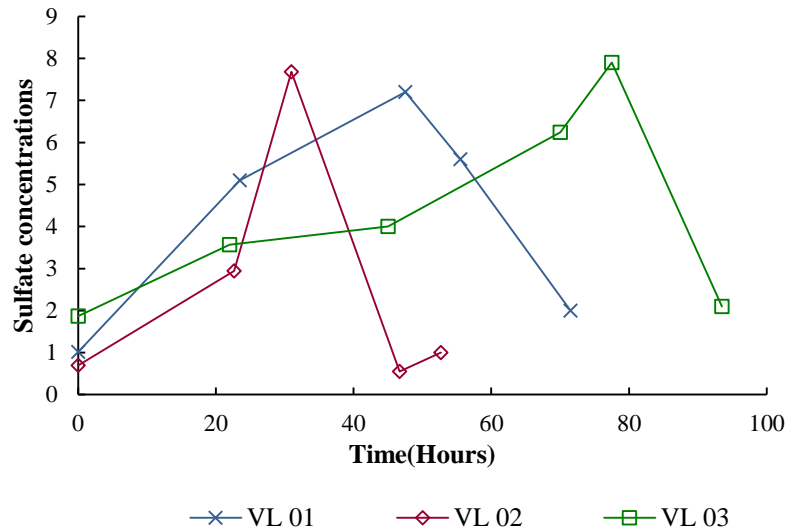
Figure 4.40: Total Dissolved Sulfide (TDS) Vs Time

Measured H_2S concentration of the digester and the total volumetric biogas generated are shown in Figure 4.41 and Figure 4.42 respectively.
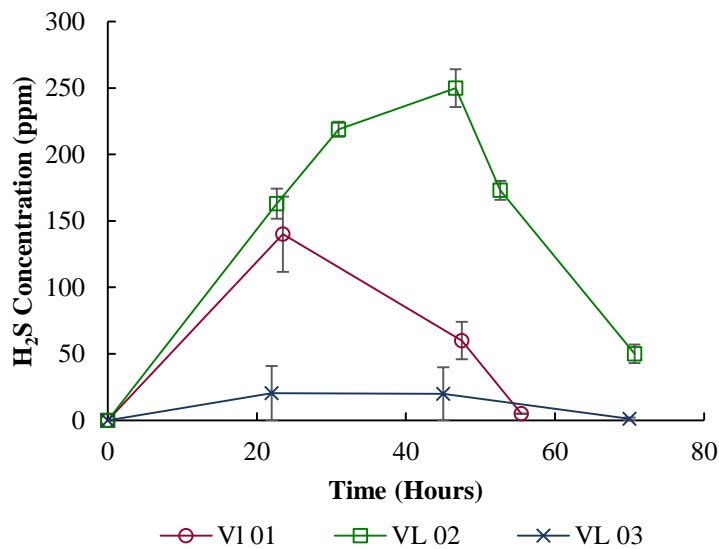
Figure 4.41: Gaseous H_2S Concentration Vs Time

H_2S concentrations were also greater in VL 02 sample than VL 01. But when the sulfate loading was further increased to VL03, gaseous H_2S concentration also

decreased. It is due to less breakage of sulfate in the beginning, and dilution of H_2S with sudden increase of gaseous biogas production. However, biogas produced on samples VL 02 and VL 03 are comparatively higher than VL 01. Because the influent substance load is high in VL 02 and VL 03. But if a component analysis of biogas including ammonia and methane had been carried out, it would have been more understanding on the bulk liquid phase reactions.
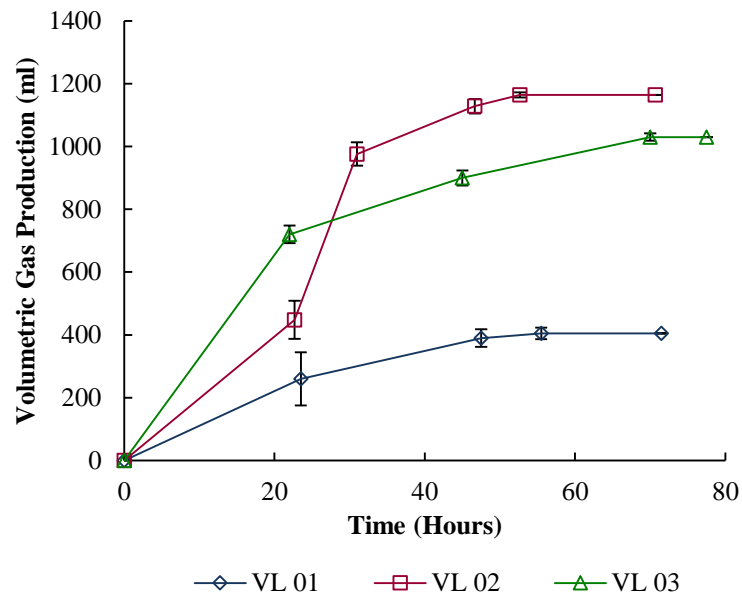
Figure 4.42: Volumetric biogas production Vs Time

From all observations presented above, it is clear that the most efficient sulfate reduction is for volumetric load of VL 02 which the sulfate loading rate was 0.15 $kgSO_4^{-2}/m^3.d$. For VL 03, 0.30 $kgSO_4^{-2}/m^3.d$ load, the digester showed an instability and long lag period for efficient sulfate reduction. The behaviour of the sulfurous compounds is clearly understood with the study of ammonia variation inside the digester which is discussed under section 4.3.1.
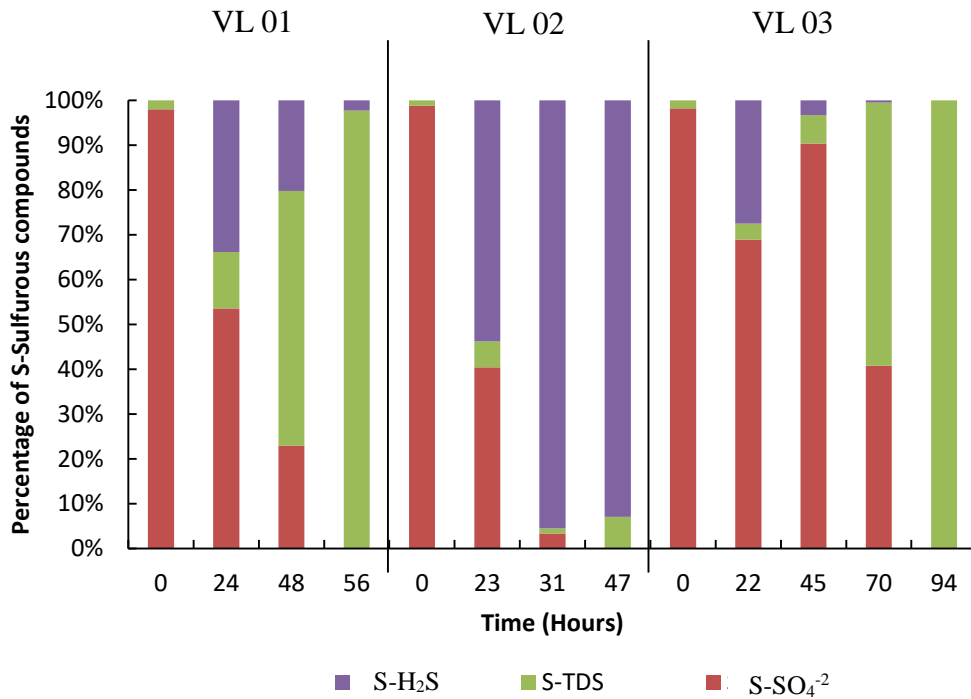
Figure 4.43: Summary of sulfurous compounds in the reactor Vs Time

In the digester $S-SO_4^{-2}$ present as remaining $S-SO_4^{-2}(aq)$, the total aqueous sulfide (HS^-, S^{-2}, $H_2S(aq)$) and gaseous $S-H_2S(g)$. The percentage prevalence of the S-sulfurous compounds in different forms in the anaerobic digester is summarized as follows in the Figure 4.43.

4.3.1 Effect of influent volumetric loading on pH

Although the sulfate reduction was the targeted substance of the experiment series, effect of TAN concentration on the anaerobic reactor performance also played an important role as influent TAN and protein loading to the reactor also simultaneously increased with volumetric loading. The ratio of TAN/Initial TAN concentration vs time after feeding is shown in the Figure 4.4. Protein presence in the influent

hydrolysed into amino acids and further increased the ammonia concentration in the system as explained in detail under section 4.1.
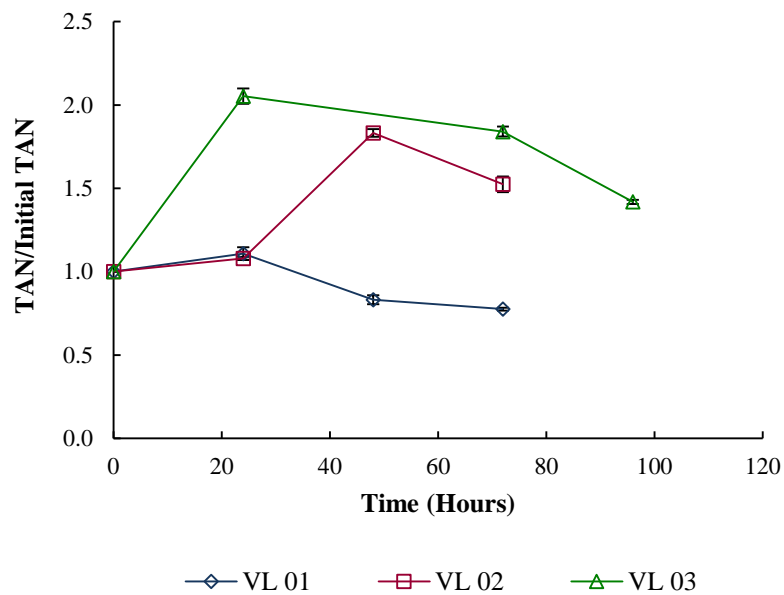
Figure 4.44: TAN/Initial TAN Vs Time

Ammonia is an essential nutrient for the micro-organisms including SRB. As recorded in past literature, the optimum , ammonia concentration up to 200 mg/l ensures adequate supply of nitrogen as nutrient substance for anaerobic bacteria [23]. On the other hand, high ammonia concentrations exceeding certain critical threshold limit, may badly inhibits directly the microorganisms including SRB and even indirectly inhibit SRB when acidogenesis bacteria are inhibited. SRB consume partially degraded carbonic substance which are the end products of acidogenesis stage. High Ammonia concentration adversely affect the acidogenic stages of carbonic substance degradation process. When these degradation stages are inhibited, the rate of sulfate reduction decreases due to lack of food for SRB. In aqueous anaerobic medium both Ammonium ions (NH_4^+) and Free Ammonia (NH_3) present. But FAN has been suggested to be the main cause of inhibition due to its high permeability[23],[24]. Although the pathways which FAN inhibit methanogens are explained as discussed in section 4.1, literature on adverse pathways of FAN affect SRB are still lacking.

The original COD/TKN ratio of SLW was 10.1 and it was increased to 18.5 while increasing the COD/SO_4^{-2} ratio to 5, whereas different research studies have found different values as optimal COD/TKN ratios for various wastewaters.

In VL 01 and VL 02 Kjeldahl nitrogen loadings were 33.8 g/m^3.d, 50.9 g/m^3.d which are comparatively moderate compared to VL 03 which has 101.9 g/m^3.d. The protein content also proportionately increased in the order of VL 01 to VL03. For VL01 and VL 02, there has not been a sudden increment in TAN or protein at the beginning of the period when sulfate reduction is efficient, but at the latter part there was an increment in TAN to the end of the sulfate reduction around 47 hours after feeding. However, it was not observed any adverse effect of TAN on the sulfate reduction in VL 01 and VL 02. But the sulfate reduction rate seems to be adversely affected in VL 03, in which time taken for complete sulfate reduction was increased by 77.5%. The percentage sulfate reduction of VL 02 after 22 hours was 64% but in VL 03 it was only 35%. As a result of the high rate of ammonia formation in VL 03, the system became unstable and as a result, it affected the rate of sulfate reduction as well as the overall percentage sulfate reduction generating a long lag phase for sulfate reduction, unlike in VL 01 or VL02. But latter part with the diminishing of the aqueous TAN inside the digester as emission of gaseous NH_3, rate of sulfate reduction increased.

C. Polizzi et al. [22] also observed that batch systems were more sensitive to overload conditions and accumulation of different toxic compounds. It can be concluded that both overloading, and ammonia inhibition adversely contributed the low performance of VL 03. However, the reported C/N ratio by Shanmugan and Horan [22]varied from 3.2 to 30[58], whereas process feasibility at very low C/N ratios of sole tannery fleshing proven for C/N < 10.

In continuous anaerobic reactors, this kind of initial shock loading is not observed as in batch reactors. Because of the slow and evenly distributed feed flowrate throughout the day of continuous anaerobic digesters, they are not usually subjected to sudden changes inside the digester, unlike in batch reactors in which corresponding total substrates are fed at once. Thus, the increase of feed sample of batch anaerobic reactors is limited. F. Straka [24] and his team also have observed the increase of ammonia concentrations at high loading rates of protein rich wastewater.
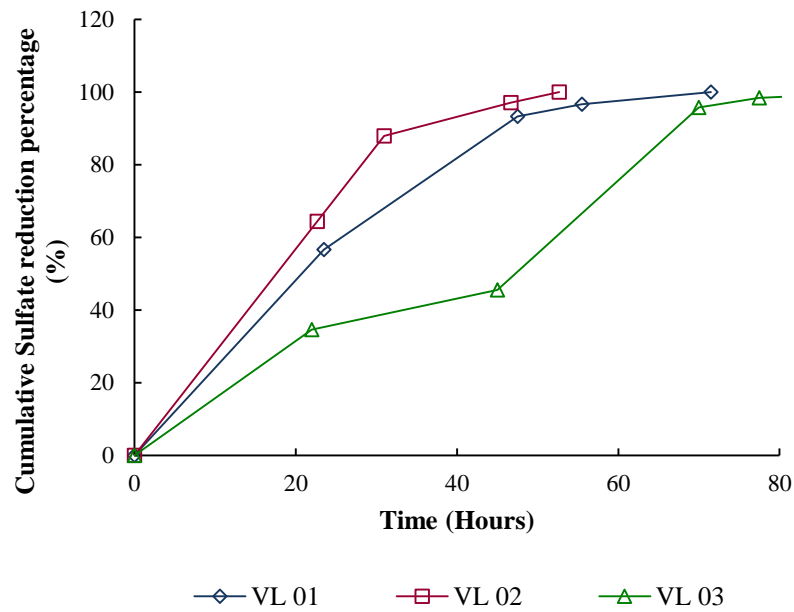
Figure 4.45: Cumulative percentage of SO_4^{-2} reduction Vs Time

It is evidenced that the high volumetric load of natural SLW wastewater adversely affected the sulfate reduction due to high ammonia generation within the system. It is further convinced from the Cumulative percentage sulfate reduction vs time after feeding curve (Figure 4.45) and the rate of sulfate reduction curve vs time after feeding (Figure 4.46). As per Figure 4.45, the cumulative sulfate reduction curve of VL02 lies above both curves corresponding to VL01 and VL03. Therefore, the cumulative percentage sulfate reduction recorded for VL 02 was higher than both the other two specific volumetric loadings of VL 01 and VL03, through-out the whole period. During VL02, time taken for 100% sulfate reduction was only 53 hours after feeding Cumulative Sulfate reduction curve of VL 01 lies below the VL 02, then finally the curve of VL 03 is even below the VL01. Thus, it has taken 93.5 hours after feeding for complete sulfate reduction of VL 03 which was the maximum time.
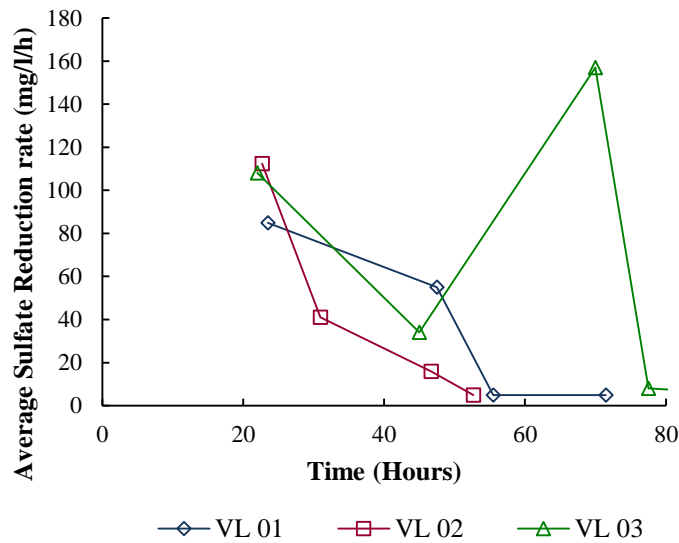
Figure 4.46: Average SO_4^{-2} reduction rate Vs Time

General behaviour of the average rate of sulfate reduction have to be maximum at the beginning and decrease with the time or else the balance sulfate remaining in the anaerobic reactor to react. But for VL 03 with highest specific volumetric loading, the rate of sulfate reduction deviates significantly with a minimum in the beginning showing serious overload and inhibition condition, then gradually increased to a maximum and reduction of TAN with the time again.

Measured tVFA was in the range less than 20mg/l which is less than the inhibitory limits. It was comparatively less due to high consumption of SRB in sulfate reduction.

4.3.2 Conclusions Derived from the Experiment C

With respect to the results of the experiment, it was clearly understood that with highest influent volumetric loading, 100 l/m³ the semi-batch anaerobic digester was severely affected and inhibited by high ammonia concentrations. Ammonia generation by protein hydrolysis enhanced the toxicity further. Nevertheless, both sulfate reduction was also affected with high rate of ammonia generation inside the reactor. But under moderate influent volumetric loading of 50 l/m³, sulfate reduction was efficient, and inhibition was not observed.

4.4 Effect of type of electron donor on sulfate reduction (Experiment D)

4.4.1 Effect of type of electron donor on sulfate reduction

Measured average sulfate concentrations were plotted against time after feeding is shown in Figure 4.47. During the experiment, anaerobic bulk liquid showed sufficient buffer capacity. Hence the measured pH was in the range of 7.4 -7.65 and it was not observed any significant pH variation in both phases with COD/SO_4^{-2} ratio adjusted with either acetate or ethanol. The measured ORP values of both phases with acetate and ethanol were -434.77 ± 17.6mV and -408 ± 4.24mV respectively. Hence both phases performed effectively under anaerobic condition. Further the observed TAN values were always below the inhibition level (Data not shown).

According to the graph (Figure 4.47), the highest degradation pattern with the steepest gradient was observed with ethanol than acetate. Nevertheless, the fastest sulfate reduction also taken place with ethanol than acetate.
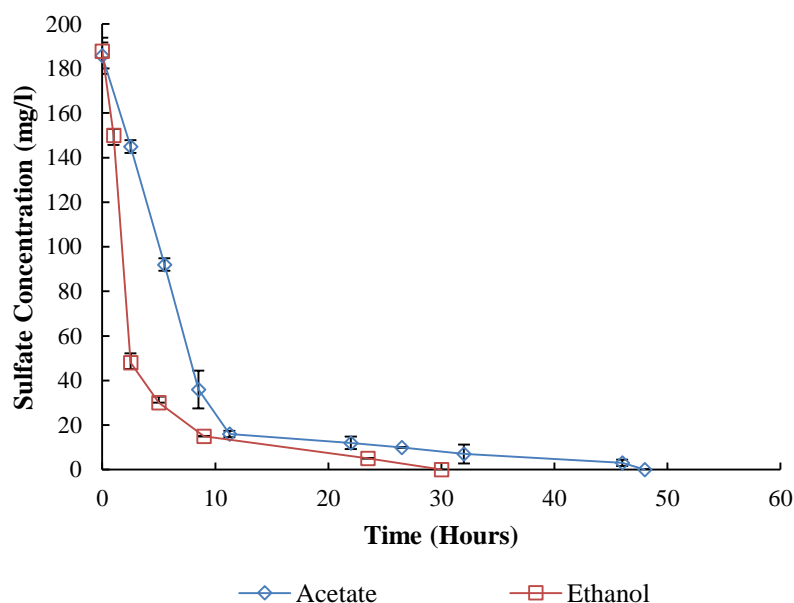
Figure 4.47: Sulfate Concentration Vs Time

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Complete reduction of sulfate with alcohol achieved at 30 hours after feeding while with acetate it was 48 hours. The highest rate of sulfate reduction with alcohol was 51.9 mg/l.hr whereas with acetate it was 28.3 mg/l.hr. It was occurred just after feeding the reactor.
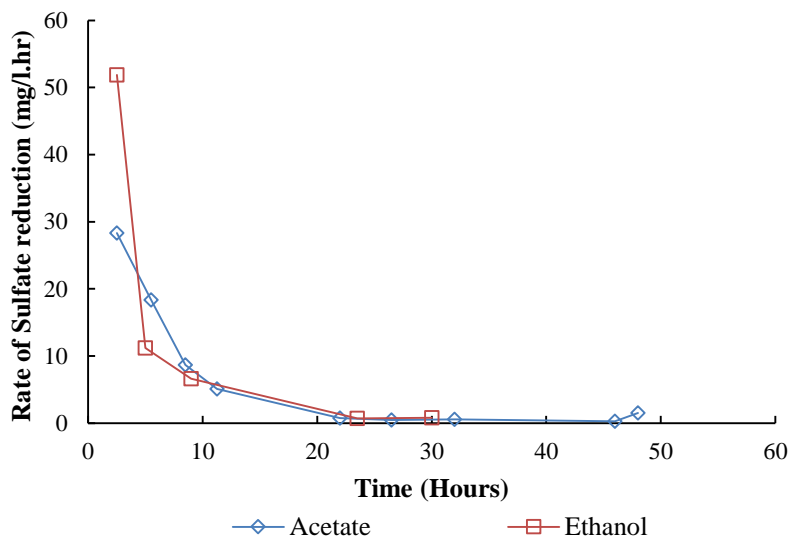
Figure 4.48: Rate of sulfate reduction Vs Time

At the beginning of the experiment, high rate of sulfate reduction was recorded with Acetate. But with the time the sulfate reduction rate was comparatively less. The rates of sulfate reduction are shown in Figure 4.48 and Percentage cumulative sulfate reduction is shown in Figure 4.49.
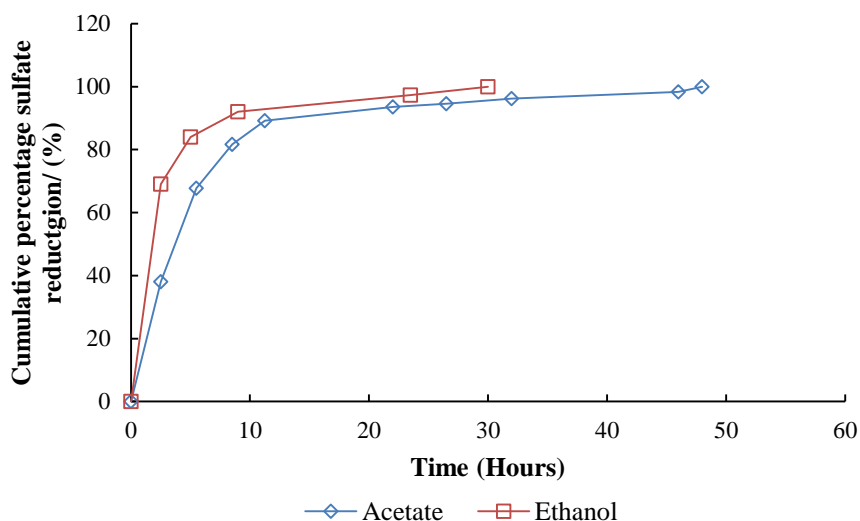
Figure 4.49: Cumulative percentage sulfate reduction Vs Time

According to the Figure 4.5, curve corresponding to ethanol lies always above the acetate curve. Thus, Cumulative percentage sulfate reduction of ethanol is higher than the acetate. Although at the beginning of the sulfate degradation, soon after feeding until 5hrs, the Cumulative sulfate percentage reductions lies nearly close by, after 5hrs the gap between both curves increased rapidly.

Although little information is available about the effect of ethanol and acetate on sulfate reduction, similar results were observed by several researches and it can be well explained by theory as well. Hence Type of electron donor whether it is a partial oxidizer, or the complete oxidizer highly affects the sulfate reduction. Although both ethanol and acetate are electron donors, Ethanol is considered as a partial oxidizer whereas acetate is a complete oxidizing agent. SRB converts Sulfate to sulfide easily and directly transforming ethanol to acetate in the first stage.

Table 4.2: Reaction by SRB and MB on ethanol, acetate and hydrogen

Substrate	Bacteria	Reaction	ΔG° (kJ)
Ethanol	SRB	$SO_4^{-2} + 2Ethanol \rightarrow HS^- + 2H_2O + 2Acetate^- + H^+$	-132.7
	HAB	$Ethanol + H_2O \rightarrow Acetate^- + H^+ + 2H_2$	9.6
Acetate	SRB	$SO_4^{-2} + Acetate^- \rightarrow HS^- + 2HCO_3^-$	-47.3
	MB	$Acetate^- + H_2O \rightarrow CH_4 + 2HCO_3^-$	-31
Hydrogen	SRB	$4H_2 + H^+ + SO_4^{-2} \rightarrow HS^- + 4 H_2O$	-152.4
	MB	$4H_2 + H^+ + HCO_3^- \rightarrow CH_4 + 3 H_2O$	-135.6

(Extracted from Y. Hu et al. [107])

It is also evidenced from the Gibb's free energy values that the Gibb's free energy for sulfate reduction using ethanol is -132.7 kJ/mol which is the most negative than acetate which is -47 kJ/mol. Therefore, sulfate reduction is easy and fast with partial oxidizer

such as ethanol. Nevertheless, B. Lui and his team has found also that addition of ethanol promoted sulfate reduction rate as well as facilitated good synergetic metabolism of sulfate reducing and methane producing bacteria[7].
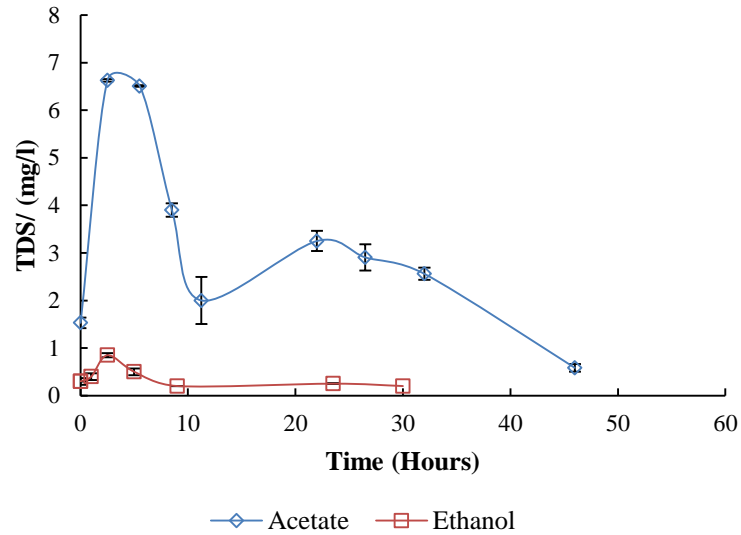
Figure 4.50:Total Dissolved Sulfide Vs Time

Above sulfate degradation results were further convinced from the measured TDS and gaseous H_2S. The other major difference observed in ethanol fed phase and acetate fed phase was that TDS concentration was high in liquid phase when COD/SO_4^{-2} ratio was adjusted with acetate as presented in Figure 4.5. The average TDS concentration of acetate fed and ethanol fed anaerobic reactors were 3.54 ± 2.1 mg/l and 0.4 ± 0.26 mg/l respectively.
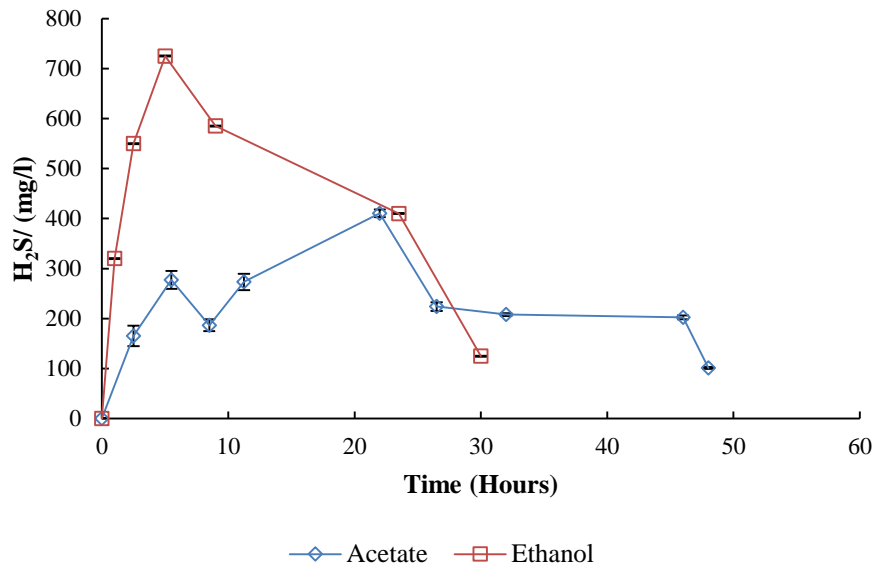
Figure 4.51: H_2S Concentration in biogas Vs Time

However the gaseous H_2S concentration was high when COD/SO_4^{-2} ratio was adjusted using ethanol. It is shown in Figure 4.51. This variation of various sulfurous compunds in the acetate fed and ethanol fed anearobic reactor phases are summarized in Figure 4.52 and Figure 4.53 respectively.
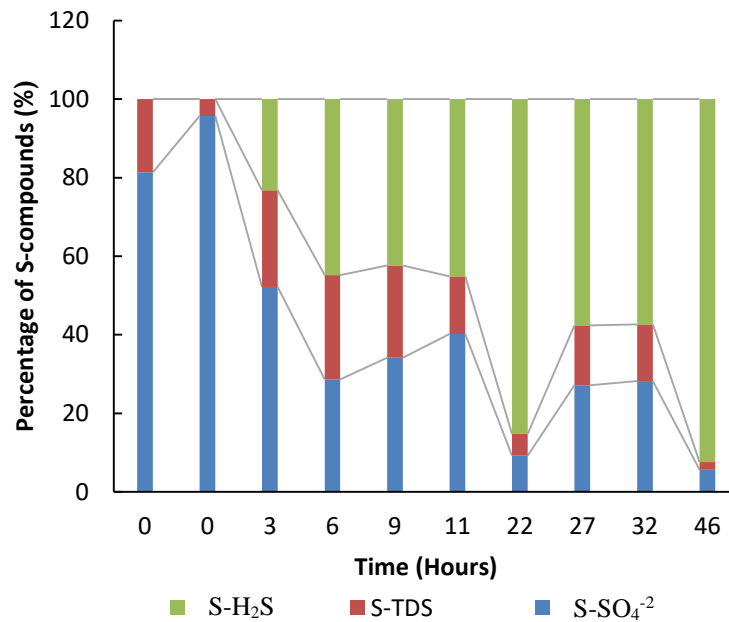
Figure 4.52: Percentage S-compounds Vs Time after feeding of anaerobic reactor at COD/SO_4^{-2} ratio adjusted using Acetate

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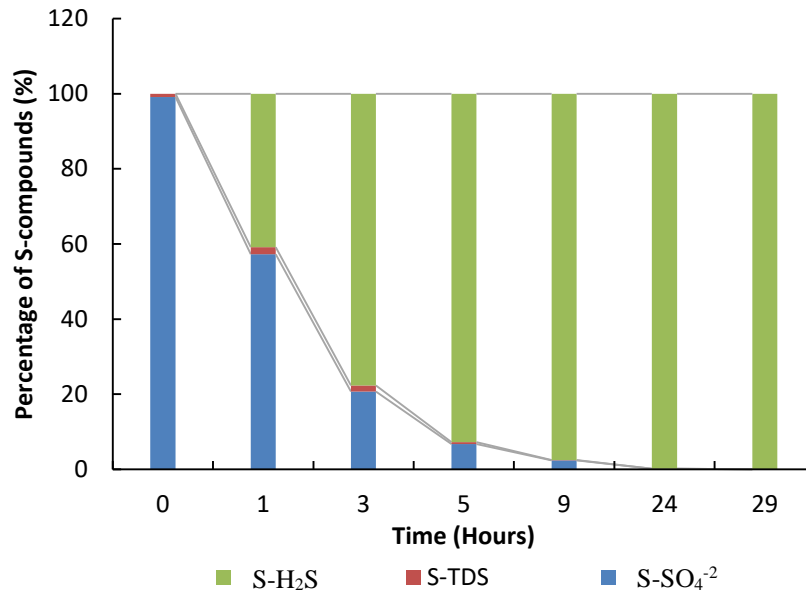
Figure 4.53: Percentage S-compounds Vs Time after feeding of anaerobic reactor of COD/SO_4^{-2} ratio adjusted using Ethanol

When both the aquoues sulfide (HS$^-$, S^{-2}) concentration and gaseous hydrogensulfide concentration were compared for ethanol added phase, aqueous sulfide concentration were low whereas the gaseous H$_2$S concentrations were high. But for acetate added system it was vise versa. pH values of both phases are nearly same. It can be assumed that because of the high rate of biogas production as well as sulfate reductions rate with ethanol added phase, most sulfide escaped as hydrogen sulfide bubbling through the liquid medium.

4.4.2 Effect of type of electron donor on Methane production and COD reduction

COD/SO_4^{-2} ratio adjustment with Ethanol not only enhanced the sulfate reduction but also the methane production. The biogas production of both phases with addition of acetate and ethanol are shown in Figure 4.54.
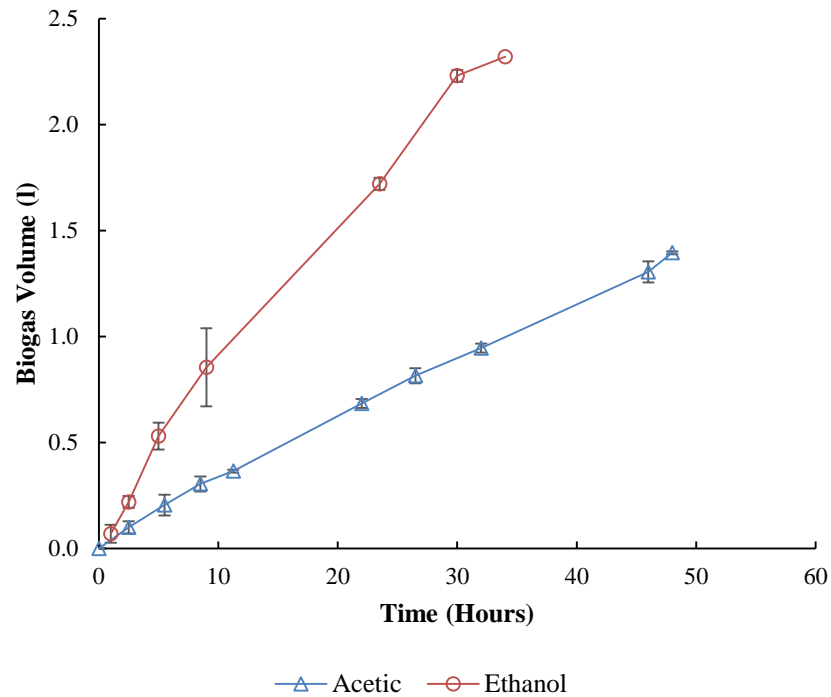
Figure 4.54: Cumulative Biogas production Vs Time

According to the results, volumetric biogas production was higher for ethanol than the acetate added phase at the beginning. At the end of the degradation period with ethanol, probably at the end of the sulfate reduction there was a significant increase of biogas generation. But the biogas generation of the acetate added anaerobic reactor, was steady.

Average methane composition of biogas of ethanol added phase was comparatively higher than acetate added phase with methane composition been $81.4 \pm 3.4\%$ and $41.5 \pm 12.8\%$ respectively. Measured methane composition of the generated biogas is shown in Figure 4.55. However, biogas production was observed even after sulfate reduction was completed. Thus, carbon substance degradation rate seems to be lower than sulfate reduction.
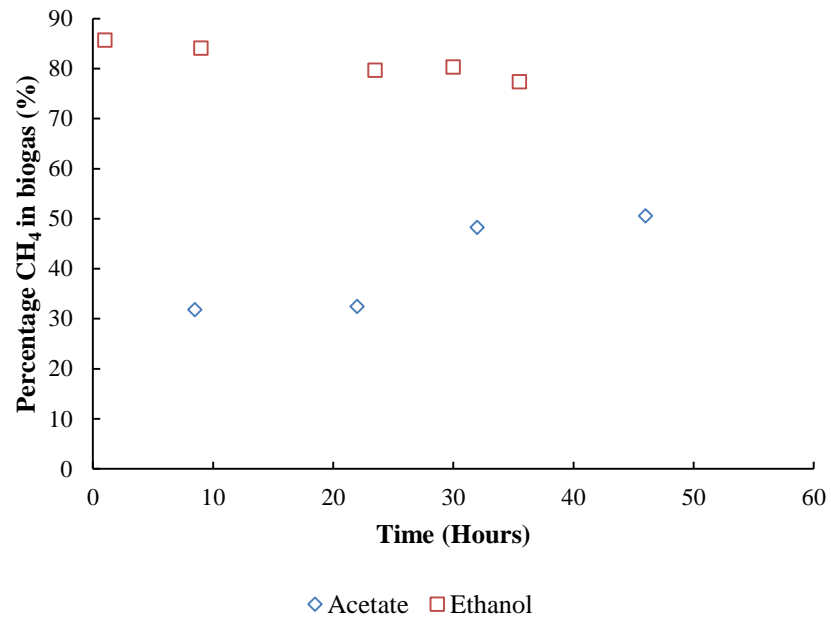
Figure 4.55: Percentage methane composition in bio gas Vs Time

However, the average CO_2/CH_4 ratio as in Figure 4.56 was less in acetate added phase than ethanol added phase. It might be because of the high sulfate reduction in the ethanol added phase and more CO_2 is production in sulfate reduction.
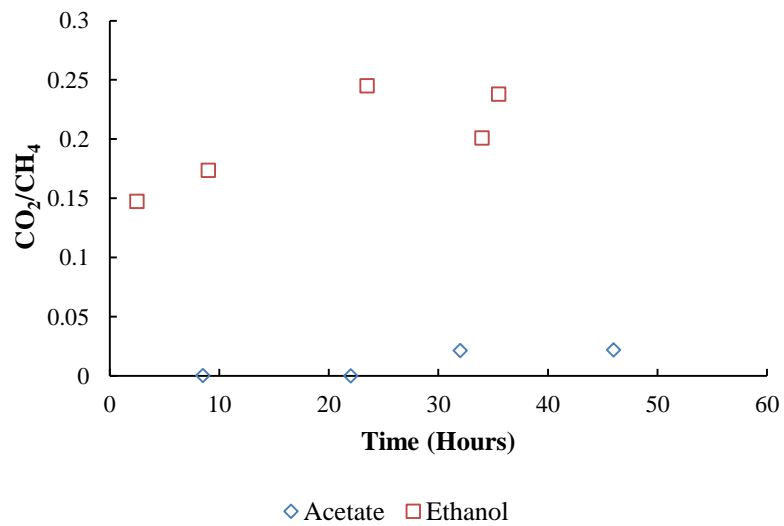
Figure 4.56: Percentage methane composition in bio gas Vs Time

The above-mentioned results with the methane production can be further confirmed with the tCOD reduction in both phases of each reactors. tCOD reduction was rose

from 45.8% to 85.0% by adjusting the COD/SO_4^{-2} ratio with ethanol than acetic. A. Sarti [12] also successively reduced both DS with 98% and COD with 86% using Anaerobic Sequencing batch biofilm reactor (ASBBR) with ethanol as the electron donor.

It was evidenced that, not only sulfate reduction and methane production, but also the COD reduction was enhanced with COD/SO_4^{-2} ratio adjustment with ethanol than acetate. B. Lui [7] and his team has observed similar results to our experiment that addition of partial oxidizer ethanol improve sulfate reduction, methanogenic activities and increase efficiency of COD removal.

The resulting fraction of sulfate reduction, COD removal and methane production can be explained based on the several factors such as competition of SRB and MB, Gibb's free energy, type of SRB species available, population fraction of SRB: MB and other environmental factors.

The competition exists among SRB and MB for sulfate rich wastewater as the influent. SRB compete with MB for the available substrate for sulfate reduction and methane production. The fraction of sulfate reduction and methane production depend on this competition. As SRB and MB are highly competitive at low COD/SO_4^{-2} ratios leading to decrease methane production and even failure of treatment process, but less competitive with sufficiently high COD/SO_4^{-2} ratios[108].

SRB preferred to utilize partial oxidizers such as ethanol, lactate or propionate more effectively in sulfate reduction whereas MB mainly use Acetate and H_2/CO_2 as the source for methane production[85],[109]. It can be further seen from the Gibb's free energy values available on Table 4.2. However, when the digester contained high concentrations of sulfate and was in lack of preferential substrate, SRB compete against MB for acetate and H_2.

However once sufficient preferential substrates are available for SRB, that is ethanol or propionate and butyrate, it would be unnecessary for SRB to compete for other carbon source like acetate. In addition, M.V.G. Vallero et al. [110] reported that SRB had a higher affinity for ethanol than acetate. Hence, inhibition results from competition between SRB and MB can be avoided, promoting sulfate reduction as well as facilitating MB to use acetate for methane production. When the electron donor is a partial oxidizer, the other advantage is that when SRB utilize ethanol for sulfate

reduction, acetate is generated. These generated acetates can be again utilized by MB in methane production. Thus, a substrate chain of co-metabolism was formed among SRB and MB. As a result, all three processes; sulfate reduction, methane production as well as COD removal process effectively enhanced when the type of the electron donor used is ethanol than acetate.

But it has been reported that a process failure when partial oxidizers are added in some specific conditions, because of sudden increments in dissolved sulfide from high rate of sulfate reduction. G.F. Parkin[53] and his team has observed sulfate reduction under both propionate and acetate. He observed 65%,83%, 95% and 88% sulfate reduction for COD/S ratios of 10,20,40 and 60 respectively for organic loading rate of 0.27gCOD/l.d. Percentage sulfate reductions of 98% and 97% achieved for COD/S ratios of 40 and 60 at organic loading rate of 0.39 0.27gCOD/l.d. Further he has documented that although sulfate conversion rate is more effective and faster with partial oxidizing electron donor ethanol than acetate, propionate systems failed sooner due to rapid built up of dissolved sulfide up to inhibitory levels sooner. Jing Z. et al. also has also observed rapid sulfide inhibition and accumulation of acetate when ethanol and acetate inclusive synthetic wastewater was added in UASB reactor at low HRT[109]. At HRT of 2h, electron flow was mainly utilized by SRB and free sulfide increased above inhibition level of 110 mg/l due to rapid sulfate conversion to TDS. They reported dominance of MB at 6h HRT having COD removal and methane yield above 80%, whereas sulfate reduction was only 30%.

As per the findings of Z. Jing[109] sulfate conversion rates and percentages are further affected by the type of SRB and MB species available and percentage of populations in the reactor as well. When MB was dominant in the above said UASB reactor, there was only 17.6% were SRB. Nevertheless, both Y. Hu et al. [107] and Z. Jing et al. has reported that the most kinds of SRB species are also incomplete oxidizers which cannot utilize acetate directly as electron donors for sulfate reduction at both high and low COD/SO_4^{-2} ratios, where some of the SRB species like Desulfovibrio found to play a dominant role in ethanol degradation at low COD/SO_4^{-2} ratios[107]. However, C. O'Reilly and E. Colleran [111].has documented that SRB species could not out-compete MB species for acetate at influent COD/SO_4^{-2} ratios ranging from 2 to 16. These diverse findings may be related to differences in composition of carbon source,

sulfate concentrations, Type of SRB micro-organisms present and other environmental factors.

4.4.3   Conclusions Derived from the Experiment D

After analysing all results obtained from the experiment D, it is evidenced that the rate of sulfate reduction increased more when partial oxidizers such as ethanol was added than adding complete oxidizers such as acetate. Not only sulfate reduction but also the COD reduction and methane formation enhanced with COD/SO_4^{-2} ratio adjustment with Ethanol than Acetate. When, ethanol was added as the external electron donor, the aqueous sulfide concentration were low whereas the gaseous H_2S concentrations were high. when acetate is added, it was vise versa. pH values of both phases are nearly same. It is expected that most sulfide ions escape as hydrogen sulfide bubbling through the bulk liquid of the reactor due to high rate of biogas production as well as sulfate reductions.

4.5   Effect of micro-aeration method on simultaneous Hydrogen sulfide emission reduction and elemental sulfur formation in synthetic wastewater (Experiment E)

Effect of air feeding mechanism on sulfide removal and elemental sulfur formation was studied under this experiment. In this experiment only O_2/S ratio of all phases was kept at constant value of 0.5. Specially practicability of feeding air and the effect on hydrogen sulfide removal and elemental sulfur formation was studied from this experiment, before conducting the experiment series for skim latex wastewater.

4.5.1   Effect of the gaseous H_2S emitted

The measured gaseous H_2S concentration of all phases are shown in Figure 4.57. phase I in which the complete anaerobic condition was maintained in the reactor, average H_2S concentration was 440 ± 195 ppm. With air fed to the reactor through the bulk liquid to the head space of the reactor, the gaseous H_2S concentration was able to be reduced via elemental sulfur formation.

The maximum gaseous H_2S reduction was observed in phase IV, with average of 47±46 ppm. It was 89% reduction compared to the complete anaerobic phase. Using this technique of air supply, air washout was able to be minimized during which the sample was fed at very low flow rate for 2 hours. However, the amount of air planned to be kept in the liquid media as dissolved oxygen also expected to be high.

In phase II, although the initially emitted H_2S concentration is lower, around 10 hours after the feed, Gaseous H_2S concentration showed a sudden increase. Although 188 ml of head space gas sample was removed before adding fresh air sample, because the sample was fed at higher rate (air sample fed in within 2 mins), some amount of air seems to be washout with the head space gas volume. Therefore, the expected amount of O_2 could not be supplied using this air feeding technique.
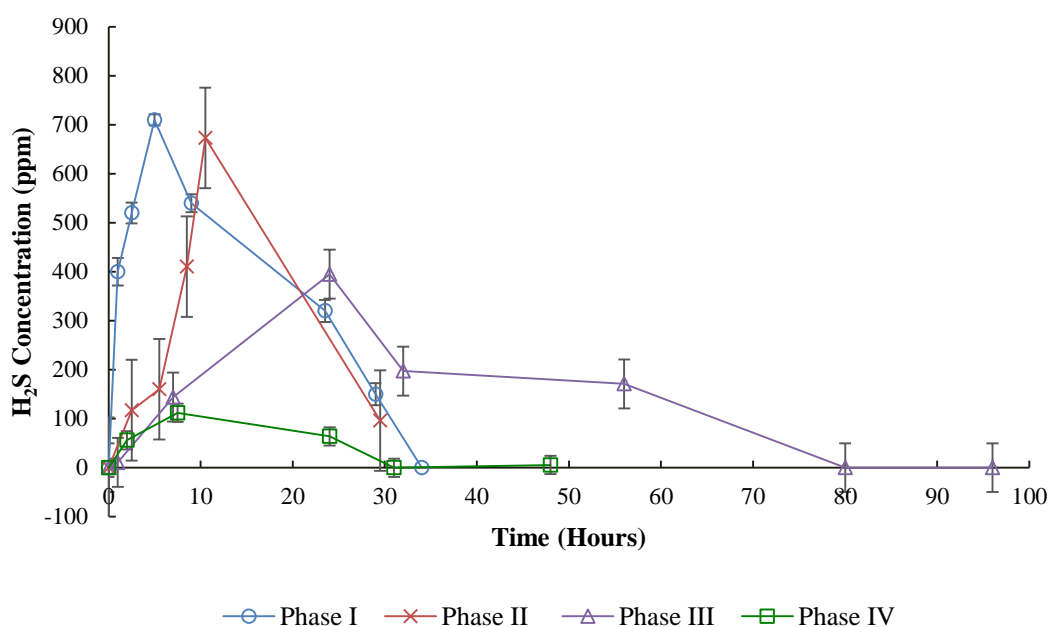
Figure 4.57: Gaseous H_2S composition in bio gas Vs Time

During phase III, continuous micro aeration was conducted for 48 hours with 0.065 l/min and the average gaseous H_2S emission was able to be reduced to 153±145 ppm which was 64%. But it seems that the rate of air supply is not sufficient compared with the rate of reduction of sulfate or else the rate of formation of sulfide at the initial stage. Thus, more gaseous H_2S escaped without converting to elemental sulfur. Sufficient amount of O_2 had to be maintained to convert most of the sulfide to elemental sulfur

by SOB. But in the latter stage of phase III, O_2 seemed to be higher, thus formation of sulfate was also higher (Figure 4.59).

4.5.2 Variation of sulfurous compounds in each phase with time

The dominant sulfurous compounds, sulfate concentrations, Total Dissolved Sulfide concentration (HS^-, S^{-2}, $H_2S(aq)$), gaseous H_2S, were observed from phase I to IV and elemental sulfur amount was calculated using material balance of sulfurous compounds in the microaerobic reactor as performed by most of the other researches [62]. Sulfur balance was done by considering the in balance of influent and effluent sulfurous compounds as elemental sulfur. The dominant sulfurous compounds considered in sulfur balance were Sulfate, Aqueous total sulfide (H_2S (aq), HS^-, S^{-2}), gaseous H_2S only. From the research carried out to study the elemental sulfur formation from simultaneous sulfate reduction and sulfide oxidation, by Stefess G.C. the major sulfurous compounds generated were only H_2S, aqueous sulfide (at higher pH S^{-2}, at pH< 8 – HS^- [71]) and elemental sulfur. But he has not detected any thiosulfate formation[79]. C.J.N. Buisman and his research team[112] has found that thiosulfate is generated as a result of chemical oxidation not from a biological oxidation. Thiosulfate generation takes place for influent wastewater which contains high sulfide concentrations not sulfate and direct chemical oxidation to thiosulfate easily takes place for high sulfide loading [13]. A.J.H. Jassen [19]and his team also observed only sulfate and elemental sulfur and during biological oxidation of sulfide in Fed-batch reactor. Krishnakumar B. [13] has observed 25%-35% thiosulfate and only 2% sulfate at higher pH such as 9-9.5 Reverse fluidized loop reactor. He has further reported that undesired products like $S_2O_3^{-2}$ and polysulfide which reduced the efficiency of micro-aerobic reactor occurred only at decline bacterial activity such as high pH or high sulfide loading like above 10 mg/l only. Therefore, further it was confirmed that it is reasonable to assume the dominant components as sulfate, DS, Gaseous H_2S and elemental sulfur which the sulfide loading is below high values. In this experiment the formed elemental sulfur during phase II to IV was not only in the bulk liquid but also walls of the head space of the reactor. During micro-aerobic phases elemental sulfur on the walls of the head space was clearly seen whereas on suspension a light turbid of white colour appeared.

Considering the phase II results, sulfate concentration has shown a reducing pattern until 19 hours, after the wastewater fed to the reactor, but increases gradually thereafter as shown in Figure 4.58. There wasn't any significant difference observed in DS concentrations. The maximum elemental sulfur was measured at around 4 hours which was only 0.8 mmol and diminishes very faster. Therefore, the stability of formed elemental sulfur was very low. The emitted H_2S has increased steeply at around 10 hours. When studying the variation of sulfurous compounds in the reactor the biologically degraded elemental sulfur has removed with the biogas as gaseous H_2S. When O_2 in the reactor was consumed to produce elemental sulfur, O_2 in the reactor diminishes, thus creating a condition more towards anaerobic. Hence sulfur reducing bacteria seems to be activated degrading the elemental sulfur and producing H_2S gas. From the results it was convinced that rate of air supply with 94 ml/min in 2 min was high, thus the probability of O_2 flushed away from the head space due to sudden shock loading was also high. Due to insufficient O_2 available in the reactor, elemental sulfur formation was low. Nevertheless, the stability of formed sulfur was also low.
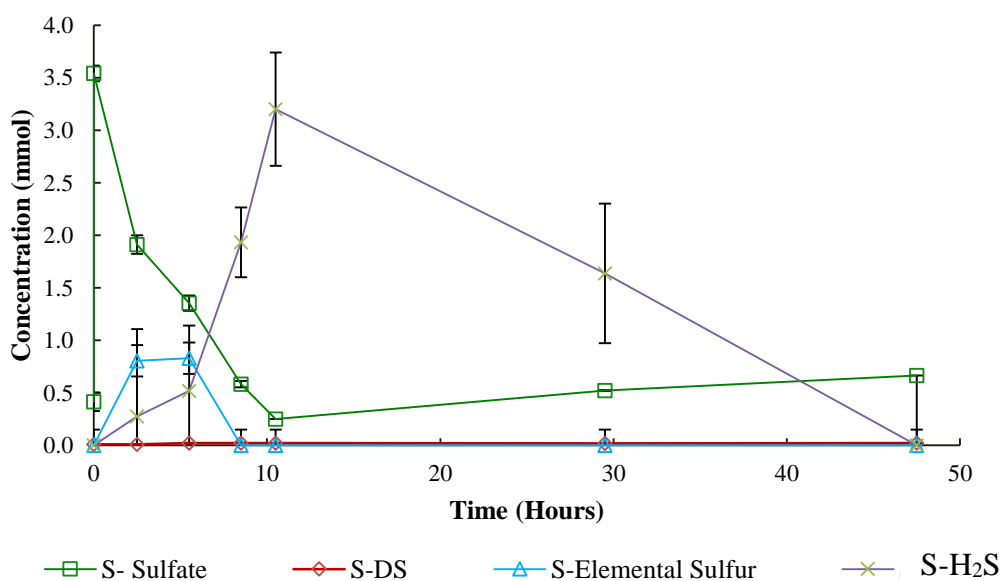
Figure 4.58: Sulfurous compounds in phase II Vs Time

Therefore, in phase III, it was decided to feed corresponding 188 ml air at slow rate of 0.065 ml/min over 48 hours. According to the observed sulfurous compounds in the reactor as shown in Figure 4.59 only about 0.4 mmol of elemental sulfur seems to be formed at initial stage at time about 4 hours after feeding during this phase and it also

seemed to be degraded faster, emitting highest H_2S concentration at 24 hours after feeding. But thereafter the sulfate concentration increased gradually. With continuous feed of O_2 till 48 hours after feeding, excess O_2 seems to be present in the reactor compared to sulfurous compounds present, converting head space H_2S to Sulfate in the latter stage. while in the initial stage more H_2S has emitted because of the lack of O_2 for sulfide conversion to elemental sulfur. Therefore, rate of supply of air at 0.065ml/min was to be quite low at the beginning which resulted in high sulfate reduction rates, but at latter part the rate of air supply was higher. Therefore, it is preferable that at the initial stage, the rate of supply of air is higher while it had to be diminished with the time, but as such controlling of air to the reactor were not possible, with the facility available.
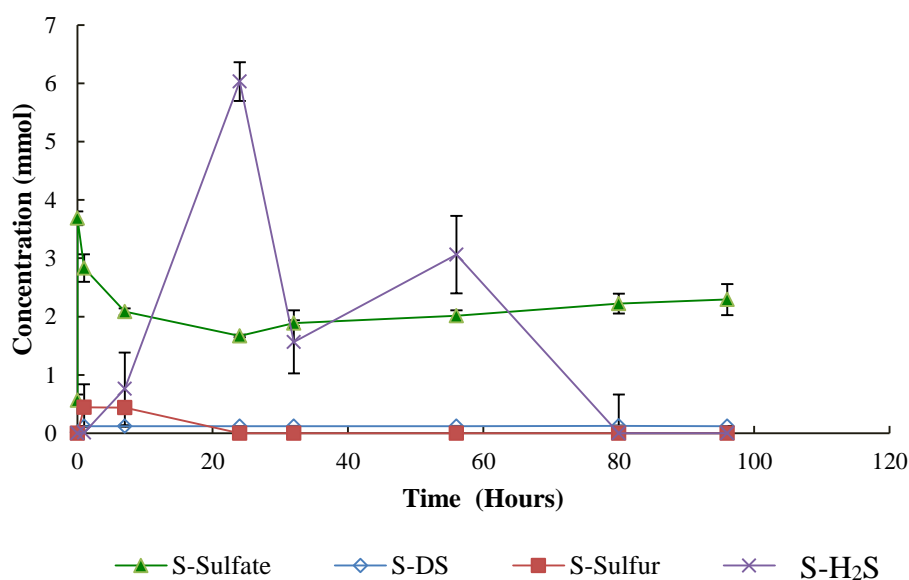
Figure 4.59: Sulfurous compounds in phase III Vs Time

Different air supplying strategy was followed at phase IV, i.e. the rate of supply of air was in between phase II and phase III. Under this strategy the air was supplied directly into the liquid phase through a diffuser. In phase IV, air was fed at 1.6 ml/min for 2 hours and supplied air volume was 188 ml at O_2/S ratio of 0.5. Further air was fed to the reactor, half an hour after feeding the waste sample to the micro-aerobic reactor. This prevented head space air flushed away due to shock loading of air and supplied sufficient air during initial stage which the sulfate reduction rate was high.

During the period of two hours SOBs in the liquid phase were able to directly consume oxygen supplied to the liquid phase and some amount of oxygen might have retained in the liquid media as dissolved oxygen and other oxygen was allowed to accumulate in the head space. As the bulk liquid phase inside the reactor was continuously stirred, generated aqueous sulfide and hydrogen sulfide from breakdown of sulfate further expected to gain oxygen for elemental sulfur formation through the head space as per the requirement. Thus, to maintain the dominant reaction to produce more elemental sulfur and increase the stability period of generated elemental sulfur without reversing the direction to form sulfate again with excess oxygen. The analysis of all sulfurous compounds in phase IV is shown in the Figure 4.60.

I. Diaz[62] have reported that SOBs are preferentially present in the head space as well gas-liquid interphase. They convert H_2S also to elemental sulfur. There are several reactions taking place in the reactor simultaneously inside a micro aerobic reactor. SRB reduces sulfate to sulfide and the SOB consume generated sulfide to elemental sulfur. If again the O_2 inside the reactor reduces than a limiting value, formed elemental sulfur again reduced back to sulfide by SRBs. However, if the O_2 inside the reactor is high, the formed elemental sulfur can be turned back to sulfate. The conditions inside the reactor have to be maintained to establish the dominant reaction to be to reduced sulfate to produce elemental sulfur.

The maximum elemental sulfur amount was produced in the phase IV, which was 2.1 mmol at around 10 hours after feeding. Then stability of the generated elemental sulfur was also high during which the rate of depletion of elemental sulfur was low. The simultaneous level of H_2S emitted was also low and it was only 47±46 ppm. It was 89% reduction compared to the completely anaerobic phase.
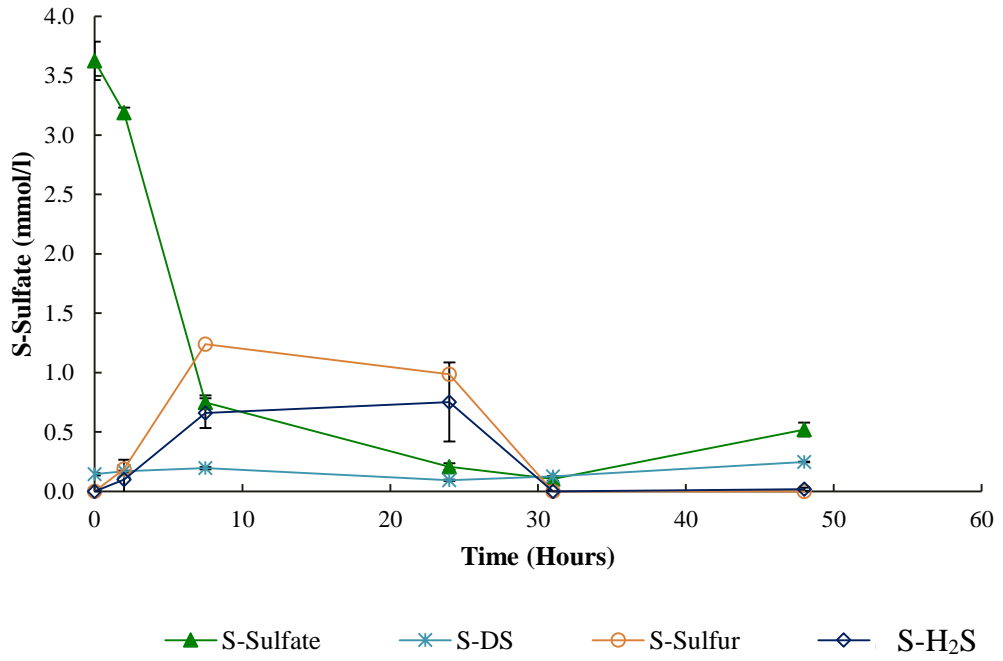
Figure 4.60: Sulfurous compounds in phase IV Vs Time

4.5.3   Elemental Sulfur formation vs air feeding technique
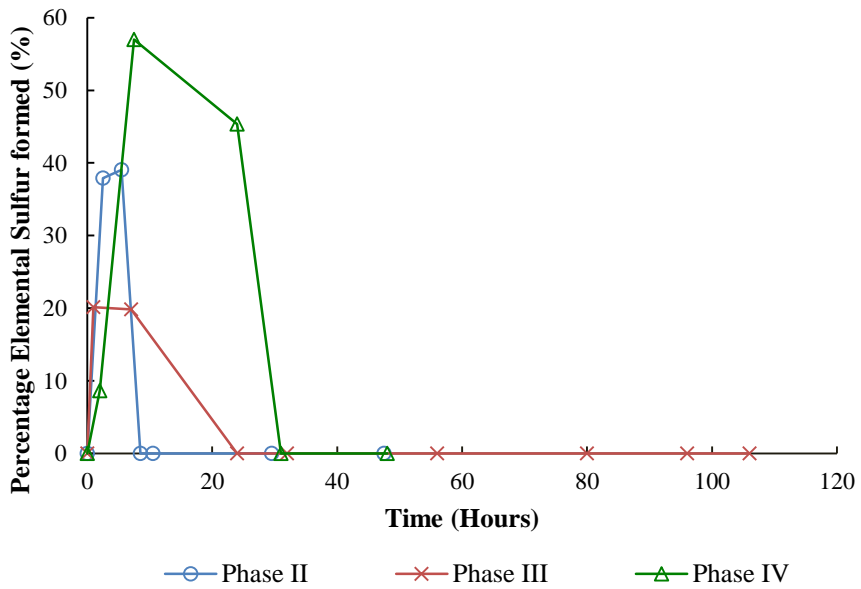
Figure 4.61: Generated elemental sulfur Vs Time

The generated percentage elemental sulfur quantities under different micro aeration techniques are summarized in the Figure 4.61 and calculation of the elemental quantities considering the mass balance of sulfurous compounds is attached in the annexure B. As per the Figure the highest elemental sulfur formation as well as the stability of formed elemental sulfur is higher in phase IV in which the air was fed half an hour after feeding. Therefore, the same air feeding technique followed for further studies.

4.5.4   Effect of air feeding technique on the sulfate reduction

The degradation pattern of sulfate inside the micro aerobic reactor can be observed from Figure 4.62. Apart from anaerobic digestion phase (Phase I), Phase II has shown the highest sulfate reduction, whereas the most adverse effect on sulfate reduction was observed in phase III in which air supply was at 0.065ml/min rate for 48 hours. As explained earlier in phase III, sulfate reduction rate was high during initial stage in which the sulfate concentration was highest, soon after feeding and at that time sufficient amount of O_2 was not present, more sulfide went out of the system as H_2S. Therefore, at the latter stage due to excess O_2 present in the system, SRB which are strict anaerobes were more affected.
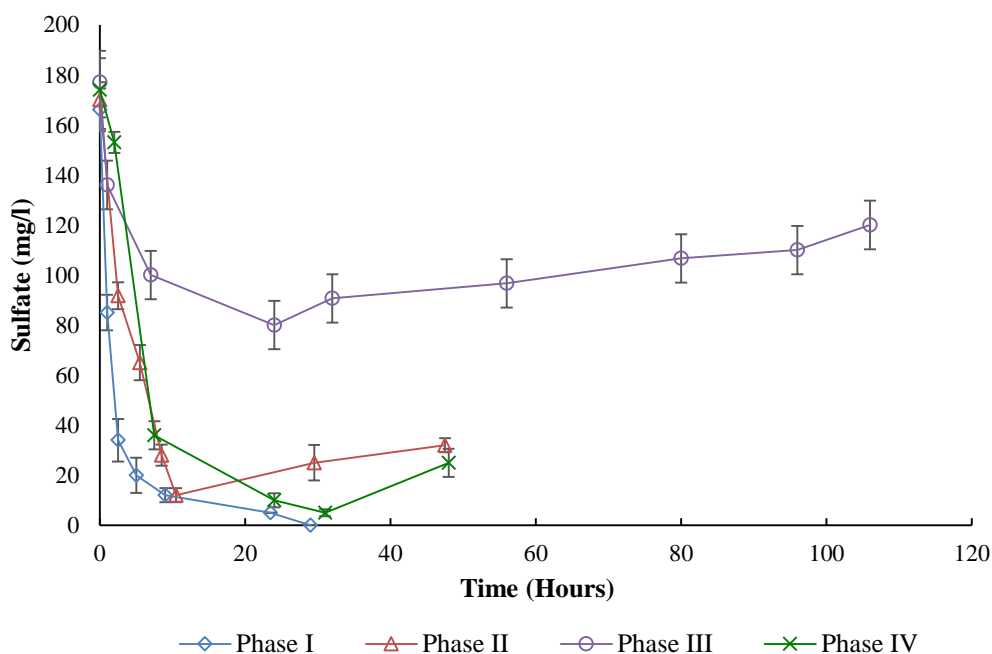
Figure 4.62: Sulfate concentration Vs Time

The suitable technique of air feeding to the micro-aerobic reactor is analysed using the following parameters i.e. quantity and stability of formed elemental sulfur, emitted H_2S concentration and effect of air feeding to the sulfate reduction process.

Phase IV results which is corresponding to air feeding method which air sample fed in half an hour after feeding at a rate of 1.6 ml/min over 2 hours was found to be the most suitable method to feed air to the semi-batch micro-aerobic reactor for sulfate conversion to elemental sulfur. Thus, this air feeding technique used to feed air sample into the micro-aerobic reactor in other experiments on sulfate conversion to elemental sulfur in semi-batch reactors.

4.5.5 Conclusions Derived from the Experiment

During this experiment, micro aeration was performed using 3 different methods. The air sample fed to the reactor was kept constant at 188 ml which is corresponding to O_2/S ratio of 0.5.

With introduction of air into anaerobic reactors, both sulfate reduction to sulfide step as well as the sulfide conversion to elemental sulfur step was able to be achieved in a single reactor. From the results, it can be concluded that with introduction of air into anaerobic reactors, sulfate reduction, H_2S emission as well as elemental sulfur formation were achieved for all three phases, but the degree of each reaction varies with the method of air supply. Out of the three air feeding methods, air supply to the bulk liquid of the reactor at a rate of 0.16 ml/min for two hours, half an hour after feeding the wastewater sample was found to be the best air feeding method. Half an hour delay to provide air to the reactor after feeding the wastewater sample was to enhance the first step of sulfate reduction to sulfide which is a strict anaerobic process. With this air feeding method, both maximum gaseous H_2S concentration reduction which was 89% with respect to complete anaerobic condition and the maximum elemental sulfur formation has taken place. Gaseous H_2S concentration decreased from 440 ± 195 ppm to 47 ± 46 ppm. During this new air feeding method, some amount of oxygen might have directly consumed for conversion of sulfide to elemental sulfur and some oxygen might have retained in the bulk liquid via dissolved oxygen whereas remaining oxygen was let to accumulate in the head space. As the bulk liquid inside the reactor was continuously stirred, generated aqueous sulfide and hydrogen sulfide

transferred to the head space as required to maintain the dominant reaction of the reactor to produce more elemental sulfur and increase the stability period of generated elemental sulfur without reversing the direction to form sulfate again. Nevertheless, elemental sulfur formation in the head space was observed. Therefore, it was convinced that the presence of Sulfur Oxidizing Bacteria in the head space and elemental sulfur formation in the head space are possible as reported in past literature. Formed elemental sulfur in every phase retained in the reactor for some time and completely diminished with time beyond the measurable range using the available analysing method. When oxygen in the reactor was consumed for elemental sulfur formation and other reactions, the micro-aerobic condition in the reactor reduced generating more anaerobic condition inside the reactor. Therefore, the dominant reaction reverses to break down formed elemental sulfur to gaseous H_2S as observed in phase II and phase IV.

Continuous supply of air to the bulk liquid phase was not suitable for semi-batch fed micro-aerobic reactor, because once the sulfate depletes with the sulfate reduction, generated sulfide and elemental sulfur reverse the direction of the dominant reaction of the reactor to generate sulfate again.

4.6 Effect of O_2/S ratio on elemental sulfur formation and sulfate reduction in Single Stage Sulfate-removal Micro-aerated Anaerobic Digester (SSMAD) feeding synthetic wastewater (Experiment F)

During this experiment, the measured pH did not show significant variation, but was in the range of 8.01 – 8.36. The measured ORP value of all phases varied in the range of 404.0 mV – 446.3mV.

4.6.1 Effect of O_2/S ratio on Hydrogen Sulfide removal in biogas

H_2S concentration measured in each anaerobic and micro-aerated phase are shown in Figure 4.63. According to the measured values, H_2S concentration of anaerobic phase was 423 ± 170ppm. It was evidenced that with the gradual increase of O_2/S ratio to the micro-aerated reactor, the concentration of emitted H_2S also decreased gradually. O_2/S ratio at 0.25 and 0.5 it was only 218 ± 201 ppm and 47 ± 46 ppm respectively, at O_2/S

ratio of 1 and 1.5 it was significantly further reduced to 11 ± 12 ppm and 2 ± 3 ppm. The H_2S concentration reduction in each micro-aerobic phase with respect to H_2S concentration during anaerobic phase were 48.5%, 88.9%, 97.4% and 99.5% for O_2/S ratios of 0.25, 0.5, 1.0 and 1.5 respectively.
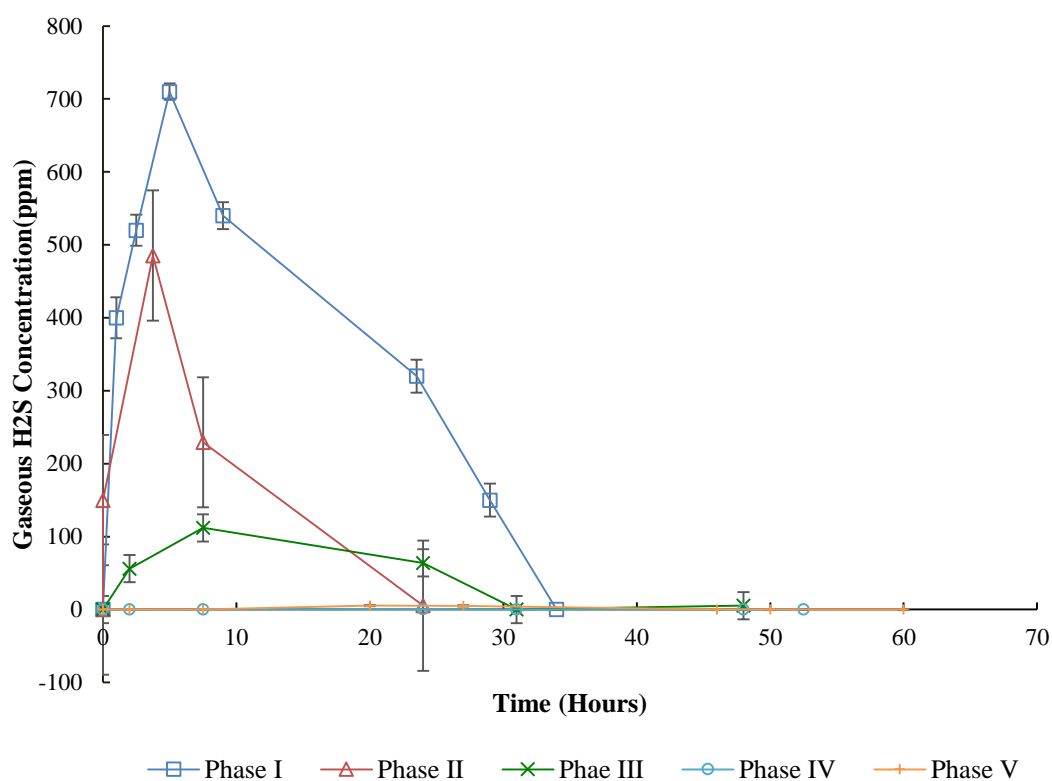
Figure 4.63: Gaseous H_2S concentration Vs Time

4.6.2 Effect of O_2/S ratio on elemental sulfur formation

The liquid phase samples were analysed for elemental sulfur and the results were as shown in Figure 4.65. When air was fed to the reactor, some cream colour turbid layer formed on top of the liquid-gas interphase. With the time some light cream layer formed on the transparent glass walls of head space as well. As $S-SO_4^{-2}$ quantity fed to the batch reactor was less, only a very thin turbid layer formed. According to the Figure 4.65, maximum sulfur generation occurred at O_2/S ratio at 12 hours after feeding. The detectable level of elemental sulfur analysis method used in the

experiment was 0.1 mg which was followed and described by G.C. Stefess et al.[79]. The elemental sulfur analysing method is explained in section 3.10. Thus, the any elemental sulfur formed at very low level could not be detected. But it has given clear variation of the results with the stability of formed elemental sulfur was less at O_2/S ratio 0.5 than 1. But with O_2/S ratio 1.5 the generated elemental sulfur was less. At O_2/S ratio 0.25 (Phase I) only a small elemental formation humped at around 4 hours and formed elemental sulfur seems to be broken down faster. At 0.25 it was clearly shown an oxygen deficiency. Because the formed elemental sulfur was broken down suddenly became unstable. At O_2/S ratio of 1.5, the rate of formation of elemental sulfur decreased but the stability of the formed elemental sulfur was comparatively high.

Figure 4.64: Elemental sulfur formed on the walls of the head space of the reactor
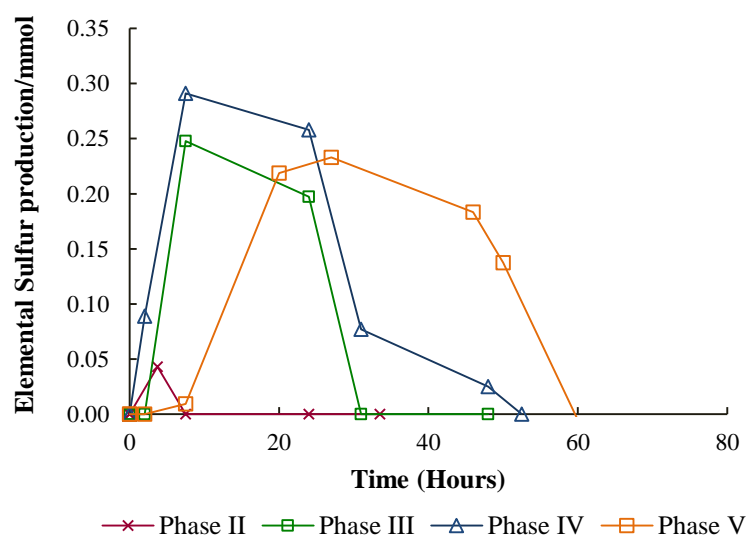
Figure 4.65: Liquid phase elemental sulfur vs Time

Figure 4.66: Pale yellow elemental sulfur formed on the gas-bulk liquid interphase

In Figure 4.65, only the liquid phase elemental sulfur was shown. But it was observed that some amount of elemental sulfur formed on the head space wall which was difficult to quantitatively analyse. From the sulfur balance, conducted for the system the corresponding total elemental sulfur produced can be calculated. Sulfur balance was performed considering the gap of influent and effluent sulfurous compounds as elemental sulfur. The dominant sulfurous compounds considered in sulfur balance were Sulfate, Aqueous total sulfide (H$_2$S (aq), HS$^-$, S^{-2}), gaseous H$_2$S only. From the research carried out to study the elemental sulfur formation from simultaneous sulfate reduction and sulfide oxidation, by G.C. Stefess the major sulfurous compounds generated were only H$_2$S, aqueous sulfide (at higher pH S^{-2}, at pH< 8 – HS$^-$ [71]) and elemental sulfur. But he had not detected any thiosulfate formation [79]. Investigations conducted by X. Xu. et al. [2] also investigated thiosulfate formation in their SR+SO integrated single reactor conversion of influent to elemental sulfur in continuous Expanded granular fluidized Bed reactor[18], whereas L. Krayzelova studies confirmed only negligible amount of thiosulfate was observed in micro-aeration in UASB reactor[15]. C.J.N. Buisman [13]and his research team has found that thiosulfate is generated as a result of chemical oxidation not from a biological oxidation which takes place under high sulfide loading which occurred especially direct influent sulfide oxidation to elemental sulfur not influenced by sulfate. A.J.H Janssen and his team also observed only sulfate and elemental sulfur during biological oxidation of sulfide in Fed-batch reactor[19]. B. Krishnakumar has observed 25%-

35% thiosulfate and only 2% sulfate at higher pH as 9-9.5 in Reverse fluidized loop reactor[13]. He has further reported that undesired products like $S_2O_3^{-2}$ and polysulfide reduced the efficiency of micro-aerobic reactor which has taken place only at declination of bacterial activity such as at high pH or high direct sulfide loading in the influent like above 10 mg/l only. Thus, further it was confirmed that it is reasonable to assume that thiosulfate has not formed as the influent in our experiment was only sulfate not direct sulfide and the sulfide formation after breaking down of sulfate to sulfide concentration was also is less.

When the elemental sulfur found from sulfur balance and compared with measured liquid phase elemental sulfur there was a gap about 40% which can be reasonably assume that this might be the elemental sulfur which has formed on the head space and some loss occurred with bulk liquid biomass. Many researches have observed this effect of sulfur formation on the headspace wall. A. Sarti and his research team has recorded such sulfur accumulation on top of the liquid-gas interphase as well as on the walls of the Anaerobic sequencing batch biofilm reactor operated under micro aerophilic condition to produce elemental sulfur from sulfide[12]. Nevertheless A.J.H. Jassen et al. also reported that there existed difference between measured and calculated elemental sulfur formed due to sulfur generated on the reactor wall. The responsible bacteria for sulfide to elemental sulfur formation is Thiobacillus [12], [19]. I. Diaz et al. [62] also has reported that SOB bacterium is preferably exist in the head space producing elemental sulfur reducing H_2S emission.

At higher oxygen concentrations backward reaction of breaking down formed elemental sulfur and sulfide back to sulfate takes place[113]. But in the researches done to investigate the sulfurous compound formation in direct sulfide oxidation to other oxidized compounds have observed thiosulfate formation due to auto chemical oxidation at higher sulfide/oxygen ratios. A.L.H. Janssen and team[19] has reported that the polysulfide formation at the beginning of some of the experiments and this greenish colour always disappears within 3 hours. Then the suspension become whitish due to sulfur formation. However, during this experiment, greenish colour effect was not observed, it might be because sulfide was not directly fed to the system in high concentrations but only sulfide was formed gradually anaerobically degrading sulfate,

Thus the sulfide loading was not suddenly increased in the system which leads to various other sulfurous components.

4.6.3 O$_2$/S ratio on the sulfate reduction

As per the above-mentioned results, it was convinced that influent sulfate is possible to be converted to elemental sulfur accompanying both steps of sulfate reduction and sulfide oxidation to elemental sulfur simultaneously by feeding air at a controlled manner in semi-batch micro-aerobic reactor.

Although both sulfate reduction and elemental sulfur formation are possible under micro-aerobic condition, the rate of sulfate reduction was decreased with O$_2$/S ratio feed into the system. The sulfate reduction was done by sulfur reducing bacteria (SRB) and most of them are obligate anaerobic micro-organisms. Therefore, with air fed to the reactor, the rate of sulfate degradation was inhibited up to certain extent, but not completely inhibited. As per the results shown in Figure 5.50, the O$_2$/S ratios 0.25 to 1, sulfate reduction was only less affected showing lesser variation of gradient, but at O$_2$/S ratio of 1.5 the sulfate reduction has shown significantly adverse effect with higher reduction of gradient.
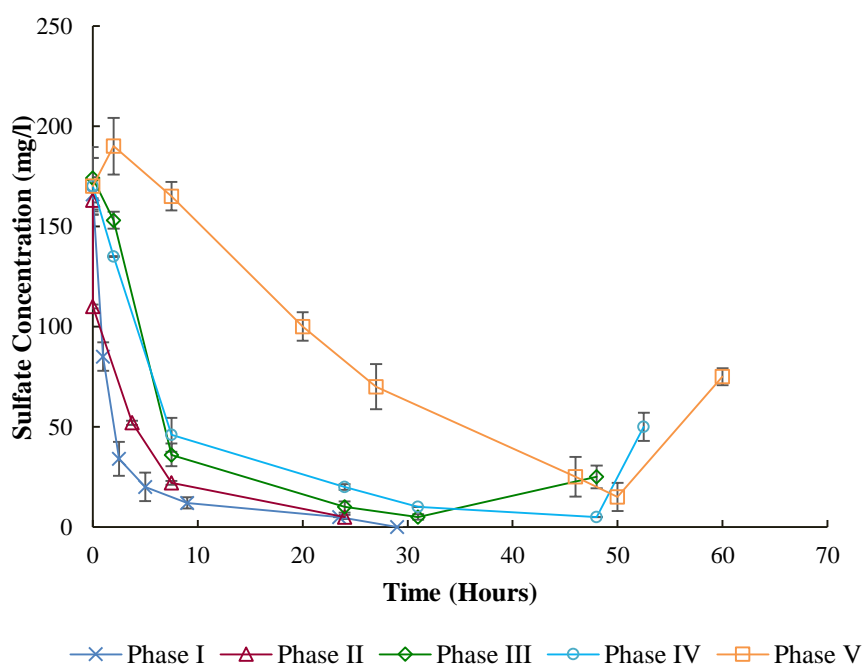
Figure 4.67: Sulfate Concentration Vs Time

Quantitative Sulfurous product variation with time after feeding was plotted at different feed air volumes with O_2/S ratios of 0.25, 0.5, 1.0 and 1.5 as shown on Figure 4.68 to Figure 4.71 respectively.
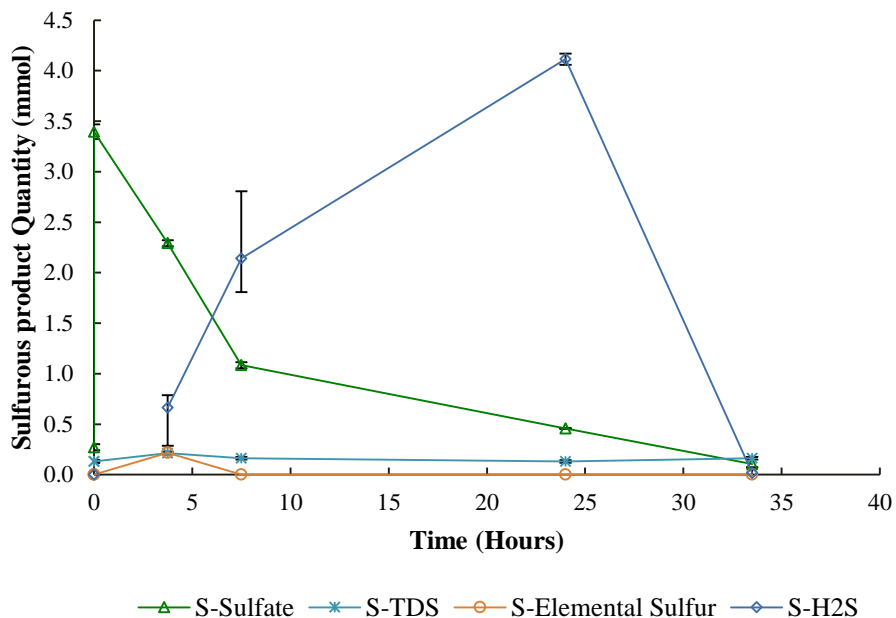
Figure 4.68: Quantitative sulfurous product vs Time at air volume of O_2/S ratio 0.25 (Phase II)
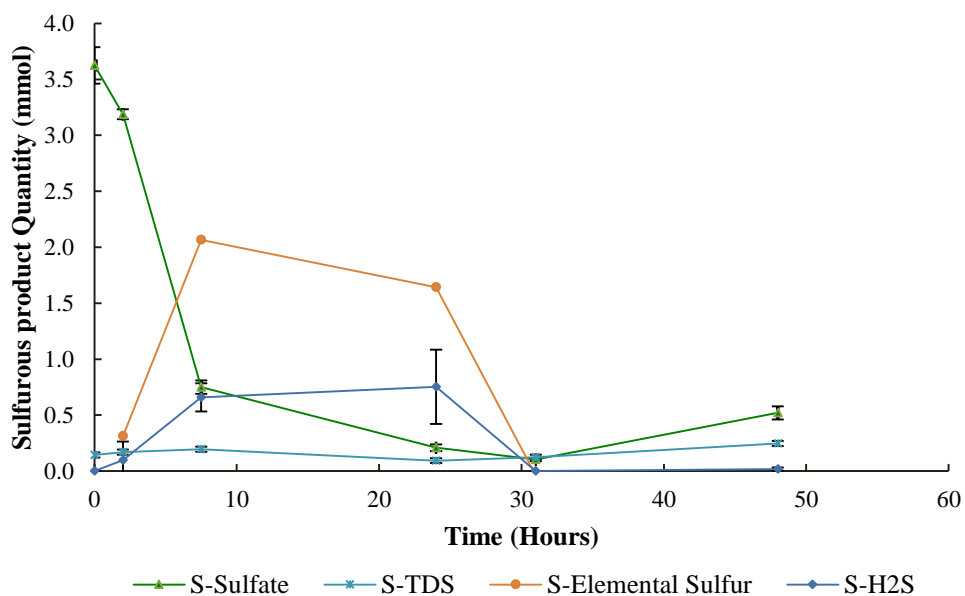
Figure 4.69:Quantitative sulfurous product vs Time at air volume of O_2/S ratio 0.5 (Phase III)
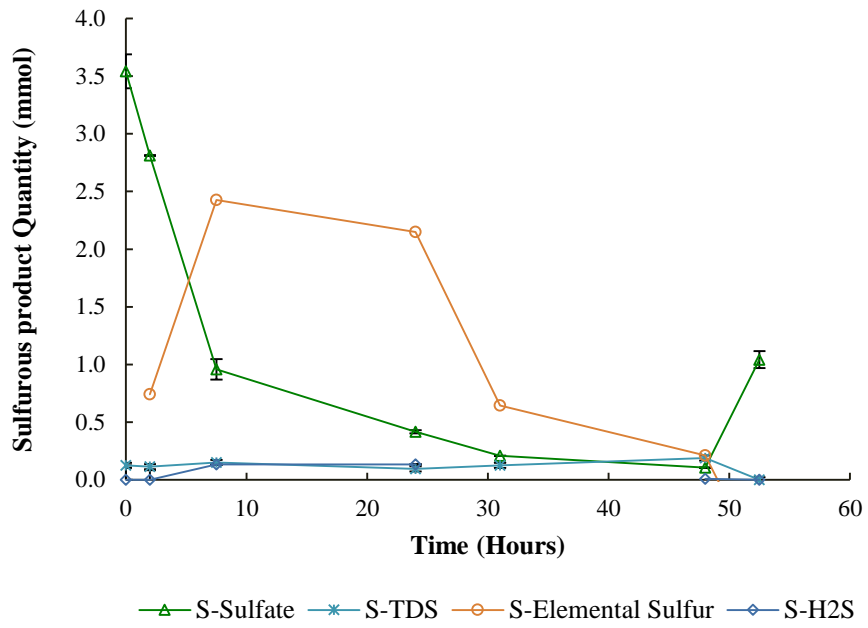
Figure 4.70:Quantitative sulfurous product vs Time at air volume of O_2/S ratio 1.0 (Phase IV)
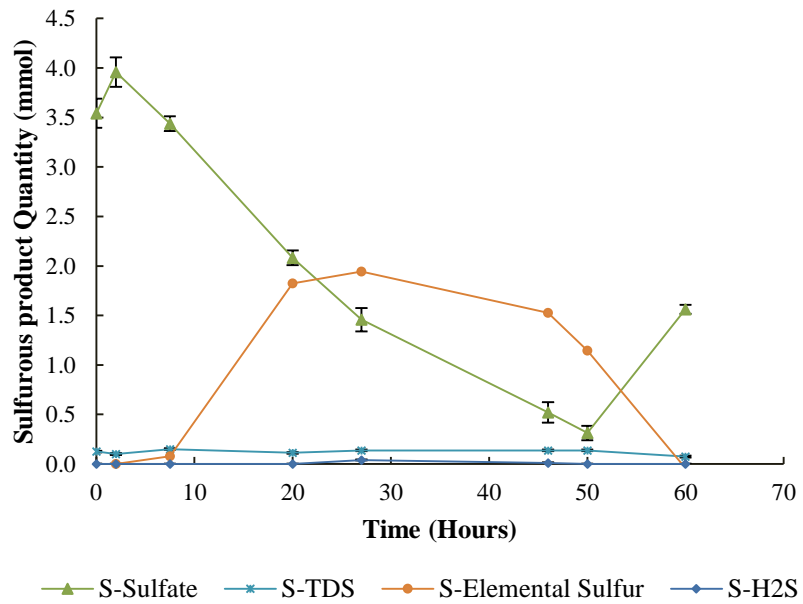
Figure 4.71: Quantitative sulfurous product vs Time at air volume of O_2/S ratio 1 (Phase V)

In all the above four phases, simultaneous sulfate reduction, gaseous H_2S emission, total dissolved sulfide generation as well as elemental sulfur formation has taken place. With different O_2/S ratios, quantity of these products varies from one phase to the other.

At O_2/S ratio 0.25 only very less amount of elemental sulfur was observed, whereas gaseous H_2S emission was high. But with the increase volume of O_2/S ratio at 0.5 the gaseous H_2S emission has come down to 47 ± 46 ppm which is 89% reduction compared to complete anaerobic condition. The amount of elemental sulfur formation also increased from 86%. The degree of micro-aeration was high at 0.5 than 0.25, which increased the quantitative elemental sulfur production as well as stability of formed elemental sulfur. When the degree of micro-aeration is not high as in 0.25, the generated elemental sulfur soon turned back to sulfide compounds by sulfur reducing bacteria.

With further increase of O_2/S ratio fed into the reactor to 1.0, it further enhanced the gaseous H_2S reduction by elemental sulfur production to 11 ± 12ppm which was 97% compared to complete anaerobic condition and elemental sulfur formation was only increased by 17% than O_2/S ratio 0.5. However, the stability of generated elemental sulfur was further improved with increase of O_2/S ratio to 1.0 because the time taken to degrade the elemental sulfur was high in O_2/S ratio of 1.0. The maximum elemental sulfur formation was recorded at O_2/S ratio of 1. Although the H_2S reduction was only 2 ± 3 ppm which was 99.5% at O_2/S ratio of 1.5, elemental sulfur production decreased from 20% than O_2/S ratio of 0.5. when exposed to high oxygen concentration, rate of sulfate reduction decreases, because sulfate reduction is done by strict sulfate reducer which are complete anaerobic bacteria. The time taken for maximum elemental sulfur formation also delayed than with O_2/S ratio of 0.5 and 1. As shown in Figure 4.72, both sulfate reduction and elemental sulfur formation optimized at O_2/S ratio of 0.8 to 1.0. But according to the Figure 4.72, the simultaneous effect at 0.8 and 1.0 was not significantly different. Research findings of A.J.H. Jassen [29] also explained the optimum elemental sulfur production takes place between O_2/S ratio of 0.6 – 1.0 not the stoichiometric value of 0.5.
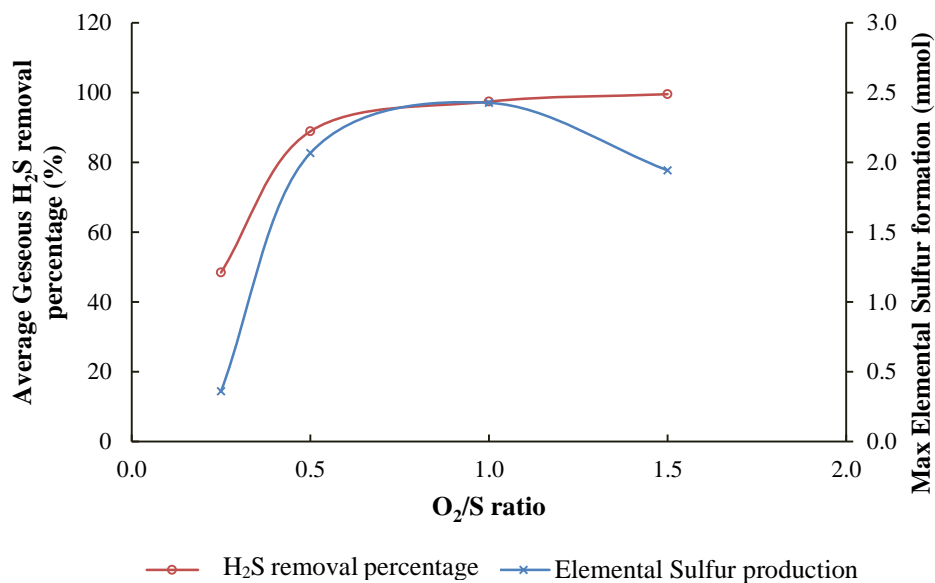
Figure 4.72: Maximum sulfur production percentage, Gaseous H_2S removal percentage Vs O_2/S ratio

4.6.4 The variation of formed elemental sulfur with the time

When considering the variation of sulfurous compounds in the micro-aerobic reactor with the time, the generated elemental sulfur was very small at O_2/S ratio of 0.25 due to insufficient O_2 fed to the reactor. When O_2 was consumed to produce elemental sulfur, O_2 concentration in the reactor decreased creating the anaerobic condition again. Thus, the sulfur reducers might have activated again. That might be the reason the formed elemental has degraded faster emitting gaseous H_2S at the later stage. Similar kind of results have been modelled and observed by X. Xu[113]. and his team, which at the latter stage of micro-aerobic reactor, formed elemental sulfur was broken back again to Sulfide. Although his predictions of elemental sulfur broken down to sulfide when oxygen is depleting in the reactor was valid for all O_2/S ratios, even for O_2/S ratio of 2.5, it is Contradictory according to this results because at greater O_2/S ratios, the probability of elemental sulfur been converted to sulfate by SOB was higher than its been converted to sulfide by SRB.

According to these results, elemental sulfur conversion to sulfide was not observed in other phases where O_2/S ratio is equal or greater than 0.5. In those reactors also generated elemental sulfur has shown a degradation, whereas only sulfate concentration was increased but H_2S or DS concentrations were not increased significantly. The theoretical stoichiometric requirement of O_2/S ratio in sulfur conversion is 0.5 when equation (3) is considered. There might have been remaining unreacted O_2 with in the reactor and with the time formed sulfur was seemed to be reacted with this remaining oxygen and produce sulfate back again when produced elemental sulfur was kept in the reactor for long time. This phenomenon can be explained from Gibb's free energy as well. The Gibb's free energy of sulfate production is lesser than elemental sulfur production. Similar results of freshly formed sulfur particles oxidized back to sulfate were observed by C.J.N. Buisman et al. for influent sulfide fed micro-aerobic CSTR reactors[8] and by A.J.H. Jassen and his team with Fed-Batch reactor[19]. Further he has seen that SOBs are capable of switching within 2 hours from sulfur to sulfate and vice versa. For elemental sulfur production maintaining the DO concentration less than 0.1mg/l is important.

According to the results of experiment F, it was evidenced that gaseous H_2S concentration progressively reduced while the O_2/S ratio increased from 0.25 to 1.5.

4.6.5 Conclusions Derived from the Experiment F

Single stage Sulfate-removal Micro-aerated Anaerobic (SSMAD) reactors can be used to convert influent sulfate to elemental sulfur with supply of control level air in a single reactor. The fraction of sulfate degradation, gaseous H_2S reduction and elemental sulfur formation varies with O_2/S ratio fed to the reactor.

The optimum simultaneous gaseous hydrogen sulfide removal as well as elemental sulfur formation has taken place between at O_2/S ratio of 0.8 to 1.0 around 12 hours after feeding wastewater sample.

In every phase, with introduction of air into the reactor, initially sulfate reduction, elemental sulfur formation simultaneously occurred. Then when oxygen concentration depletes in the head space of the reactor with consumption of oxygen to elemental sulfur formation or any biological transformation, generated elemental sulfur convert back to gaseous H_2S, dominating the SRB reactions of SRB at the anaerobic condition.

At O_2/S ratio of 0.25, the formed fresh elemental sulfur converted into gaseous Hydrogen sulfide where as O_2/S ratio of 0.5-1.5 the freshly formed elemental sulfur oxidized back to sulfate with the time. It is because following formation of elemental sulfur even if there exist more oxygen in the head space, Sulfur Oxidizing Bacteria further oxidise the generated elemental sulfur to sulfate. Therefore, proper removal of mechanism has to be deployed to take elemental sulfur out of the reactor once it is formed before re-biological oxidation of formed elemental sulfur. The freshly formed elemental sulfur accumulates accumulated on the gas-liquid interphase as well as the headspace reactor walls.

4.7 Effect of O_2/S ratio on elemental sulfur formation and sulfate reduction in Single Stage Sulfate-removal Micro-aerated Anaerobic Digester (SSMAD) feeding SLW (Experiment G)

During this experiment, the measured pH was maintained in the range of 7.5-8.0 feeding 3M HCl. pH variation was not significantly varied in the micro-aeration experiment as the completely anaerobic experiments due to the presence of sufficient buffer capacity in the micro-aerobic reactors. Sulfurous compounds in the anaerobic and micro aerobic phases were measured and analysed, while only initial and final COD values were monitored at each feed cycle of 48 hours after feeding during which complete sulfate reduction was taken place. However, TAN was measured intermittently to check the prevalence of ammonia inhibition in the reactor.

4.7.1 Effect of O_2/S ratio on H_2S emission

The emitted gaseous H_2S concentration decreased with the introduction of O_2 via air. The average H_2S concentration of the biogas in completely anaerobic condition was 168 ± 128 ppm. However, it was reduced to 48 ± 61ppm, 10 ± 12ppm, 4 ± 4ppm with variation of O_2/S ratio 0.5 (Phase II), 1.0 (Phase III) and 1.5 (Phase IV) respectively whereas the influent COD/SO_4^{-2} remain constant at 5 in each phase. The percentage of H_2S concentration reduction in micro-aerobic phases of phase II to phase IV were observed to be 71.4%, 94.0% and 97.6% with respect to completely anaerobic condition (Figure 4.73). Although the minimum gaseous H_2S was observed for O_2/S

ratio of 1.5 and influent COD/SO_4^{-2} ratio 5, when the influent COD/SO_4^{-2} ratio increased to 10 while the O_2/S ratio was kept constant at 1.5, again the gaseous H_2S concentration increased to 96 ± 97 ppm.

As shown in Figure 4.74, above results were analysed using a surface plot which gaseous H_2S concentration variation is with time and O_2/S ratio. From the graph, it is clearly evidenced that during the phase I, in which O_2/S ratio is 0 under complete anaerobic condition H_2S emission was highest and it was gradually decreased with introduction of more oxygen into the system with increasing O_2/S ratio of 0.5, 1.0 and 1.5.
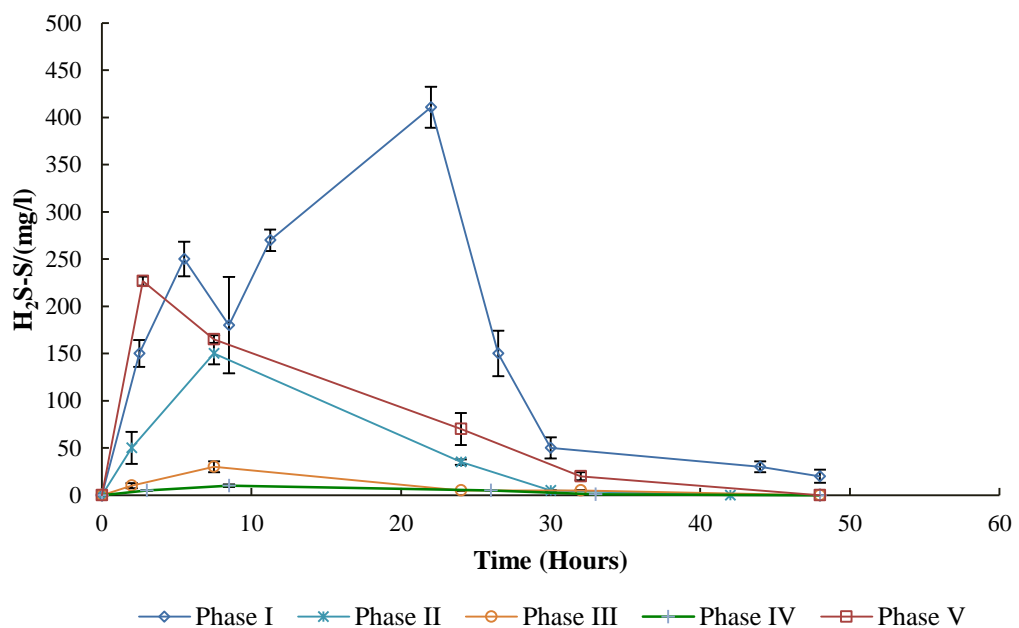
Figure 4.73:H_2S Concentration in the biogas Vs Time
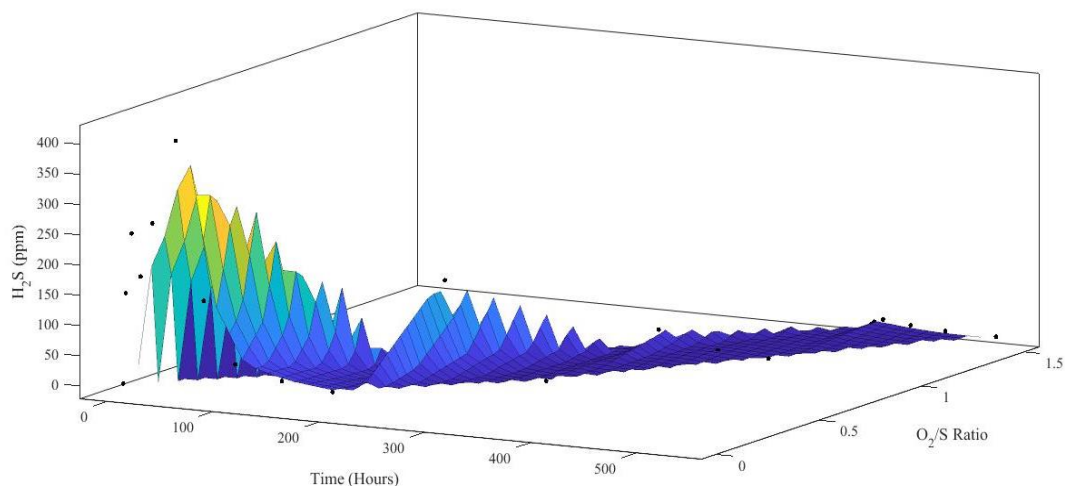
Figure 4.74: Surface plot of H$_2$S Vs O$_2$/S ratio Vs Time

4.7.2 Effect of O$_2$/S ratio on sulfate reduction

Although the H$_2$S concentration in the biogas stream decreased with the O$_2$/S ratio, the sulfate reduction was affected with increasing O$_2$/S ratio. The specific sulfate concentration with respect to influent sulfate concentration variation is shown on Figure 4.75. Although in completely anaerobic (phase I) and O$_2$/S ratio 0.5 (phase II) phases the sulfate concentration was gradually decreasing throughout the experiment, in other two micro-aerobic reactors the sulfate concentration slightly increased initially and then decreased gradually.
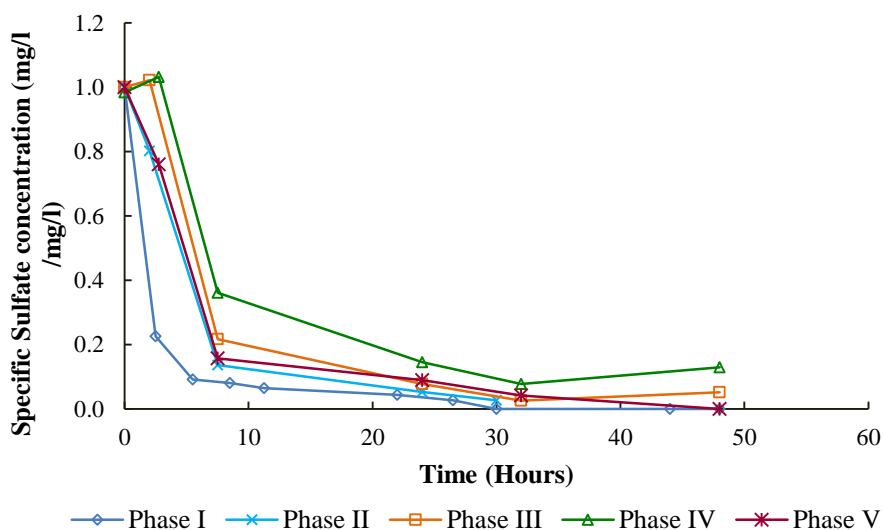
Figure 4.75:Sulfate Concentration in bulk liquid Vs Time

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As per the Figure 4.75, the highest rate of sulfate reduction was achieved at complete anaerobic condition. Highest rate of sulfate reduction was recorded for anaerobic phase of 57.50 mg/l.hr. However, the rate of sulfate reduction was affected with introduction of air. It is also further evidenced in Percentage cumulative sulfate reduction Vs Time as shown in Figure 4.76. The first day sulfate reduction recorded in each phase was 96.5%, 95%, 94% and 86% form phase I to phase IV respectively. When the complete sulfate reduction was considered, sulfate degradation at O_2/S ratio 1.5 is only more affected with introduction of air. Complete sulfate reduction was not observed in phase IV while sulfate concentration was only reduced to 15mg/l at 33 hours after the feed and increase the sulfate concentration again to 25 mg/l at 48th hour. Nevertheless, minimum sulfate reduction rate also observed for phase IV. This may be due to oxidation of formed elemental sulfur further to sulfate with presence of excess air available in the head space.
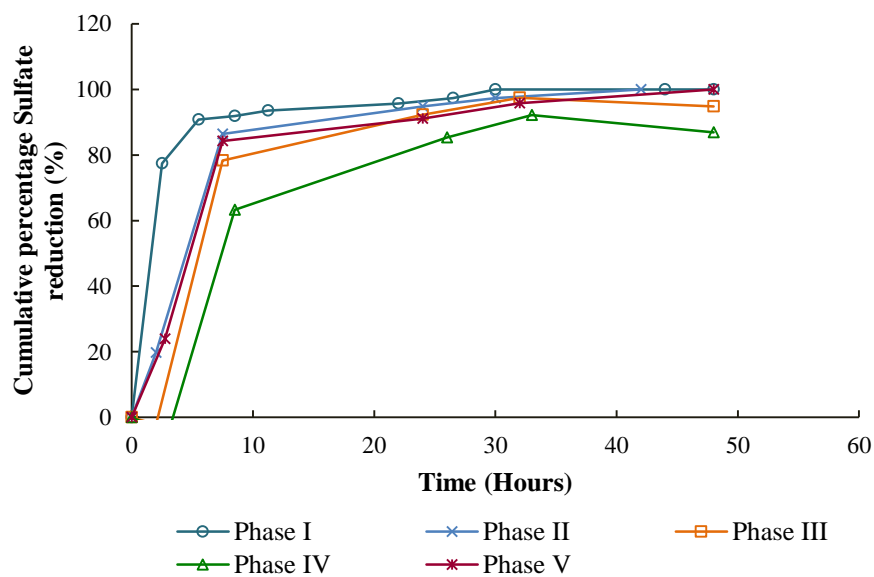
Figure 4.76: Cumulative percentage sulfate reduction Vs Time

Adverse effect on sulfate reduction was observed by X. Xu and his team[113] with introduction of excess oxygen whereas he has measured the oxygen content via DO. In the range of DO 0.08 – 0.26 mg/l the sulfate reduction rate was 19 ± 1 mmol $SO_4^{-2}g^{-1}$VSS d^{-1}, but the SOB was enhanced and increased oxidation activities to 8.75 mmol

$S^{-1}g^{-1}VSS\ d^{-1}$. Nevertheless, at DO concentration higher than 0.30m g/l h, complete failure of SRB and SOB in a single EGSB reactor.

4.7.3 Effect of O_2/S ratio on Elemental sulfur formation

The measured elemental sulfur concentration in micro-aerobic phases are as shown in Figure 4.77. The highest elemental sulfur concentration was measured with in phase III with O_2/S ratio of 1.0. at 20 hours after feeding whereas for phase IV with O_2/S ratio of 1.5 at 22 hours after feeding. However, the maximum elemental sulfur formation for phase II with O_2/S ratio of 0.5 was observed at 8 hours after feeding. The results explained above is summarized in corresponding surface graph of Elemental sulfur formation with time and O_2/S ratio for phase I to IV is shown in Figure 4.77. From Figure 4.78, it is much clear that maximum elemental sulfur formation occurred at O_2/S ratio of 1.0.

Undissolved elemental sulfur produced in the reactor was carried up by the generated biogas and accumulating on the interphase of the bulk liquid and the head space. Sulfur particles on this layer was not disrupted by turbulence. Light cloudiness was observed on the interphase between bulk liquid and the headspace and the walls of the reactor headspace when elemental sulfur was formed, because of the less concentration used in the experiment. But when after the series of micro aerobic experiments were conducted a light cream colour elemental sulfur could be clearly observed been attached on the reactor walls, upper lid and gas outlet tubes. Further it was observed that when these micro-aerobic reactors were not fed with substrate for a long time without biogas generation, these formed elemental sulfur solid sediment down to the reactor. The generation of elemental sulfur (S^0) on the bulk liquid and head space interphase was observed in other past experiments on micro-aeration as well [19], [12].
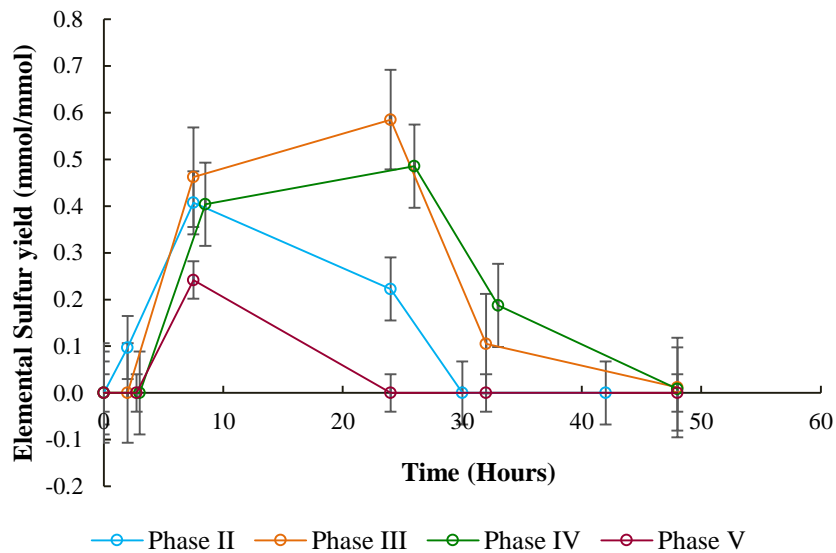
Figure 4.77: Elemental sulfur yield with respect to influent Sulfate Vs Time
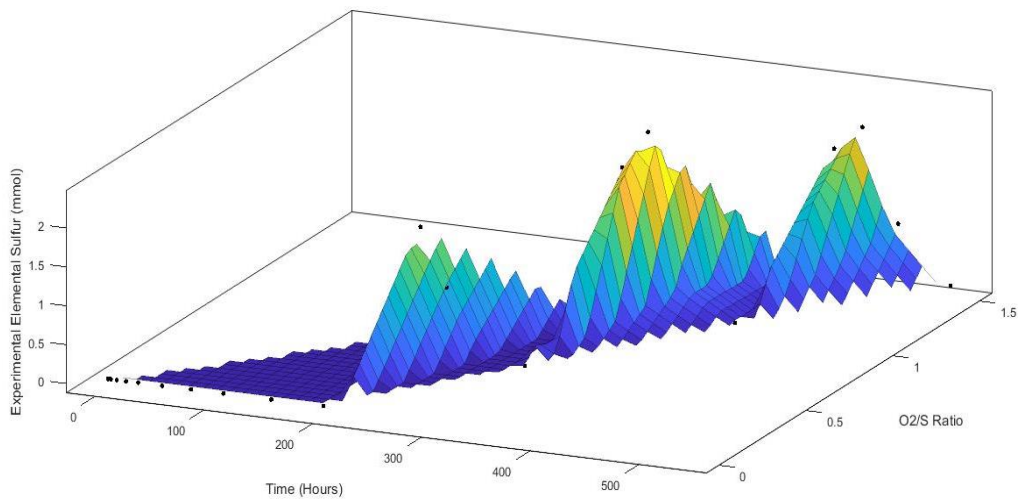
Figure 4.78: Surface plot of experimental elemental sulfur formation with Time and O_2/S ratio

The Dissolved Oxygen amount measured was less than 0.1 mg/l in the bulk phase of the micro-aerobic reactor. As per the past literature also under oxygen limited condition with dissolved oxygen below 0.1 mg/l, the S^0 is the major end product of sulfide oxidation, while sulfate is the major end product at sulfide limiting

condition[19],[12]. Further, X.J. Xu et al. has found that level of dissolved oxygen (DO) is an effective parameter to regulate activities of SRB and SOB[114]. At high DO concentrations the activities of SRB were inhibited, Thus the integrated SRB + SOB reactors fail and even the S^0 recovery percentage declines because sulfide was oxidized by free oxygen. X.J. Xu and his team[113] has investigated that the optimum DO concentration was 0.10-0.12 mg/l, since activities of SOB enhanced neither SRB were inhibited.

There was a time lag for formation of elemental sulfur when in phase IV with O_2/S ratio is 1.5, because the sulfate degradation process was slow down with introduction of high content of air into the micro-aerobic reactors with the partial inhibition of SRB which a strict anaerobic bacterium. Stability of the generated elemental sulfur was high in both O_2/S ratio of 1 and 1.5, whereas at O_2/S ratio of 0.5 formed elemental sulfur was rapidly degraded. Because of the dominance of anaerobic condition with the oxygen consumption for various biological processes in the reactor including formation of elemental sulfur.

Although the Gibb's free energy for sulfate formation as explained in section 2.2.2 is higher than elemental formation, biological oxidation of sulfide to sulfur proceeds much faster than oxidation of sulfide to sulfate from the obtained results under limited oxidation because of the difficulty in maintaining the electron flux constant as explained by S. Okabe and Buisman et al. as per equation 8 and equation 9 [8], [114].

$$HS^- \rightarrow S^0 + H^+ + 2e \qquad \text{……………………. (8)}$$

$$HS^- + 4H_2O \rightarrow SO_4^{-2} + 9H^+ + 8e \qquad \text{…………... (9)}$$

Figure 4.79: Elemental sulfur formed on the wall of the head space and the gas-bulk liquid inter-phase
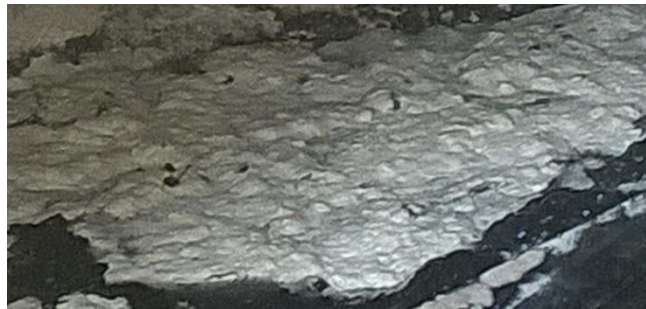
Figure 4.80: Pale yellow Elemental sulfur formed generated on the gas-bulk liquid interphase

When theoretical elemental sulfur amounts were calculated using the mass balance for sulfurous compounds (Sulfate, DS, Gaseous H_2S) in the reactor as explained in-detail in section 4.7, and the measured quantities were compared, the measured elemental sulfur amount was about 45% less. This may be because only elemental sulfur generated in bulk phase were measured and elemental sulfur formed by head space SOB, on the head space of the reactor were not accounted in the measured elemental sulfur amount and the loss of some elemental sulfur with biomass as the samples for

elemental sulfur was taken from the interphase between bulk liquid and headspace. From the utilized analytical method, elemental sulfur concentration high points were only detected, and lesser amount of elemental sulfur generated at the beginning and the later part was not detected. However elemental sulfur generating in the headspace and the wall of the reactor headspace difficult to be measured practically. L. Krayzelova. and the researchers team has observed that micro-aerobic UASB reactor, 74% sulfur was detected in the effluent (41% being sulfide and 33% being elemental sulfur), only 10% accumulated in headspace as elemental sulfur and 9% escaped in biogas as hydrogen sulfide[15].

Many researches have observed this effect of sulfur formation on the headspace wall and biogas outlet tubing[15]. Sarti A. and his research team has recorded such sulfur accumulation on top of the liquid-gas interphase as well as on the walls of the Anaerobic sequencing batch biofilm reactor operated under micro aerophilic condition to produce elemental sulfur from sulfide[12]. Nevertheless A.J.H. Jassen et al. also reported that there existed difference between measured and calculated elemental sulfur formed due to sulfur generated on the reactor wall. The responsible bacteria for sulfide to elemental sulfur is chemolitho autotrophic bacteria belonging to the genus Thiobacillus [12], [19]. I. Diaz et al. [62] also has reported that SOB bacterium is preferably exist in the head space producing elemental sulfur reducing H_2S emission during this experiment.

There are many researches carried out to investigate for direct sulfide conversion to elemental sulfur for various types of synthetic as well as natural wastewater prevent air pollution due to emission of hydrogen sulfide. Some researchers have investigations on sulfate reductions using two reactors, which initially the sulfate was broken down to sulfide in an anaerobic reactor, then use another micro-aerobic reactor to convert sulfide into elemental sulfur. A. Sarti[115] and his team used two bench scale anaerobic sequencing batch biofilm reactors (ASBBR) which contain coal as inert support to treat sulfate rich wastewater. However from the first reactor sulfate was reduced to sulfide, and with the second reactor which maintained under oxygen limited condition, partially treated wastewater with high sulfide concentrations was converted to elemental sulfur by aerobic sulfide oxidizing process[12]. He further has found that there was 57% and 98% DS removal efficiency of respective reactors. His

and L.W. Hulshoff-Pol investigations further justified the use of ethanol in sulfate reducing systems increase sulfate reduction[71]. In our study also, SLW was pre-treated using ethanol adjusting the COD/SO_4^{-2} for optimum value of 5. The common drawback of using two reactors is that it increases capital cost and operational cost[18]. Very few literatures are available on using a single reactor to treat sulfate rich wastewater with integration of the SRB processes of sulfate reduction to sulfide and SOB step of reduced sulfide converted to elemental sulfur[61]. X.J. Xu et al. [18] has shown successful recovery of S^0 using a single reactor with enhanced activities of SOB with limited oxygen to peak recovery of S^0 from sulfate. J.T. De Sousa[20] et has used an Anaerobic Hybrid reactor comprising anaerobic zone in the below and micro-aerated UASB reactor zone with an aeration device above for biological conversion of sulfide to elemental sulfur. The variation of specific sulfurous compounds; S-Sulfate, S-DS, S-Gaseous H_2S, S-Experimental Elemental sulfur in the reactor is present from Figure 4.81 to Figure 4.83.
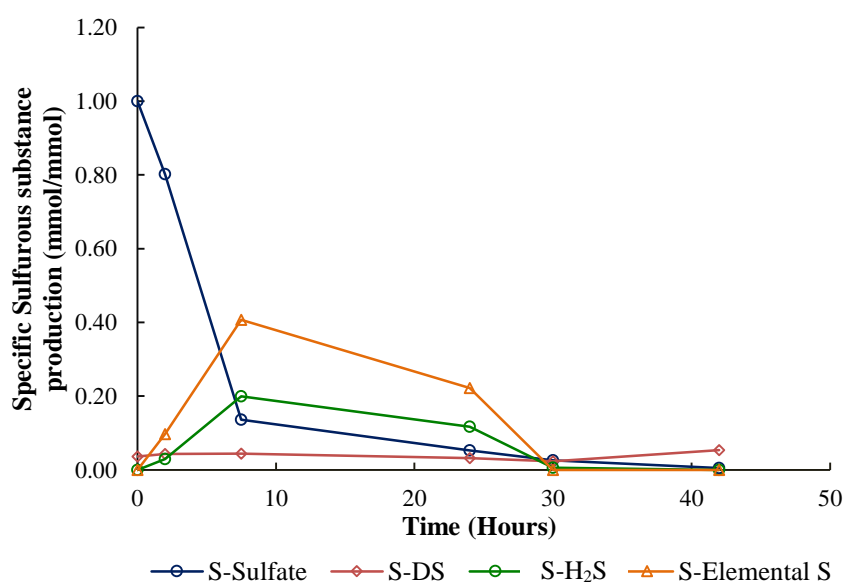
Figure 4.81: Specific sulfurous compound production with respect to influent S-sulfate of phase II Vs Time
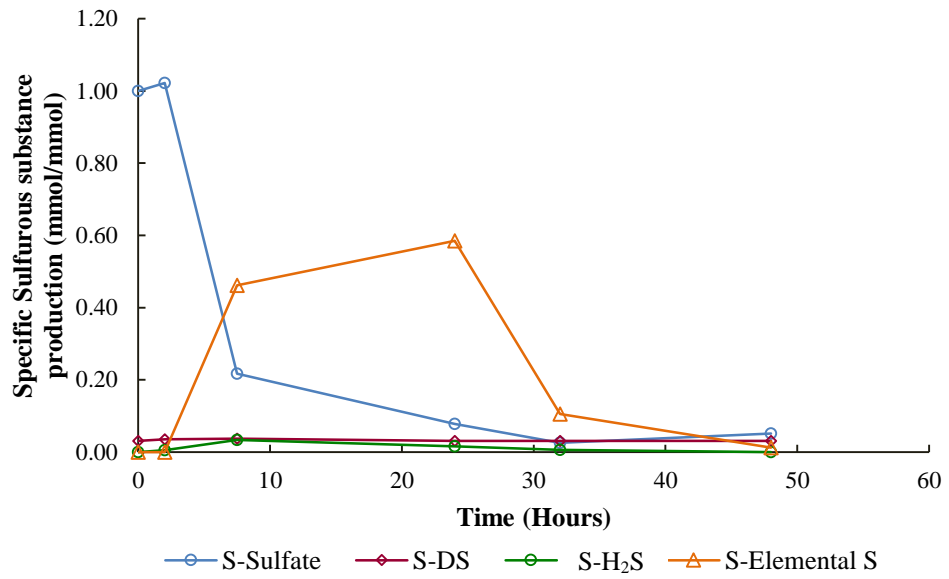
Figure 4.82: Specific sulfurous compound productıon with respect to influent S-sulfate of phase III Vs Time
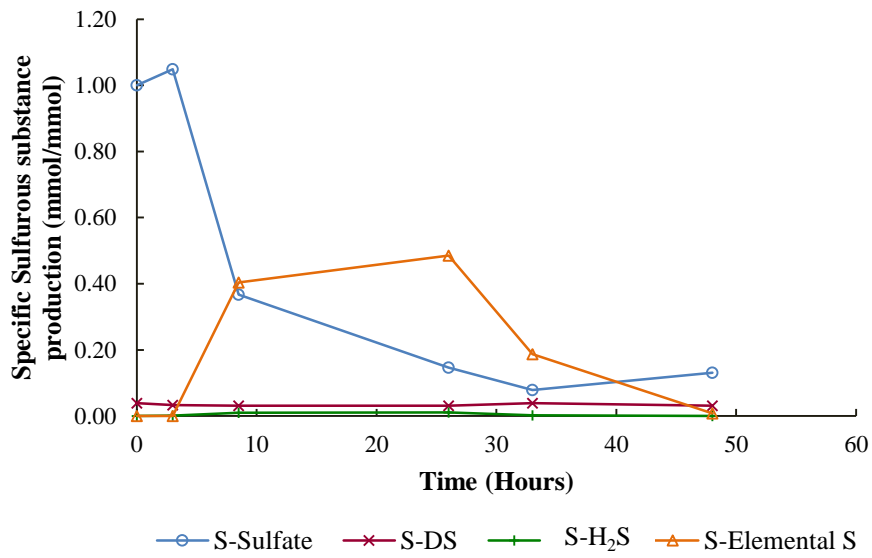
Figure 4.83:Specific sulfurous compound production with respect to influent S-sulfate of phase IV Vs Time

In all the above phases from II to IV, simultaneous sulfate reduction, gaseous H_2S emission, total dissolved sulfide generation as well as elemental sulfur formation has taken place. With different O_2/S ratios, quantity of these sulfurous products varies from one phase to the other.

At phase II with O_2/S ratio 0.5, very less amount of elemental sulfur was observed at about 10 hours after the feed, whereas gaseous H_2S emission is still high. But with the increase of O_2/S ratio to 1.0 the gaseous H_2S emission has come down to 10 ± 12 ppm which is 94% reduction compared to complete anaerobic condition. In phase III the elemental sulfur production has risen by 47% compared to phase II.

The maximum elemental sulfur yield was observed in phase III with O_2/S ratio of 1, but the time taken for it has increased to about 24 hours unlike for synthetic wastewater with acetate and ethanol in the above experiment in section 4.7 which the maximum elemental sulfur recorded at 12 hours after feeding. It is because unlike using simple organic compounds with synthetic wastewater, in this experiment carbon compound hydrolysis and acidogenesis acts as rate limiting step for sulfate reduction providing simple organic compounds for SRB. Therefore, the elemental sulfur concentration inside the phase III micro-aerobic reactor has gradually built up. When the degree of micro-aeration was increased from 0.5 to 1.0, quantitative elemental sulfur production as well as stability of the formed elemental sulfur has increased. Because the generated elemental sulfur in phase II has degraded back to sulfide compounds by activation of SRB when the O_2 concentration in the phase II reactor decrease down a certain threshold value with the consumption of oxygen for some other biological reactions and formation of elemental sulfur. Elemental sulfur bio-reduction back to sulfide in a stirred reactor under limited mesophilic condition was observed by Escobar C. Nevertheless C. Escobar were able to be found that it the responsible bacteria is Desulfovibrio Desulfuricans which is a SRB[116]. However F.P. Van der Zee has reported reappearance of sulfide from S^0 after complete depletion of oxygen[61]. Even with mathematical modelling of X. Xu [113]suggested S^0 been broken down back to sulfide for all O_2/S ratios, but it was only observed in phase II with low O_2/S ratio, while for high O_2/S ratios in phase III and IV, S^0 converted to sulfate, not sulfide.

In phase IV the degree of micro aeration has further increased to O_2/S ratio of 1.5. The H_2S concentration was only 4 ± 4ppm which is 97.6% reduction compared to phase I. But the elemental sulfur production has decreased by 6% in phase IV. when exposed to high oxygen concentration, rate of sulfate reduction decreases, because sulfate reduction is done by strict sulfate reducer which are complete anaerobic bacteria. The time for maximum elemental sulfur formation got delayed from phase IV to phase III respectively.

However, towards the later part of the phase IV the sulfate concentration has increased with decrease of elemental sulfur. Thus, the elemental sulfur seems to be further converted to sulfate because of the remaining excess O_2 in the head space. Microaerophilic mixed-population biofilms were grown in fully submerged rotating disk reactors (RDRs) with SR +SO process, Reduced influent sulfate converted to H_2S and S^0 both can be converted back to SO_4^{-2} by chemolitho autotrophic sulfur oxidizing bacterium in excess oxygen condition [114].

In many papers and experiments the sulfate formation with higher O_2/S ratios were observed. Mathematical model was presented for SR+SO reactions on micro-aerophilic treatment with maximum sulfur recovery for sulfate rich wastewaters by X. Xijun [113]. However, the model was validated for SR+SO systems with denitrifying sulfide removal systems. The curves drawn for specific sulfurous compound of each O_2/S ratio for all phases have similar pattern as his model with some deviations. With his model, the sulfate reduction curves shown a negative impact with introduction of oxygen into the system. The rate of sulfate reduction decreases with time and time required for sulfate reduction has increased from O_2/S ratio of 0.25 to 2.5.Unlike the explanation of his mathematical model, which for all the O_2/S ratios, complete sulfate reduction seen, in our real experiments as well as X.J. Xu experiments, sulfate reduction has not reached complete sulfate reduction at higher O_2/S ratios, but the rates were similarly decreased. The characteristics of elemental sulfur, S^0 curves behaves in a similar manner with increase of stability with increase of O_2/S ratio, gradually increase the formed S^0 with a maximum and again gradually reduce with time. Yet, the maximum elemental sulfur formation was presented at O_2/S ratio 2.5, where as in our experiment it was at 1.0 as in Figure 4.82. In his plots, with gradual reduction of elemental sulfur, sulfide has generated, whereas in our experiments, sulfate

concentration increases at higher O_2/S ratios which shown excess oxidation of elemental sulfur to sulfate. At higher oxygen intensity, elemental sulfur reduction to sulfate was observed in a sulfide fed batch fed reactor[19].

4.7.4 The effect of increasing influent COD/SO_4^{-2} ratio from 5 to 10 when O_2/S ratio of 1

It has been observed that the maximum elemental sulfur yield taking place at O_2/S ratio of 1 while the influent COD/SO_4^{-2} ratio was 5. Therefore, the influent COD/SO_4^{-2} ratio was further increased to 10 at O_2/S ratio of 1 in phase V. As shown in Figure 4.75 and Figure 4.76 the sulfate reduction was as other phases. But as per Figure 4.77, the elemental sulfur production as gone down with increasing COD/SO_4^{-2} ratio to 10 resulting adverse effect on elemental sulfur formation. Ethanol is a strong partial electron donor for sulfate reduction which enhance the elemental sulfur formation up to COD/SO_4^{-2} ratio of 5 but addition of excess ethanol seems to support rapid sulfate reduction and more gaseous H_2S escaped with the high biogas volumetric production without producing more elemental sulfur and even the produced elemental sulfur were broken back to sulfide due to flushing of head space oxygen with high volumetric bio gas production. It is evidenced from the gaseous H_2S concentration which has increased to 96 ± 97 ppm as in Figure 4.74.

4.7.5 Effect of O_2/S ratio on Sulfate reduction, Gaseous H_2S production and Elemental sulfur formation.
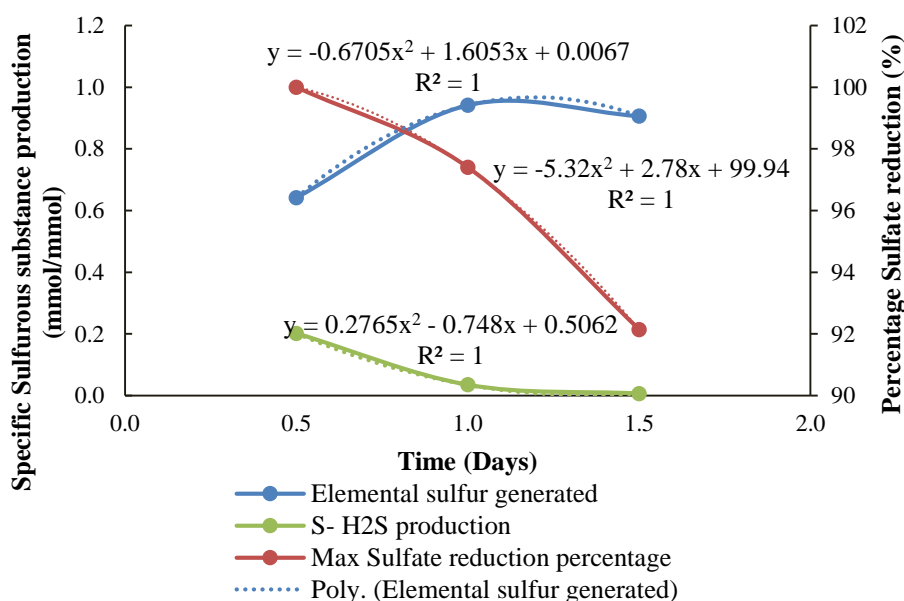
Figure 4.84:Elemental sulfur, Maximum sulfate reduction percentage and average H_2S production Vs Time

The most essential and influential factors for simultaneous sulfate reduction, H_2S elimination and maximize elemental sulfur production are summarized in the Figure 4.84. According to the above figure, O_2/S ratio of 0.5 is not sufficient as the minimum elemental sulfur production and even the highest H_2S production takes place at O_2/S ratio of 0.5. Anyhow the maximum sulfate reduction occurs at the same ratio. At O_2/S ratio of 1.5 elemental sulfur production was high and expected sulfate reduction was low, while the sulfate reduction was affected adversely. The trend lines for each parameter were drawn as shown in the Figure 4.84. These results were more clearly emphasised with the surface plot drawn for elemental sulfur production with sulfate reduction and O_2/S ratio in Figure 7.85.

According to the drawn trend lines, the maximum elemental sulfur production lies at O_2/S of 1.18. At O_2/S of 1.18, specific H_2S formation was less than 0.2 mmol/mmol while the sulfate reduction was 95.8%. In a continuously operated Expanded Granular Sludge Bed (EGSB) reactor which the influent was sulfate the peak sulfate removal

efficiency and recovery of elemental sulfur was 81.5% and 71.8% at DO concentration 0.10-0.12 mg/l.
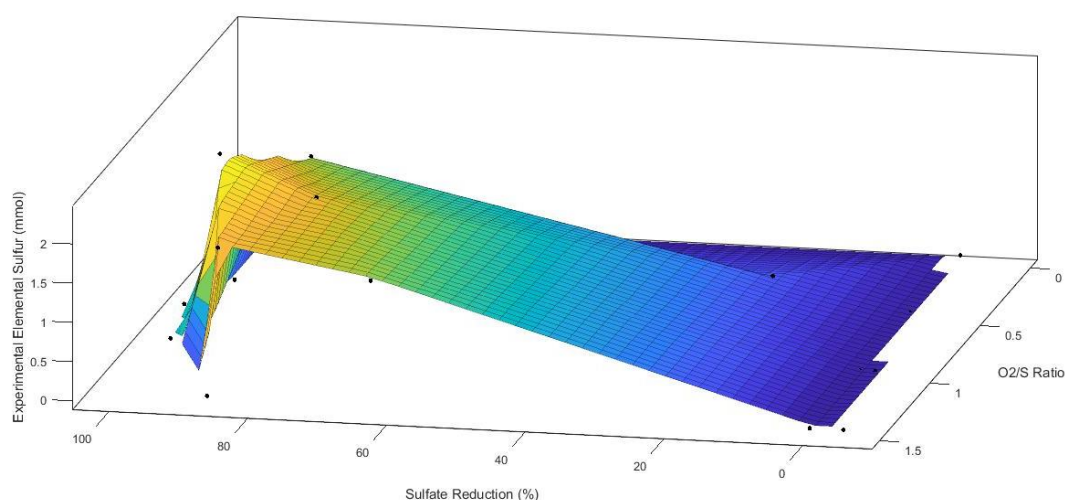
Figure 4.85: Surface plot of elemental sulfur production with sulfate reduction and O_2/S ratio

In the range of O_2/S ratio of 1.0 to 1.2, elemental sulfur production was high, without limiting in to only one value of 1.18. The maximum elemental sulfur production has taken place for synthetic wastewater at 0.8-1.0 while for SLW it was at 1.18. Hence there was 18% rise in the optimum O_2/S ratio for synthetic wastewater with acetate and ethanol mixture, compared to natural skim latex wastewater. This could be due to consumption of oxygen to some other biological processes such as protein and carbonaceous substance hydrolysis as reported by D. Botheju and R. Bakke [117]. According to the results derived through this experiment, optimum O_2/S ratio lies in a narrower range.

Although the stoichiometric O_2/S ratio is 0.5, maximal sulfur formation is obtained at an O_2/S ratio of between 0.6 and 1.0 for sulfide fed CSTR at pH 8.0 with $73\pm10\%$ maximum sulfur yield[19]. Batch experiments carried out by van der F.P. Zee et al. [61] proved that O_2/S ratio was 0.52 -0.53 mmol/mmol for influent sulfide concentrations. Nevertheless, he has carried out continuous micro-aerobic fluidized bed reactor with airflow of 0.7-0.9 $m^3 m^{-3} d^{-1}$ which correspond to O_2/S ratio of 8-10

with respect to influent sulfide, which the O_2/COD ratio was only 0.05g O_2/ g influent COD which reduce the H_2S level to below threshold value of 0.02%.

As suggested by Okabe, although the sulfide oxidation in a single SR and SO integrated reactor, the S^0 production is depend mainly on rate of sulfate reduction or sulfide production by SRB, some other factors like the availability of electron acceptors (O_2 or NO_3^-), the size and type of SOB population also affect the S^0 formation[114]. It was demonstrated that micro-aerobic sludge had higher sulfide oxidizing activity than the original anaerobic reactor sludge after anaerobic sludge was used in micro-aerobic reactor, but in addition, the reappearance of sulfide or re-reduction of oxidized sulfur species was also faster with micro-aerobic sludge. [61].

Several techniques are already utilized for separation of formed biological elemental sulfur in industrially biological reactors, such as sedimentation, centrifugation, filtration, extraction and membrane separation[118], [12]. In comparison with other techniques, gravity sedimentation is the cheapest and technically attractive. However, for gravity sedimentation to be applied, formation of easily settle able sulfur is essential. Sulfur slurry separated from a separator inside the continuous reactor was dewatered using a decanter centrifuge resulting in a sulfur cake of 60-65% dry solids content. After centrifugation the sulfur purity is 95-98%. While the remaining 2-5% is organic material and trace salts. If this sulfur reused by the industry or agriculture, generated sulfur has to be further processed by washing, drying and smelting. Thus the purity of sulfur is increased up to 99.9%[119]. P.R. Camiloti [32]and his team has successfully used membrane separation for separating elemental sulfur. Formed biological sulfur was deposited on top of the surface of the tubular silicone rubber membrane to feed and control air supply to the reactor.

4.7.6 Energy Dispersive X-ray (EDX) analysis with Scanning Electron Microscope (SME) for biological elemental sulfur
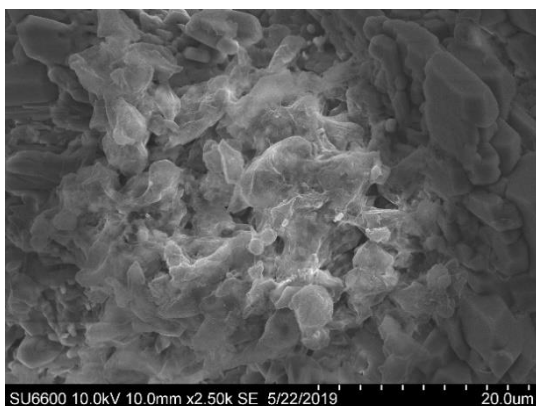
Figure 4.86: SEM image of elemental sulfur site1 at 20.00 μm
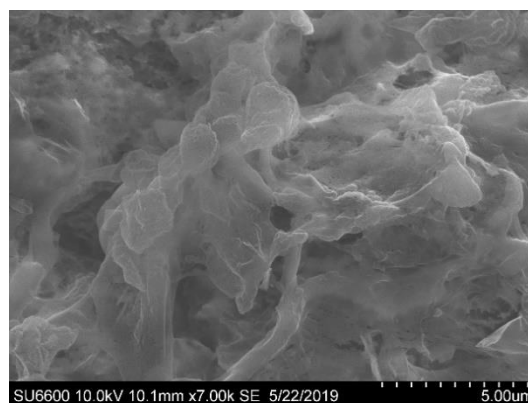
Figure 4.87: SEM image of elemental sulfur site1 at 5.00 μm
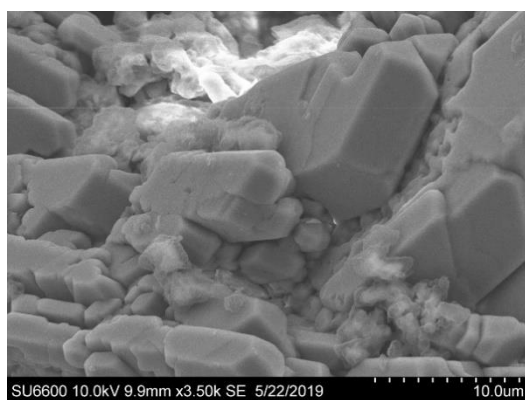
Figure 4.88: SEM image of elemental sulfur site2 at 10.00 μm
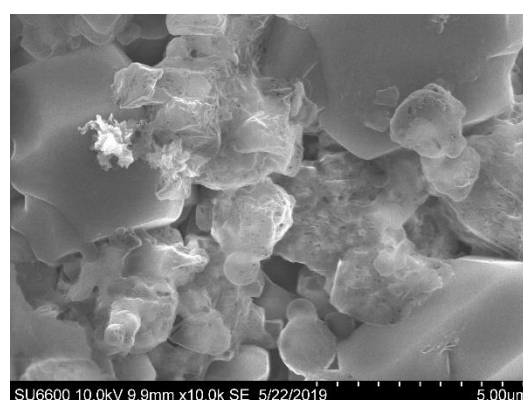
Figure 4.89: SEM image of elemental sulfur site2 at 5.00 μm

Energy Dispersive X-ray (EDX) analysis was carried out with Scanning Electron Microscope (SME) to identify the elemental sulfur distribution within the granules. The sample subjected to SEM-EDX analysis was a biological elemental sulfur sample separated from the elemental sulfur gathered at the interphase of the head space and the bulk liquid and dried at 40°C. Images taken from the Scanning Electron Microscopic in four different sites of the sample. Some of the images taken are shown in Figure 4.86 and 4.87. It was evidenced that the most dominating substance is the elementary sulfur and in most of the sites, elemental sulfur was found to be evenly

distributed in the sample, whereas in some sites high concentrated elemental sulfur gathered into small areas. It was observed in whitish colour in the images.

From the EDX analysis, the main distribution substance was identified as element sulfur. As per the EDX analysis report, there is carbon also present in the sample. may occur as a result of biomass mixed with the elemental sulfur sample when taken from the reactor. However, there are trace amount of oxygen, calcium and sodium as well. In Figure 4.90 and Figure 4.91, elemental sulfur distribution is shown in red colour, carbon is shown in yellow, oxygen in green and calcium in blue colour.
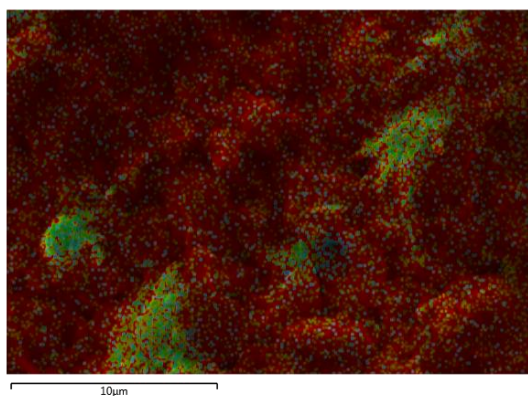
Figure 4.90: All substance distribution map as per EDX analysis of site1 at 10.00 μm
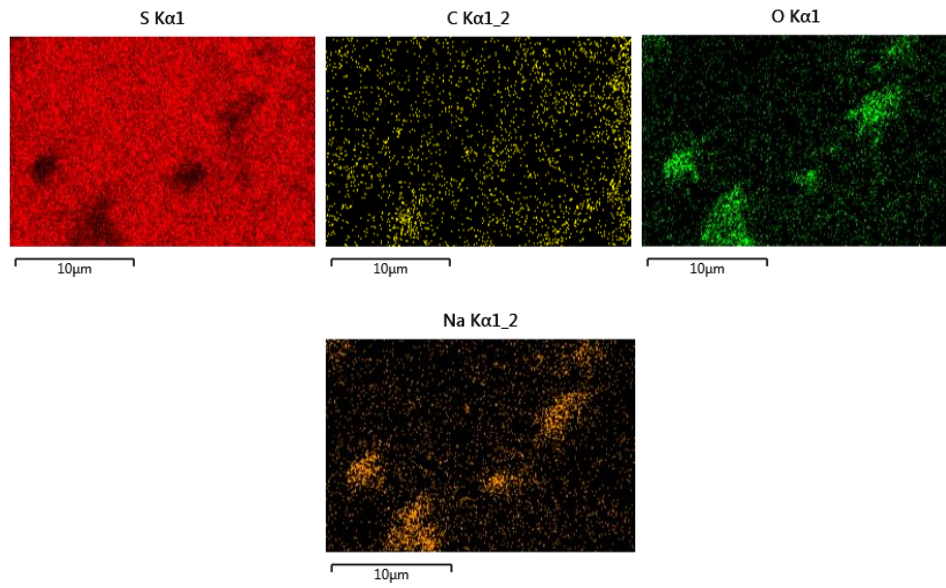
Figure 4.91: Substance wise distribution of site1 at 10.00 μm

(Sulfur-Red, Carbon – Yellow, Oxygen – Green, Calcium – Blue)
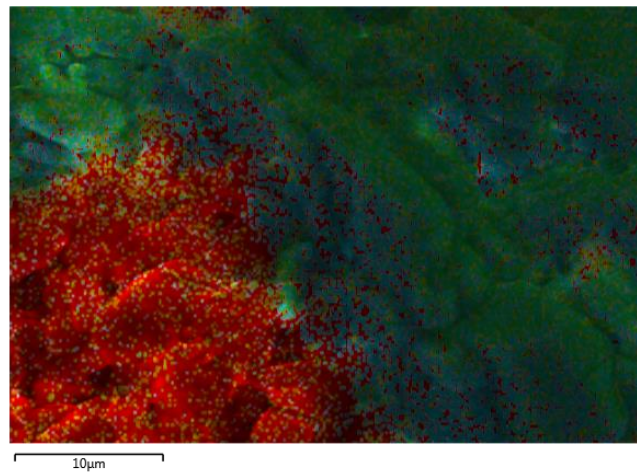
Figure 4.92: All substance distribution map as per EDX analysis of site2 at 10.00 μm
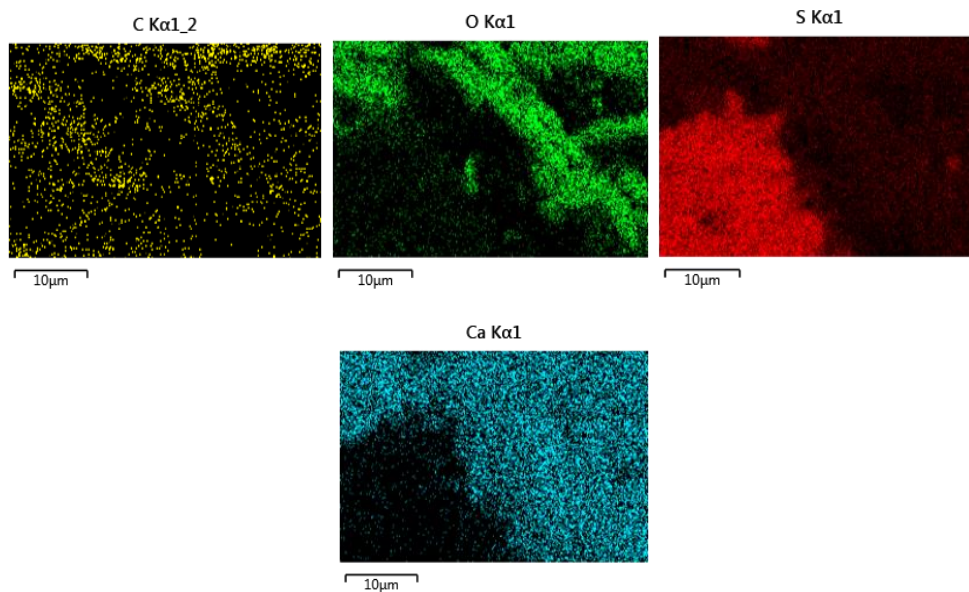
Figure 4.93: Substance wise distribution of site2 at 10.00 μm

(Sulfur-Red, Carbon – Yellow, Oxygen – Green, Calcium – Blue)

EDX spectrum for elemental sulfur sample is shown in Figure 4.94 and its highest peak correspond to elemental sulfur. As per the analysis 98%wt of elemental sulfur gather around some sites, but some areas it is only 58%wt when it mixed with biomass. Thus, the sulfur distribution observed for the analysed sample was not uniform. Biologically elemental sulfur produced in hybrid reactor with anaerobic and micro-aerobic compartments amount for 98%wt of the precipitate [75].
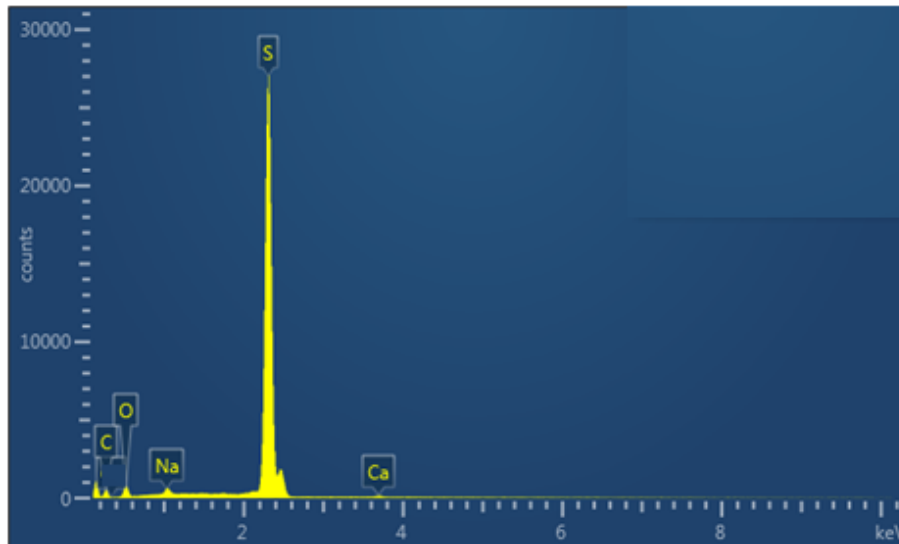
Figure 4.94: EDX spectrum

The biologically formed sulfur granules are nontoxic, noncorrosive and contain high sulfur content. It is widely utilized for fertilizer and as a raw material for some manufacturing processes like bioleaching process and agricultural production[120], [121]. Dry solid sulfur can be used for industrial production of sulfuric acid (99% purity) and can also be converted into pure sulfur (99.9%)[122]. Elemental sulfur produced via biological technologies, is used for bio bleaching of metal-polluted soils and sediments [122]. The biologically formed sulfur can be also used as an adsorbent to remove heavy metals in wastewater[123].

4.7.7 Effect of micro-aeration on COD reduction and methanogenic activity

The measured tCOD of each phase after 48 hours from phase I to phase V is shown in Figure 4.90. As per the summary results, complete anaerobic reactor showed the maximum specific tCOD reduction. But with increasing O_2/S ratio the percentage maximum tCOD reduction from phase I to phase III decrease slightly in a narrow range; 0.51, 0.49, 0.48, kgCOD/m^3.gCOD, whereas the percentage COD reduction was only 65.6%, 63.6% and 62.1% respectively. Although it has shown some negative effect on carbonic compound with introduction of air in micro aerobic phases, the impact was less compared with the complete anaerobic phase. But in phase IV with O_2/S ratio of 1.5, the impact on the COD reduction of the reactor system was comparatively high. the percentage COD reduction was only 59.6%. MB are strict

anaerobic bacteria and might be affected with the introduction of air. Strict anaerobes in an Anaerobic Digestion environment may survive and function under limited aeration with no or minor effects. But primarily rapid oxygen consumption ability of facultative fermentative organisms can protect other organisms by scavenging on dissolved oxygen[124]. Microbial aggregates like flocs, granules and bio films may shield organisms living deep inside by diffusion barrier which stop the full penetration by oxygen. Steep oxygen gradients are created through microbial aggregates due to the diffusion limitation and also oxygen consumption by the facultative or aerobic organisms thriving closer to the surface of the aggregates/ biofilms[125], [124].
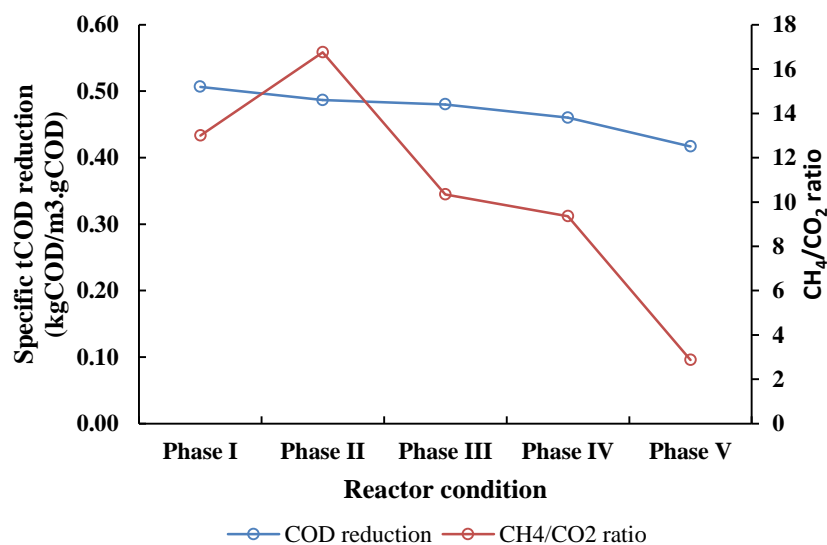
Figure 4.95:specific tCOD reduction and methane yield at 48 hours Vs phase

As per Figure 4.91, there aren't much significant effect on the volumetric biogas generation, but the CH_4/CO_2 ratio rises at phase II and decreases due to generation of more gaseous CO_2 with increasing bio gasification. In phase II the O_2/COD percentage is about 6.4% (g/g). Limited oxygen supply enhance the higher hydrolysis rates of complex organic matter [126], [127]. D. Botheju and R. Bakke also observed enhancement of hydrolysing stages at limited oxygenation[117]. They have found that the hydrolysing optimized at O_2 load of 2.5%. That might be the reason for the rise in CH_4/CO_2 ratio in phase II. The COD reduction percentage and methane enhancement was observed in an micro-aerobic continuous UASB reactor as well for O_2/S ratio of 0.5[15]. The highest cumulative biogas volume was recorded for phase V, because the

high influent tCOD with COD/SO_4^{-2} ratio of 10. But both the tCOD reduction and CH_4/CO_2 ratio were low due to high O_2 load applied. From the studies of the Xu S. et al. also it was confirmed that low levels of oxygen enhances the hydrolysis of carbohydrate and protein in food waste by 21-27% and 38-64% with respect of anaerobic phase, high levels were undesirable as methane yield reduces with carbon oxidation to carbon dioxide[128].
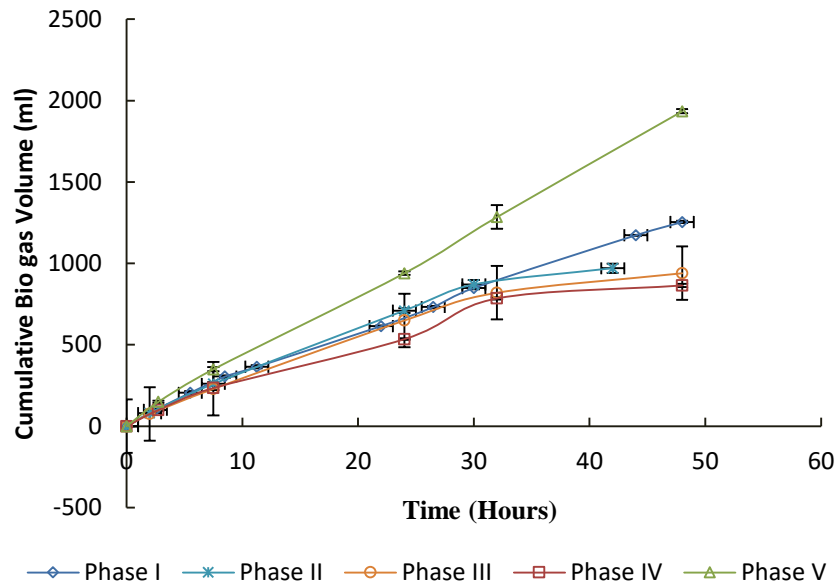
Figure 4.96:Cumulative volumetric bio gas generation Vs Time

F.P. Van der Zee et al.[61] has observed that micro-aeration done in fluidized bed reactor has not shown significant reduction of COD removal efficiencies 86.3%± 0.7% and 86.6%± 0.7% for anaerobic and micro-aerobic phases even under O_2/S ratio of 8-10. But there the influent COD concentration was high, Thus the O_2/COD ratio was 0.02[61]. Limited aeration caused no oxygen inhibition of the anaerobic microorganisms in a UASB reactor, but only led to sulfide oxidation, according to a study by Zhou et al. [129] Granular sludge in the UASB reactor could shield the methanogenic organisms from oxygen exposure, since they mainly grow inside the granules. At O_2/S ratio of 0.5, L. Krayzelova [15] and his team also observed that there aren't decrease in COD removal efficiency or methanogenic activities of the micro-aerobic reactors compare to the same reactor operated under complete anaerobic phase which simultaneously remove sulfate in influent by generation of elemental sulfur. But

it was after one years of operation in micro-aerobic phase. Studies have shown that at least some of the methanogenic organisms have built up their capability of tolerating limited oxygen with enough adaptation or exposure to oxygen environment for extended time period[130], [131]. Therefore, there are many factors which affect the survival of MB in environment of oxygen, thus the inhibition level of reactors depends on the level of the aerated conditions.

4.7.8 Conclusions Derived from the Experiment

During this experiment, SSMAD reactors were successfully used to investigate the transformation of sulfurous compounds under micro-aerobic condition and investigated the optimum condition for removal of sulfate as elemental sulfur of SLW. SSMAD reactor simultaneously reduced high concentrations of influent sulfate of SLW to a minimum sulfate concentration in the effluent, while reducing the hydrogen sulfide emission via micro aeration, it has been transformed hydrogen sulfide to elemental sulfur.

To achieve the optimum sulfate reduction as well as elemental sulfur yield, following controlled conditions were essentially maintained during the semi-batch experiment. SLW are not only rich in sulfate but also TAN and protein compounds which breakdown to Ammonia during anaerobic degradation process. Therefore, sufficient precautions were taken to increase the C/N ratio from 3.8 to 6.9, maintain pH of the reactor at 7.5-8.0 and maintain volumetric loading at 50 l/m^3.d to prevent ammonia inhibition in the reactor. COD/SO_4^{-2} ratio of natural SLW which was used in the study was 2.7 adjusted to 5 using ethanol as the electron donor and carbon source. Corresponding air samples were fed direct to bulk liquid after half an hour of the feeding for two hours was given the maximum elemental sulfur yield. When conducting the semi-batch experiment under above mentioned controlled condition, it was observed that the maximum cumulative sulfate reduction varies 100%,97% and 92% and reduced gaseous H_2S concentration to 48±61ppm, 10±12ppm, 4±4ppm with introduction of air from O_2/S ratio from 0.5 to 1.5 which was 71.4%, 94.0% and 97.6% reduction with respect to complete anaerobic condition with average H_2S emission was 168 ± 128 ppm.

The quantitative as well as the stability of the generated elemental sulfur was improved at O_2/S ratio of 1.0 than 0.5 or 1.5. From the in-detailed analysis carried out it was found that in the narrow range of 1.0-1.2 elemental sulfur optimization taken place to be exact it was 1.18. At O_2/S of 1.18, specific H_2S formation was less than 0.2 mmol/mmol while the sulfate reduction was 95.8%.

Although ethanol enhances the sulfate reduction, excess ethanol makes adverse impact on the micro-aerobic systems reducing generated elemental sulfur amount back to gaseous H_2S. Thus, the elemental sulfur yield has decreased by 69% in phase V compared to phase III when COD/SO_4^{-2} ratio increased from 5 to 10 using alcohol as the electron donor.

From the obtained results, it is convinced that micro-aeration technique can be successfully utilized to treat SLW in a single micro-aerobic digester simultaneously to treat high sulfate concentrations to a minimum effluent concentration, while reducing toxic H_2S gas to minimum concentration. When utilizing and further implementing this new technology to treat SLW commercially, proper mechanism must be incorporate to separate generated un-dissolved elemental sulfur at its optimum generation stage out of the reactor, otherwise generated elemental sulfur would be degraded by the bacteria present in the reactor if it remains in the reactor for long time. In this semi-batch experiment optimum time for elemental sulfur removal lies 18-24 hours range after feeding.

The maximum elemental sulfur production has taken place for synthetic wastewater at 0.8-1.0 while for SLW it was 1.0 to 1.2. Hence, there is 18% rise in the optimum O_2/S ratio for natural SLW when compared with synthetic wastewater in experiment F. This could be due to consumption of oxygen to some other biological processes such as protein and carbonaceous substance hydrolysis as reported by Botheju D. and Bakke R. [117]. According to the results derived through this experiment, optimum O_2/S ratio lies in a narrower range.

5 CONCLUSIONS AND FUTURE WORKS

5.1 Conclusions

The main objective of this research was to investigate the application of micro aeration technique to recover sulfurous pollutants as elemental sulphur from SLW using semi batch reactor. SLW not only rich in sulfate, but also protein and ammonia. However, conversion of sulfate to reusable elemental sulfur is a two-step process; firstly, sulfate reduction to sulfide occur in anaerobic condition and inhibited at high micro aeration condition, and secondly, sulfide oxidation to elemental sulfur is more likely to take place at a moderate micro aerated condition. The ultimate goal of the research was to combine both these process steps to take place in a single reactor, maintaining a suitable balanced micro aerated anaerobic condition inside the SSMAD reactor creating a suitable environment for both SRB and SOB. But necessary precautions were taken to minimize the FAN inhibition that takes place simultaneously in the SSMAD reactor.

First the investigations were carried out to study the sulfate reduction and gaseous Hydrogen sulfide emission in ammonia rich SLW under anaerobic condition.

- In this research, it was found that maintaining the pH at 7.5-8.0 of Anaerobic reactor enhances the sulfate reduction efficiency and prevent inhibition caused by free ammonia and free hydrogen sulfide which are continuously produced under anaerobic digestion of SLW at running condition including Start-up period.

- Influent COD/SO_4^{-2} ratio of the original SLW was low, around 3, which badly affects the sulfate reduction efficiency. It was evidenced that the sulfate reduction efficiency can be further increased, by increasing the influent COD/SO_4^{-2} ratio using an external electron donor, i.e. a carbon source, in this case acetate. For skim latex wastewater, influent COD/SO_4^{-2} ratio of 5 was found to be optimum for sulfate reduction than 3 and 10. Although addition of sufficient external electron donors, increases the sulfate reduction, excess electron donors with high influent COD/SO_4^{-2} ratio of 10 reduces the rate of sulfate reduction.

- The C/N ratio of the influent SLW was also low, about 3, which cause adverse effect on the biological reactions. However, adding external electron donors to the AD

reactor automatically improves the system stability over ammonia inhibition with increasing C/N ratio in the feed stock. At optimum COD/SO_4^{-2} of 5, C/N ratio rise to 7.

- Operating the digester at moderate hydraulics loadings is required to minimize ammonia or protein entering the digester with the influent which leads ammonia generation by protein hydrolysis inside the digester. Thus, moderate hydraulic loading prevents ammonia toxicity further and sustain the sulfate reduction efficiency.

After the optimum condition for sulfate reduction is identified, detailed investigations were carried out to enhance elemental sulfur formation under different micro-aeration techniques, and to optimize the elemental sulfur yield under ammonia rich environment.

- With introduction of air into single stage semi batch micro-aerobic reactors, the degree of the sulfate reduction, gaseous H_2S reduction and elemental sulfur formation varies with different methods of air feeding. The highest sulfate reduction as well as lowest H_2S formation was recorded when the reactor was micro-aerated after half an hour following feeding, at an air flow rate of 1.6 ml/min, for a two-hour period. In this method, some oxygen in the air directly dissolves in the water whereas the remaining air accumulates at the head space of the reactor. When the bulk liquid was continuously stirred, it generated aqueous sulfide and hydrogen sulfide, which gradually consumed the oxygen in the head space and this is required to maintain the dominant reaction to produce more elemental sulfur and increase the stability period of generated elemental sulfur without reversing the direction to form sulfate again.

- Elemental sulfur formation not only occurred in the bulk liquid but also in the walls of the head space and connected tubes. Formed elemental sulfur in liquid and gas phase remained in the reactor for some time and degraded with time. When oxygen in the reactor was consumed for elemental sulfur formation and other reactions, the micro-aerobic condition in the reactor diminished and generated a higher anaerobic condition inside the reactor. Therefore, the dominant reaction reversed to break down formed elemental sulfur to gaseous H_2S.

- Single-stage Sulfate-removal Micro-aerated Anaerobic Digester (SSMAD) can be used to convert influent sulfate to elemental sulfur with a supply of controlled air level in a single reactor. The fraction of sulfate degradation, gaseous H_2S reduction and elemental sulfur formation varies with influent O_2/S ratio fed to the reactor. The optimum simultaneous gaseous hydrogen sulfide removal as well as elemental sulfur formation has taken place between O_2/S ratio of 1.0 to 1.2—in the tested range of influent O_2/S ratio of 0.25 to 1.5—around 18-24 hours after feeding the wastewater sample.

- At O_2/S ratio of 0.25-05 the formed fresh elemental sulfur converted into gaseous Hydrogen sulfide whereas at a O_2/S ratio of 1.0-1.5 the freshly formed elemental sulfur oxidized back to sulfate with time. It is because even if there exist more oxygen in the head space after formation of elemental sulfur, SOB further oxidised the generated elemental sulfur to sulfate. Therefore, before re-biological oxidation of formed elemental sulfur occurs, a proper removal mechanism had to be established to remove elemental sulfur out of the reactor once it is formed.

- Although ethanol enhanced the sulfate reduction more than acetate, excess ethanol made adverse impact on the micro-aerobic systems reducing generated elemental sulfur amount back to gaseous H_2S. Thus, when using excess ethanol, the elemental sulfur formation yield reduced by 69% when the COD/SO_4^{-2} ratio increased from 5 to 10.

- Formed elemental sulfur was confirmed to be sulfur by SEM-EDX analysis. In most of the areas of the solid elemental sulfur sample, elemental sulfur was evenly distributed, but in some areas about 98%wt elemental sulfur was gathered in small areas. The average elemental sulfur of the sample was about 58%wt and carbon was up to about 30%wt. It might be because of the biomass in the sample.

- From the obtained results, it is clear that micro-aeration technique can be successfully utilized to treat SLW in a SSMAD reactor to simultaneously reduce effluent sulfate concentrations and toxic H_2S gas emissions to minimum concentration. Thus, using a single reactor is economical than using separate reactors to convert sulfate to elemental sulfur. When utilizing and further implementing this new technology to treat SLW commercially, a proper mechanism must be incorporated to

separate the generated un-dissolved elemental sulfur out of the reactor at its optimum generation stage. Otherwise generated elemental sulfur would be degraded by the bacteria present in the reactor if it remains in the reactor for a long time. In this SSMAD reactor optimum reusable elemental sulfur removal time was between 18 to 24 hours after feeding.

Finally, outcomes of this research could be, apply successfully in treating sulfur pollutants of SWL using SSMAD in the industry. This would lead to additional income generation through sulfur production that can be utilized for things such as fertilizer manufacture, while reducing air and water pollution by sulfurous compounds. Reducing pollution can also lead to companies being perceived environmentally friendly and a good public relation with the population around the industry location.

5.2   Recommendations for future studies

• Although acetate or alcohol was used as the electron donor in the laboratory scale experimental setup to enhance the sulfate reduction and minimize ammonia inhibition of Skim latex wastewater, future experiments can be conducted without adding expensive electron donors, but using organic matter rich wastewater like, sewage for co-digestion. Therefore, increase of COD/SO_4^{-2} would automatically increase the sulfate reduction. Thus, the economic viability of this strategy for industrial purpose can be more favourable.

• SSMAD reactor can be used to investigate the optimal sulfurous compound removal stage or optimal elemental sulfur formation time before deciding the hydraulic retention time of continuous reactor system.

• Proper elemental sulfur removal mechanism has to be implemented to remove the formed elemental sulfur at the optimal generation point to gain the maximum sulfurous compound removal of the influent wastewater. Otherwise generated elemental sulfur may be degraded back to sulfide or oxidized to sulfate.

• Laboratory scale setups can be improved to Pilot scale and industrial scale setups to use the findings of the research in to use for the betterment of the society.

- The elemental sulfur formed on the walls of the reactors as well as the tubing of the micro aerobic reactor not only at the head space-bulk liquid interphase, thus it is advisable to clean the walls of the reactor as well as the tubing to prevent unnecessary blockages in the tubing.

- Proper microbial identification of responsible SRB and SOB can be carried out for further improving the microbial operation.

- After identifying the time for optimum elemental sulfur generation, Experiment must be conducted by feeding continuously.

- Ammonia reducing step can also be conducted before the SRB, SOB single reactor in a separate treatment unit.

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APPENDIX:

APPENDIX A: Calculation of air quantities for micro-aeration

Oxygen requirement at O_2/S ratio = 0.25

Volume of the reactor	=	2000	ml
Daily substrate feeding rate	=	100	ml
Relevant HRT	=	20	days
sulfate of the sample feeding	=	3000	mg/l
sulfate of the sample feeding	=	300	mg
Oxygen/Sulfur molar ratio	=	0.25	
Oxygen requirement for sample fed	=	0.78125	mmols

$P = 1$ atm, $R = 8.314$ $JK^{-1}mol^{-1}$, 0.08206 Latmmol-1K-1

R	=	0.08206	L atm K-1 mol-1
Temperature, T	=	35	°C
		308	K
Pressure, P	=	1	atm
No of mols, n	=	0.000781	mols

Using PV=nRT,

V	=	nRT/P	
		0.019746	L
		19.74569	ml

As the oxygen and the nitrogen ratio of air is , N_2=78.09%, O_2= 20.95%

Required amount of air	=	94.25149	ml

Oxygen requirement at O_2/S ratio = 0.5

Volume of the reactor	=	2000	ml
Daily substrate feeding rate	=	100	ml
Relevant HRT	=	20	days
sulfate of the sample feeding	=	3000	mg/l
sulfate of the sample feeding	=	300	mg
Oxygen/Sulfur molar ratio	=	0.5	
Oxygen requirement for sample fed	=	1.5625	mmols

$P = 1$ atm, $R = 8.314$ $JK^{-1}mol^{-1}$, 0.08206 Latmmol-1K-1

R	=	0.08206	L atm K-1 mol-1
Temperature, T	=	35	°C
		308	K
Pressure, P	=	1	atm
No of mols, n	=	0.001563	mols

Using PV=nRT,

V	=	nRT/P	
		0.039491	L
		39.49138	ml

As the oxygen and the nitrogen ratio of air is , N_2=78.09%, O_2= 20.95%

Required amount of air	=	188.503	ml

Oxygen requirement at O_2/S ratio = 1.0

Volume of the reactor	=	2000	ml
Daily substrate feeding rate	=	100	ml
Relevant HRT	=	20	days
sulfate of the sample feeding	=	3000	mg/l
sulfate of the sample feeding	=	300	mg

Oxygen/Sulfur molar ratio	=	1	
Oxygen requirement for sample fed	=	3.125	mmols

P = 1atm, R = 8.314 $JK^{-1}mol^{-1}$, 0.08206 $Latmmol^{-1}K^{-1}$

R	=	0.08206	L atm K-1 mol-1
Temperature, T	=	35	°C
		308	K
Pressure, P	=	1	atm
No of mols, n	=	0.003125	mols

Using PV=nRT,

V	=	nRT/P	
	=	0.078983	L
	=	78.98275	ml

As the oxygen and the nitrogen ratio of air is , N_2=78.09%, O_2= 20.95%

Required amount of air	=	377.006	ml

Space in the head space	=	500	ml

Oxygen requirement at O$_2$/S ratio = 1.5

Volume of the reactor	=	2000	ml
Daily substrate feeding rate	=	100	ml
Relevant HRT	=	20	days
sulfate of the sample feeding	=	3000	mg/l
sulfate of the sample feeding	=	300	mg
Oxygen/Sulfur molar ratio	=	1.5	
Oxygen requirement for sample fed	=	4.6875	mmols

P = 1atm, R = 8.314 JK^{-1}mol^{-1} , 0.08206 Latmmol-1K-1

R	=	0.08206	L atm K-1 mol-1	
Temperature, T	=	35	°C	
		308	K	
Pressure, P	=	1	atm	pH
No of mols, n	=	0.004688	mols	

Using PV=nRT,

V	=	nRT/P	
	=	0.118474	L
	=	118.4741	ml

As the oxygen and the nitrogen ratio of air is, N$_2$=78.09%, O$_2$= 20.95%

Required amount of air	=	566	ml
Space in the head space	=	500	ml

APPENDIX B: Material balance for sulfurous compounds

Time (Hrs)	Accumulated Time (Hrs)	O_2/S Ratio	Type of Electron donor	Gas volume (ml)	Gaseous H_2S (ppm)	$S-SO_4^{-2}$ (mmol)	S-Total Dissolved Sulfide (mmol)	$S - H_2S$ (mmol)	Elemental Sulfur moles (mmol)	Theoretical elemental sulfur yield from feed (mmol/mmol)	Theoretical elemental sulfur yield from feed (mg/mmol)
0.00	0.00	0.00	Acetic	0.00	0.00	3.87	0.10	0.00	0.00	0.00	0.00
2.50	2.50	0.00	Acetic	100.00	150.00	0.88	0.10	0.44	0.00	0.00	0.00
5.50	8.00	0.00	Acetic	105.00	250.00	0.35	0.17	0.77	0.00	0.00	0.00
8.50	16.50	0.00	Acetic	100.00	180.00	0.31	0.24	0.53	0.00	0.00	0.00
11.25	27.75	0.00	Acetic	60.00	270.00	0.25	0.13	0.48	0.00	0.00	0.00
22.00	49.75	0.00	Acetic	250.00	410.67	0.17	0.20	3.02	0.00	0.00	0.00
26.50	76.25	0.00	Acetic	117.00	150.00	0.10	0.18	0.46	0.00	0.00	0.00
30.00	106.25	0.00	Acetic	117.00	50.00	0.00	0.16	0.15	0.00	0.00	0.00
44.00	150.25	0.00	Acetic	324.00	30.00	0.00	0.05	0.26	0.00	0.00	0.00
48.00	198.25	0.00	Acetic	81.00	20.00	0.00	0.09	0.04	0.00	0.00	0.00
0.00	198.25	0.50	Acetic	0.00	0.00	3.97	0.14	0.00	0.00	0.00	0.00
2.00	200.25	0.50	Acetic	80.00	50.00	3.19	0.17	0.12	0.64	0.16	5.17
7.50	207.75	0.50	Acetic	180.00	150.00	0.54	0.18	0.79	2.49	0.63	20.04
24.00	231.75	0.50	Acetic	450.00	35.00	0.21	0.13	0.46	1.77	0.44	14.23
30.00	261.75	0.50	Acetic	160.00	5.00	0.10	0.09	0.02	0.03	0.01	0.26
42.00	303.75	0.50	Acetic	100.00	0.00	0.00	0.08	0.00	0.00	0.00	0.00
0.00	303.75	1.00	Acetic	0.00	0.00	4.04	0.13	0.00	0.00	0.00	0.00
2.00	305.75	1.00	Acetic	75.00	10.00	4.13	0.14	0.02	0.00	0.00	0.00

Time (Hrs)	Accumulated Time (Hrs)	O_2/S Ratio	Type of Electron donor	Gas volume (ml)	Gaseous H_2S (ppm)	$S-SO_4^{-2}$ (mmol)	S-Total Dissolved Sulfide (mmol)	$S-H_2S$ (mmol)	Elemental Sulfur moles (mmol)	Theoretical elemental sulfur yield from feed (mmol/mmol)	Theoretical elemental sulfur yield from feed (mg/mmol)
7.50	313.25	1.00	Acetic	155.00	30.00	0.88	0.15	0.14	3.11	0.78	25.02
24.00	337.25	1.00	Acetic	420.00	5.00	0.31	0.13	0.06	3.63	0.91	29.25
32.00	369.25	1.00	Acetic	170.00	5.00	0.10	0.13	0.03	0.71	0.18	5.71
48.00	417.25	1.00	Acetic	120.00	0.00	0.21	0.13	0.00	0.08	0.02	0.64
0.00	417.25	1.50	Acetic	0.00	0.00	3.97	0.16	0.00	0.00	0.00	0.00
3.00	420.25	1.50	Acetic	40.00	5.00	4.17	0.13	0.01	0.00	0.00	0.00
8.50	428.75	1.50	Acetic	135.00	10.00	1.46	0.13	0.04	2.67	0.67	21.54
26.00	454.75	1.50	Acetic	300.00	5.00	0.58	0.13	0.04	3.51	0.88	28.23
33.00	487.75	1.50	Acetic	250.00	1.00	0.31	0.16	0.01	1.06	0.27	8.56
48.00	535.75	1.50	Acetic	80.00	0.00	0.52	0.13	0.00	0.06	0.01	0.44
0.00	535.75	1.50	Ethanol	0.00	0.00	3.97	0.10	0.00	0.00	0.00	0.00
2.75	538.50	1.50	Ethanol	150.00	226.67	3.02	0.11	1.00	0.00	0.00	0.00
7.50	546.00	1.50	Ethanol	200.00	165.00	0.63	0.11	0.97	1.37	0.35	11.05
24.00	570.00	1.50	Ethanol	590.00	70.00	0.35	0.11	1.21	0.49	0.12	3.93
32.00	602.00	1.50	Ethanol	345.00	20.00	0.17	0.10	0.20	0.02	0.08	2.54
48.00	650.00	1.50	Ethanol	650.00	0.00	0.00	0.08	0.00	0.18	0.04	1.42