Review of Biological Treatment of Wastewaters Using Sulfate Reducing Bacteria

MotazZarooq

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Lian-Shin Lin, Ph.D., P.E., Chair
Jennifer Weidhaas, Ph.D., P.E
Leslie Hopkinson, Ph.D.

Department of Civil and Environmental Engineering

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ABSTRACT

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Anaerobic biological treatment of various wastewaters containing sulfateusing sulfate reducing bacteria (SRB) can have significant energy, environmental, and economic advantages over aerobic biological treatment. This problem report summarizes a review of relevant literature on using sulfate reducing bacteria for wastewater treatment including: choice of electron donors, COD/sulfate ratios, inhibition of SRB by heavy metals (Fe), and reactors configurations. A wide range of inorganic and organic compounds could be applied as electron donors for SRB, such as hydrogen, short chain fatty acids, alcohols, and organic wastes. Various factors affecting the choice of the electron donors include energy yield from the sulfate reduction reaction, competition between different microorganisms for limited sources of food, COD/sulfate ratios, and the operating conditions (pH, temperature). The COD/sulfate ratio plays a very important role in driving selection of microorganisms in mixed culture; high ratios (i.e., 2 and above) have been reported to generate higher sulfate removal rate. However, high values of the ratios will increase competition from other microorganisms for available substrate. Iron ions in wastewater have mixing effects on SRB; ferrous ions (Fe^{+2}) enhanced SRB while ferric ions (Fe^{+3}) inhibited SRB. Reactors with different configurations to optimize removal of sulfate and organic wastes by SRB were reviewed and summarized. Anaerobic filters (UF) and upflow aerobic sludge blanket (UASB) reactors were the early reactors used in the anaerobic treatment and most of the latter reactors are modifications to those two reactors to aggregate the positives and overcome the negatives.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1: Introduction

Sulfate reducing bacteria (SRB) are various anaerobic bacterial species that facilitate the reduction of sulfate to sulfide, in which sulfate as a terminal electron acceptor with a wide range of organic and inorganic compounds serving as the electron donor (Dhillon et al,.2003). SRB are highly present in sulfate rich wastewaters and usually consist of the traditional genera such as: *Desulfovibrio* and *Desulfotomaculam* in addition to the morphologically and physiologically different genera such as *Desulfobacter*, *Desulfobulbus*, *Desulfococcus*, *Desulfonema*, and *Desulfosaccina* (Schmidt and Schaechter, 2012). Based on their carbon sources, SRB are classified into two groups: heterotrophs and autotrophs (Baumgartner et al., 2006):

1. Heterotrophs–species using organic compounds as the carbon source and electron donor.
2. Autotrophs - species usingcarbon dioxide as the carbon source and hydrogen as the electrondonor.

SRB generate energy by oxidizing different substrates while reducing sulfate. The energy obtained from the redox reactions is used by SRB in various activities such as microbial regeneration and mobility. Carbon is used to rebuild cells, for growth, and to source energy out of the redox reactions. In marine sediments, SRBs are responsible for up to 80% carbon oxidation (Canfield and Marais, 1993).

SRB can use a wide range of electron donors including low molecular weight fatty acids, alcohols, and hydrogen gas (Annachhatre and Suktrakoovait, 2001). Some of these substrates are completely degraded by SRB into carbon dioxide and water while sulfate is reduced. Some SRB species lack the complete carboxylic cycle (Schmidt and Schaechter, 2012), thus the substrate uptake by such SRB species results in incomplete oxidation to acetate, leaving it for further degradation into carbon dioxide by other species that are able to convert. Furthermore complex organic groups that are not degradable by SRB may be supplied to SRB by fermenting bacteria through oxidization of the complex organic matters to simpler compounds. There were different electron donors used by SRB (Table 1), and each has its advantages and disadvantages as detailed in later sections.

Table 1: Different compounds amenable to anaerobic biotechnology (Speece, 1983)

Acetaldehyde	Corn milling	Isopropyl alcohol	Potato
Acetic anhydride	Corn stover	Lactic acid	Pulp mill evaporate
Acetone	Dairy	Maleic acid	Resorcinol
Acrylic acid	Diacetonegulusonic acid	Meat packing	Rum distillery wastes
Adiptc acid	Dimethoxy benzoic acid	Methanol	Sec-Butanol
1 -Amino-2-propanol	Ethyl acetate	Methyl acetate	Sec-Butylamine
4-Amino butyric acid	Ethyl acrylate	Methyl acrylate	Sorbic acid
Aniline	Ferulic acid	Methyl ethyl ketone	Straw
Animal wastes	Formaldehyde	Methyl formate	Sugar cane
Bagasse	Formic acid	Nitrobenzene	Syringaldehyde
Benzoic acid	Fumaric acid	Pear wastes	Syringic acid
Brewery	Giant kelp	Peat	Succinic acid
Butanol	Glutamic acid	Pectin wastes	Tannery wastes
Butyraldehyde	Glutaric acid	Pentaerythritol	Tert-butanol
Butylene	Glycerol	Pentanol	Vanillic acid
Catechol	Guar gum wastes	Phenol	Vinyl acetate
Cheese whey	Hanoi	Phthalic acid	Water hyacinths
Cresol	Heat-treated activated sludge	Propanal	Wine distillery wastes
Croton aldehyde	Hexanoic acid	Propanol	Wood
Crotonic acid	Hydroquinone	Propionate	Yeast
Coking mill	Isobutyric acid	Propylene glycol	
Corn	Isopropanol	Protocatechuic acid	

The SRB are highly present in sulfate rich wastewaters such as in paper mills and acid main drainages. These wastewaters are usually deficient in electron donors and require external addition of electron donors in order to achieve sufficient sulfate reduction by increasing the food to sulfate ratio. The food to sulfate ratio is an important factor that affects the reduction of sulfate

(Liamleam and Annachhatre, 2007) and will be discussed later in Chapter 3 of this report. Likewise hydraulic detention time (HDT), the length of time the feed remains inside the reactor, affects available electron donorsand influences the microbial population. Some substrates allow a shorter HDT and others need a longer HDT to be degraded. Smaller HDT is advantage economically because it would result in small footprint of the biological treatment system. However many researchers reported that some SRB species have a longer lag period which make short HDT systems unattractive for sulfate treatment.

The sulfate reduction produces odor from the formation of hydrogen (bi) sulfide, H_2S/HS^- which is highly corrosive and undesirable in many processes. Over the years, various applications employing the sulfate reduction metabolic pathways for treatment have been explored and successfully implemented. Examples included treatment of sulfate laden acid mine drainages in which alkalinity production from sulfate reduction helps neutralize the acidity, and the treatment of calcium sulfate sludge produced from desulfurization of the stack gases. In these processes, sulfide precipitation of heavy metals is usually observed, resulting in highly stable and easily removed materials (Middleton and Lawrence, 1977).

Some factors affect the treatment performance of SRB in biological systems, such as the type of electron donors, COD/sulfate value, effect of metals and reactor configurations. The objectives of these report is to review and summaries the effects electron donors, COD/sulfate ratio, and reactor configurations for SRB in the engineering treatment systems.

CHAPTER 2: Electron Donors for SRB

From the treatment perspective, selection of a carbon sources and electron donors for complete sulfate removal depends on various factors and considerations such as: (1) the suitability of electron donor to reduce sulfate while minimizing other pollutants in the effluent and (2) the cost of the electron donor per unit of sulfate converted to sulfide (Van Houten et al., 1994). A wide variety of waste and sewage sludge, such as: leaf mulch, wood chips, animal manure, vegetable compost, sawdust, mushroom compost, whey, molasses, were reported to contain organic compounds that could be utilized by SRB to satisfy the two conditions listed previously (Dvorak et al., 1992, Hammack et al., 1994, Christensen et al., 1996, Waybrant et al., 1998, Annachhatre and Suktrakoovait, 2001). In addition, synthetic organic compounds such as lactate, acetate, propionate, and pyruvate have been used as electron donors to mitigate the deficiency of electron donors in wastewater for sulfate reduction (Okabe and Characklis, 1992, Visser et al., 1993, Harada et al., 1994). The addition of those organic compounds depends on the SRB species and the condition on hand that may favor one electron donor over another. For example adding acetate will require SRB species with complete tricarboexlic acid cycle in order to fully degrade acetate.

Possible SRB reduction reactions when organic compounds or inorganic compounds are used as electron donors are shown in reactions (1) and (2) (Postgate, 1984).

$$4RH_2 + SO_4^{-2} + H^+ \rightarrow 4R^{2-} + HS^- + 4H_2O \qquad (1)$$

Where R refers to different organic or inorganic compound

$$4H_2 + SO_4^{-2} + 2H^+ \rightarrow H_2S + 4H_2O \qquad \Delta G = -154.04 \text{ KJ/reaction} \qquad (2)$$

In addition to the type of SRB species, other parameters have been reported to play an important roles in the selection of electron donors such as the thermodynamics of the reaction, temperature, and pH. The theoretical energy obtained from the redox reactions plays a very important role in selecting electron donor. For engineering treatment system all species including SRB are favor certain electron donors over others depending on the amount energy that can be obtained. For example the free energy generated from hydrogen coupled with sulfate in a redox reaction made the hydrogen favored by all SRB species over other electron donors including acetate (Thauer et al., 1977, Oremland and Polcin, 1982, Parkesetal., 1984, Gibson, 1990). The energy obtained also plays a very important role in deciding the outcome from the competition between SRB and methanogenic Archaea (MA) which produce methane gas. It has been reported that SRB have high affinity towards acetate compared to MA due to the free energy generated during the reduction reaction (Winfrey and Zeikus, 1979, Thauer et al., 1977)

Acetate oxidation reaction by SRB

$CH_3COO^- + SO_4^{2-} \rightarrow 2HCO_3^- + HS^-$ ΔG = -49.5 KJ/reaction (3)

Acetate oxidation reaction by MA

$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$ ΔG = -32.5 KJ/reaction (4)

The SRBs outcompete MAs for a wide range of substrates such as hydrogen and acetate, yet MA may outcompete SRB for some substrates such as methanol, traimethylamine, and methionine (Oremland and Polcin, 1982, Parkeset al., 1984,Robinson and Tiedje, 1984, Gibson. ,1990). To obtain high efficiency treatment in any biological treatment systems the kind of substrate selected, the surrounding conditions that favor SRB over MA, and removal of SRB inhibiting need to be addressed (Stuckietal., 1993, Van Houten et al., 1994).

2.1 Hydrogen

Hydrogen is the most attractive and favored electron donor to all species, due to the large free energy that can be obtained from the coupled oxidation reduction reaction and wide range of electron acceptors hydrogen can react with. SRB consume hydrogen at lower concentrations or partial pressures and, the low hydrogen threshold concentrations for SRB allow them to outcompete MAs and homoacetoginc bacteria (AB) in anaerobic biological treatment (Thauer et al., 1977, Oremland and Polcin, 1982, Parkes et al., 1984, Robinson and Tiedje, 1984, Cord-Ruwisch et al., 1988, Gibson, 1990). The presence of SRB can inhibit the growth of MA and AB unless an excessive amount of hydrogen is available or acetate produced as intermediate product by SRB and made available for the MA and AB organism (Liamleam et al., 2007). Widdel and Pfennig, (1981) showed that the free energy generated from hydrogen favored SRB species over MA, and AB organisms and that was illustrated in the reactions below. Robinson and Tiedje (1984) also supported this finding when they showed that SRB has a higher affinity towards hydrogen.

(a) Reaction by SRB

$4H_2+H^++SO_4^{2-}\rightarrow 4H_2O+HS^-$ $\Delta G = -154.4$ kJ/reaction (5)

(b)Reaction by MA

$4H_2+CO_2\rightarrow 2H_2O+CH_4$ $\Delta G = -131.3$ kJ/reaction (6)

One way to favor selection of SRB over other organisms in an engineering treatment system containing hydrogen is to keep the partial pressure of hydrogen under the level required by other organisms (Cord-Ruwisch et al., 1988). The affinity of those organisms towards hydrogen at the limited hydrogen concentration follows this order: SRB, MA, and AB. (Weijma et al., 2002).

Novelli et al. (1987) reported extremely low hydrogen gas in anaerobic environment, suggesting that high affinity of existing microorganisms found in that environment towards hydrogen gas may naturally keep hydrogen concentration low in anaerobic environment. Hydrogen gas is a favored electron donor which can be utilized immediately when present by different organisms through interspecies hydrogen transfer (Cord-Ruwisch et al., 1988).

Organic degradation by fermentative bacteria in anaerobic environments may be a mechanism for providing hydrogen (Wolin et al., 1982). The transfer and utilizing of hydrogen depends also on the electron acceptors and the organic substrates that are degraded (Cord-Ruwisch et al., 1988). The sulfate reduction could be affected by electron donors as well as other electron acceptors present in the environment. For example nitrate or fumarate were reported to be more favorable over sulfate and that will resulted in increasing percentage of hydrogen transferring to that bacteria utilizing nitrate or fumarate. Some SRB species do not have affinity to hydrogen gas and require different electron donors such as lactate or acetate (Cord-Ruwisch et al., 1988).

2.2 Lactate

Lactate is a major electron donor for sulfate reduction (Cappenberg, 1974). Oremland and Silverman (1979) found that addition of lactate stimulated sulfate reduction for several species in a fresh water system. Lactate and a limited number of other fermentation products can be used as substrates by the SRB of the genus *Desulfovibrio*. However only a partial oxidation of the substrate can be accomplished due to the lack of a complete tricarboxylic acid cycle in these bacteria to carry out complete organics oxidation to carbon dioxide and water (LeGall and Postgate, 1973). Unless other SRB species utilize acetate, such as *Desulforhabdus* or

Desulfotomaclum to complete full oxidation of acetate to carbon dioxide, the end product of the redox reaction would be acetate as described in reaction (7):

$$2C_2H_5OCOO^- + SO_4^{-2} \rightarrow 2CH_3COO^- + 2HCO_3^- + HS^- + H^+ \quad \Delta G = -160 KJ/reaction \ (7)$$

Fermentative acidogenic bacteria such as some members of Genera *Clostridium* and *Bacteroides,* can play an important role in fermenting lactate to propionate, acetate, ethanol and hydrogen (Macy et al., 1978, Vander et al., 2002). SRB can then oxidize those products to acetate and water (Yangguo et al., 2008). Lactate may undergo different metabolic routes as illustrated in Figure 1(Yangguo et al., 2008). These reaction pathways are described as follows:

1. It is fermented first, by *Clostridium* sp and *Bacteroides* sp. to produce propionate or ethanol, which in turn serves as an electron donor for sulfate reduction by *Desulfobulbus* or *Desulfovibrio* sp (Yangguo et al., 2008).
2. There is also an alternative option, when the lactate was directly oxidized to acetate by *Desulfovibrio* sp. Some of the incomplete oxidizing product acetate would be oxidized by the acetotrophic *D. amnigena* to CO_2 and H_2O (Yangguo et al., 2008).

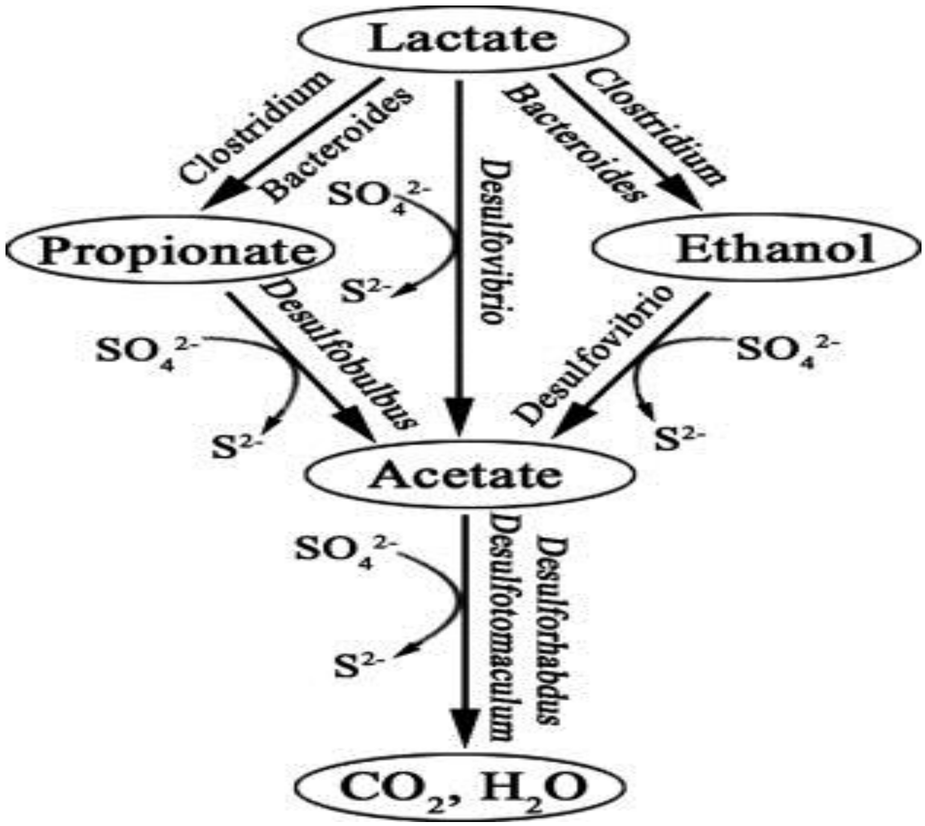

Figure 1: Presumptive lactate degradation pathways in a sulfidogenics system adapted from Yangguo et al. (2008).

2.3 Acetate

Acetate was found to be the main substrate for SRB in an estuary sediment systemin which approximately 60% of the sulfate reduction was due to acetate oxidation (Banatet al., 1981, Winfrey andWard, 1983). The finding was in agreement with another study in which acetate was the main energy substrate for the SRB in Lake Eliza's sediments (Skyring, 1988). Greben et al., (2004) showed that sulfate has a higher removal rate with acetate and propionate when the COD/sulfate ratio is ≥ 0.69 as illustrated in Table 2. The two reactors used in the experiment R_1 and R_2 with acetate and propionate respectively have almost the same feed condition. R_2 has higher sulfate removal rate compare to R_1. R_1 has higher food to sulfate ratio which could be the reason for its lower sulfate removal rate. Also that could be related to condition favor MA population consuming acetate, or because SRB needed longer time to acetate. Visser (1995) reported that SRB efficiency improved by time.

$$CH_3COO^- + SO_4^{-2} \rightarrow HS^- + 2HCO_3^- \qquad \Delta G = -49.5 KJ/reaction \qquad (8)$$

Table 2:The experimental data of the continuous operation of R_1 and R_2 (Greben et al., 2004)

Determinant	Unit	R_1 (Acetate)	R_2 (Propionate)	
Feed				
Sulfate	mg/L	1139	1242	
COD	mg/L	992	980	
Alkalinity	mg/L	2214	2469	
pH		7.69	7.72	
Treated				
Sulfate	mg/L	511	345	
COD	mg/L	512	571	
Alkalinity	mg/L	2867	3025	
Sulfide	mg/L	176	217	
pH		7.88	7.93	
Redox	mV	-131	-145	
SO_4^{2-} removal Rate	%	55.5	78	
SO_4^{2-} reduction rate	g SO_4/(L.d)	0.63	0.95	
Ratios				
(Experimental)				COD/sulfate Theoretical ratio
Feed COD/ SO_4^{2-} ratio		0.87	0.79	0.67
COD used/SO_4^{2-} rem.		0.84	0.42	

As mentioned earlier acetate is also produced as an intermediate product of some SRB species such as *Desulfovibrio* and *Desulfobulbus that* can compete against homoacetogenic bacteria and methanogens for substrate. Acetate generated could be used further for reducing sulfate with different SRB species such as *Desulforhabdus* or *Desulfotomaclum* to complete the reaction into carbon dioxide and water. Other studies showed that acetate production in sulfate reducing reactor was a setback because SRB cannot completely oxidize acetate even in excess amount of sulfate (Lens et al., 2003,Vallero et al., 2003).

2.4 Propionate and butyrate

Degradation of propionate and butyrate by SRB has been broadly investigated. The small chain fatty acids are intermediate products from carbohydrate fermentation. Their oxidation by SRB followed two routes depending on the species involved: complete degradation to carbon dioxide and water such as oxidation of fatty acids to CO_2 by *Desulfofrigus oceanense* sp. nov. strain (Knoblauch et al., 1999), Incomplete oxidation to acetate, can be done through direct oxidation by SRB species such as *Desulfobulbus* to generate acetate or by SRB syntrophy associated with acetogenic bacteria (Widdel, 1988, Yangguo et al., 2008). Another study found that the inhibition of sulfate reduction by molydate in marine sediment resulted in accumulation of propionate and butyrate as a clear proof that SRB enhanced the degradation of propionate and butyrate (Jan Sorenson et al., 1981). The complete oxidation by SRB in the mixed culture show 10 to 20% of the electron flow from propionate and butyrate to the SRB, according to reactions 9 and 10

$$C_2H_5COO^- + 1.75SO_4^{-2} \rightarrow 1.75HS^- + 0.25H^+ + 3HCO_3^- \qquad \Delta G = -118 \text{ KJ/reaction} \quad (9)$$

$$C_3H_7COO^- + 2.5SO_4^{-2} \rightarrow 2.5HS^- + 0.5H^+ + 4HCO_3^- \qquad \Delta G = -171 \text{ KJ/reaction} \quad (10)$$

Celis-Garcia et al. (2007) showed over 90% sulfate removal in a down-flow fluidized bed reactor with propionate and butyrate, and the removal rate depended on the COD/sulfate ratio. Increasing the ratio value would result in less sulfate removal, due to an increase in available food that encourages methanogenic utilization. Lowering the ratio would result in improved sulfate removal until an optimum ratio is reached beyond which the removal would start to decrease due to cell starvation from food shortage. As showed in Table 2, propionate achieved higher sulfate removal than acetate under normal conditions. Under harsh conditions such as high salinity and pH over 10, strain *Desulfobulbus alkaliphilus* sp**.** nov were able to oxidize butyrate incompletely to acetate (Sorokin et al., 2009) and that proved the ability of SRB to degrade and favor those fatty acids under different conditions. Only under sulfate limiting conditions were syntrophic propionate oxidizers reported to outcompete propionate degrading sulfate reducers. Remarkably, syntrophic butyrate oxidizers were able to compete with sulfate reducers for the available butyrate, even with an excess amount of sulfate (Visser et al., 1993).

2.5 Methanol

Methanol is an attractive electron donor for biological treatment processes because it is easily degraded by microbes and inexpensive (Dijkhuizen et al., 1985, Tsukamoto and Miller, 1999). Methanol is degradable by thermophilic and mesophillic organisms. Due to the lower freezing point methanol is favored in remote areas and in the higher elevations over other substrates because it is easily degradable in different climates (Weijma et al., 2000). The fate of methanol in anaerobic reactors is determined by the outcome of the direct competitions between MA, SRB and AB, as well as the indirect competitions with intermediate products acetate or hydrogen gas (Weijma et al., 2003) as demonstrated in the reaction below:

SRB:

$$4CH_3OH + 3SO_4^{-2} \rightarrow 4HCO_3^- + 3HS^- + 4H_2O + H^+ \qquad \Delta G = -364 \text{ kJ/reaction} \qquad (11)$$

MA:

$$4CH_3\,OH \rightarrow 3CH_4 + HCO_3^- + H_2O + H^+ \qquad \Delta G = -316 \text{ kJ/reaction} \qquad (12)$$

AB:

$$4CH_3OH + 2HCO_3 \rightarrow 2CH_3COO^- + H^+ + 4H_2O \qquad \Delta G = -220 \text{ kJ/reaction} \qquad (13)$$

The energy values obtained from the above reactions favor SRB over the methanogensis and homoacetogen. However, free energy is not the only factor determining the fate of methanol under anaerobic conditions. Many factors were found to play a role such as temperature, pH, growth kinetics, methanol loading rate, retention time, and substrate diffusion in biofilms (Weijma and Stams, 2001).

Temperature is an important factor in determining the fate of methanol. In thermophilic conditions at 65C^o, Weijmaet al. (2000) found that SRB outcompeted methanogenic archaea in methanol degradation with pH 7.5 and hydraulic detention times14 and 3.5 h. At this temperature, methanogenic archaea were inhibited and not able to grow, and SRB were accounted for 80% methanol degradation. In another study, the research group found that 90% methanol removal was related to methanogenic activity at 30 C^o (Weijma et al., 2003). The later finding was also confirmed by Greben (2000) who reported that SRB had very low methanol degradation under ambient temperatures. The two contradictory findings explained the importance of the environmental conditions in the competition between SRB and methanogens in methanol degradation and that energy obtained may be much less important (Weijma et al., 2001). Another report by Weijma et al. (2001) showed different degradation pathways of methanol by SRB as illustrated in Figure 2.

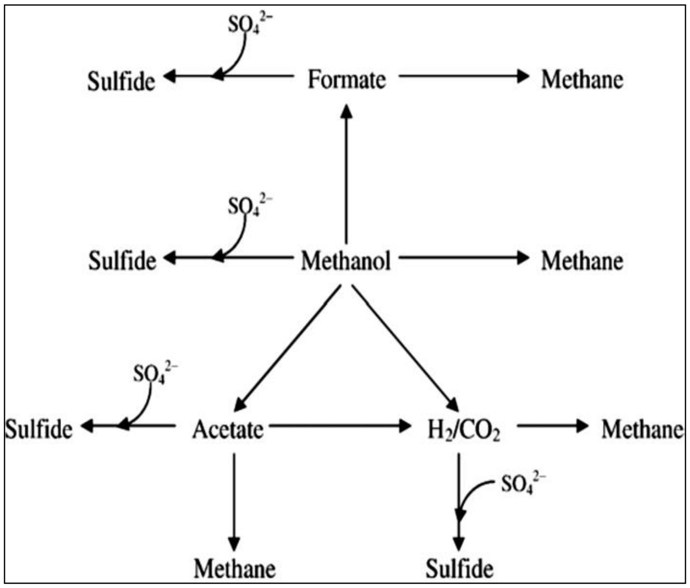

Figure 2: Anaerobic methanol degradation (Weijam et al., 2001)

2.6 Ethanol

Ethanol is an attractive electron donor for some SRB species. A total of 70% of sulfate was removed with ethanol at 32C° in an anaerobic batch reactor (Cao et al., 2012). 80% sulfate conversion was achieved under a high sulfate loading rate with ethanol (Barnes et al., 1991). The redox reactions for ethanol by the three competing organisms as reported by Cao et al. (2012) were as follows:

SRB:

$$C_2H_3OH + 0.5SO_4^{2-} \rightarrow CH_3COO^- + 0.5HS^- + 0.5H^+ + H_2O \quad \Delta G = -75 \text{ KJ/reaction} \quad (14)$$

AB:

$$C_2H_5OH + H_2O \rightarrow CH_3COO^- + 2H_2 + H^+ \quad \Delta G = -14.6 \text{ KJ/reaction} \quad (15)$$

MA:

$$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^- \quad \Delta G = -32.5 \text{ KJ/reaction} \quad (16)$$

$$H_2 + 0.25H^+ + 0.25HCO_3^- \rightarrow 0.25CH_4 + 0.75H_2O \quad \Delta G = -31.3 \text{ KJ/reaction} \quad (17)$$

Complete ethanol degradation to CO_2 occurred when sulfate was in access by the *Desulfovibrio, Desulfuricans and Desulfobacterpostgatei (*Nagpal et al., 2000). Similar to some other substrates utilized by SRB, incomplete ethanol degradation produces acetate as an intermediate product, leading to increased competition between different anaerobic organisms that utilize acetate, which may affect SRB efficiency and result in low sulfate and COD removal rates (Nagpal et al., 2000, Cao et al., 2012). Other drawbacks with ethanol as an electron donor for SRB was the low growth rate which was probably caused by relatively less energy obtained from this redox couple (Cao et al., 2012).

2.7 Organic wastes

Organic wastes can serve as an economical alternative of electron donor for biological sulfate reduction. Garcia et al. (2001) showed that SRB helped reduce sulfate to H_2S and concurrently oxidizes the organic waste to CO_2, leaving little sludge behind. Utilization of organic wastes for biological sulfate reduction offers two advantages: (1) beneficial uses of the waste and (2) metal removal through metal sulfide precipitation as shown in the following reactions (Widdel, 1988):

$$\frac{1}{4}CH_2O + \frac{1}{8}SO_4^{2-} \rightarrow \frac{1}{4}HCO_3^- + \frac{1}{8}H_2S \qquad \Delta G = -14.5 \text{ KJ/reaction} \qquad (18)$$

$$H_2S \rightarrow H^+ + HS^-, HS^- \rightarrow H^+ + S^{2-} (19)$$

$$M^{+2} + S^{-2} \rightarrow MS\downarrow \qquad (20)$$

Organic wastes such as fresh alfalfa, straw and hay, sawdust, woodchips, paper sludge, peat, spent mushroom compost, cow manure, oak leaf, poultry manure, sheep manure, cattle manure, and municipal and industrial wastewater have been used as electron donors individually and in mixtures(Amos and Younger, 2003, Gibert et al., 2004). Zagury et al. (2006) reported that a reactor containing a mixture of organic wastes resulted in high sulfate removal and greater metal precipitation, while a reactor a containing single organic waste resulted in less sulfate reduction.

Municipal wastewater (MWW) typically contains organic material such as carbohydrates, proteins, volatile fatty acids, short chain fatty acid, alcohol, cellulose, and lipids. Volatile fatty acids and short chain fatty acids are preferred substrates for SRB and undergo rapid degradation. Complex organic material such as the proteins, long chain fatty acids and lipids need to be decomposed by different fermentative bacteria and converted to simpler compounds such as acetate and H_2, alcohols or short chain fatty acids before they are utilized by SRB as discussed in the preceding paragraphs. Deng and Lin (2013) combined MWW and acid mine drainage in a batch reactor to improve the electron donor deficiency and > 80% sulfate and COD removal with COD/sulfate ratio of 0.6 and 5.6 was achieved. In the same time the pH of the mixture increased from the alkalinity produced and a higher metal removal obtain (99% Fe removal).

Cellulosic wastes such as maple wood, chips and sawdust have been used to reduce sulfate by SRB. Lignin attached with cellulose was found difficult to be degraded by known SRB species (Chang et al., 2000). In a comparison between organic and cellulosic waste, Choudhary and Sheoran, (2012) observed that the reactor containing only manure resulted in higher sulfate and metals removal than reactor containing a cellulosic material. In an opposing finding, high sulfate removal was observed (99.9%) in a reactor that contained 30% (w/w) cellulose compared to 50% removal rates in a reactor has 2-3%(w/w) cellulose (Neculita and Zagury., 2008).

Activated sludgewas found to result in high sulfate removal efficiencies and provide affordable and easy degradable organic content (Prasad et al., 1999) while municipal compost was reported to have little to no sulfate removal efficiency in an anaerobic batch reactor (Gibert et al., 2004). The low sulfate removal was attributed to the poor organic carbon and high lignin content by the authors.

Animal manures (e.g., cattle or poultry) contain high amounts of organic materials and could be a good alternative for the biological treatment of wastewater (Amos and Younger, 2003). Cattle manure contains carbohydrate, protein, volatile fatty acids (VFA), phenol, p-cresol, in dole, skatole, ammonia-N lactate and fats (Spiehs and Varel, 2009) as simple carbohydrates, and short-chain fatty acids. Complex carbohydrates, long-chain fatty acids, and proteins need to be decomposed first by fermentative bacteria before SRB can use them as electron donors (Postgate, 1984). Although SRB can utilize different electron donors, the competition with MA may be an issue due to varying affinity towards each of those compounds by those different organisms.

Gilbert et al. (2004) showed that sheep manures used in an AMD passive treatment employing SRB resulted in 99% sulfate removal and poultry litter showed 80% removal under the same conditions with hydraulic detention times 2.4 and 9 days. That was confirmed in another study showing poultry manure had lower efficiency for sulfate removal although it contained significant organic carbon (Zagury et al., 2006).

Spent mushroom compost is the residual compost waste generated by the mushroom production industry. It consists of a combination of straw, horse manure, peat, sawdust, rice bran and corncobs (Vavrina et al., 1996, Chang et al., 2000). It is a suitable substrate for SRBs for its content of nutrients available for bacterial growth and readily degradable organic matters such as fungal mycelia and plant residuals (Dvorak et al., 1992). In addition, its bulk physical properties helped retain precipitated metal sulfite. Spent mushroom compost is not widely used because it is not available in large quantities.

2.8 Sugar (sucrose, fructose, and glucose)

Sugar is a suitable carbon and electron donor for SRB, resulting in high sulfate removal in a completely mixed reactor with sugar as the sole substrate under anaerobic conditions (Mizuno et al., 1994, Greben et al., 2000). In this process, high alkalinity was produced from sucrose within the 3.6 h retention time, with hydrogen gas was the interspecies product as shown in the following reactions (Mizuno et al., 1994, Greben et al., 2000):

$$C_{12}H_{22}O_{11} + 5H_2O + 4SO_4^{2-} \rightarrow 4CO_2 + 8H_2 + 4HS^- + 8HCO_3^- + 4H^+ \qquad (21)$$

$$4H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4H_2O \qquad \Delta G = -154.4 \text{ KJ/reaction} \qquad (22)$$

Despite the relative ease of sugar degradation and the high sulfate removal as shown in the above reactions, only a few strains of SRB have been observed to grow on sugar, especially, *Desulfobulbus japonicus* sp. nov. which degrades sucrose and fructose at ambient temperatures (Suzuki et al., 2007). The *Desulfovibrio fructose vorans* sp. nov. was reported to have grown on fructose and sulfate (Olivier et al., 1988, Cord-Ruwisch et al., 1986). Growth on fructose has also been reported for geothermic *Desulfotomaculum nigrificans* (Klemps et al., 1985). Glucose can be depleted completely and easily under different conditions with pH affecting its product. Lopes et al. (2007) reported that glucose was degraded to acetate under pH 6 and to butyrate and acetate under pH 7.

2.9 Selection of electron donors for optimal treatment performance

Amongst all the different substrate or electron donors described above, not any could be described as the best for all SRB. Different factors of the engineering treatment system and waste streams must be considered before selecting an electron donor, such as temperature, pressure, competition for donor by different species, and the specific microbial species present in that environment. Also some electron donors might produce the best result, but the electron donor itself has a downside such as high producing cost. For example hydrogen is favored by most of the SRB species but it is costly.

To improve biological treatment performance, understanding the factors that affect the electron flow from the donors to the acceptor is necessary. For example temperature plays an important role in SRB removal efficiency. Most SRB species survive in wide range of temperatures, operating at optimum temperature would increase the sulfate removal efficiency due to rapid SRB growth. For example using mixture included thermophilic *Desulfotomaculum* which is spices propagated in high temperature range from 40 to 70 C^o (Ashita et al., 2003) and it may be suitable for industrial effluent matching that temperature. For the same argument using mixture included species that reproduces in low temperature may enhance colder climate waste degradation.

The compositions of species in the environment plus the ratio of sulfate to substrate are the available tools for optimizing the SRB biological treatment systems. Enough sulfate to convert organic substrates is the key to successful treatment.Wei-Min et al. (1991) found that adding 8.2 to 9.4 mM sulfate to the brewery waste contain ethanol, acetate, propionate, hydrogen, and formate was not enough to complete substrate conversion and that 60% and 28% of propionate and ethanol respectively converted into acetate when enough sulfate was supplied. They related high propionate conversion to it is high diffusivity suggesting the mass transfer rate is another factor that needs to be considered when choosing electron donors.

CHAPTER 3: Effects of COD/Sulfate Ratio and Iron

3.1 COD/sulfate effects

The ratio of COD to sulfate is animportant design factor in sulfate reduction biological treatment systems. It prescribes the balance between substrate and sulfate to achieve the treatment goal without starving the bacteria or flooding the system with excessive amount of organics. It is a sensitive actor for the competition outcome of the SRB, AB, and MA in anaerobic microbial reactors. SRB was found to outcompete AB and MA under a higher value of COD/sulfate ratio when there was sufficient food for the bacteria to completely reduce sulfate into elemental sulfur, and addition of substrate to the system enhanced MA and AB activity due to the availability of food for all competitors (Lopes et al., 2007, Wang et al., 2008). Piña-Salazar et al. (2011) reported that increasingthe ratio from 0.67 to 2.5 increased sulfate removal efficiency. O'Reilly and Colleran, (2006) found that increasing the value to 2 enhance the SRB metabolism in mesophilic anaerobic reactors (Mizuno et al., 1994). Whereas increasing the value to 6 made the MA the predominant species, in other accounts a value of 16 resulted in MA been the dominated species (Mizuno et al., 1994, O'Reilly and Colleran, 2006)

The COD/sulfate rational so has an effect on the metabolic functions of bacterial populations in anaerobic reactors. It has been reported by several authors that the ratio affected the metabolic pathways of sulfate reducing bacteria (O'Reilly and Colleran, 2006). There were varying lag periods reported for the same substrate under different ratio values (Lopes et al., 2007). They observed smaller lag periods when the ratio value was low and vice versa. The varying lag periods under different COD/SO_4^{2-}ratios in two reactors tested by Lopes et al., (2007) are showing in Table 3.Were two reactors R_1 and R_2 inoculated withsludge from a full scale mesophilic reactor treating paper mill wastewater with COD/sulfate ratio of 9.5and pH of

7. The two reactors were left in two UASB reactors for 538 days with COD/sulfate ratio of 9 and then the sludge from the two reactors was used to test the effect of COD/sulfate ratio on SRB metabolism using ratio of 4 and 1 for reactor 1 and 2 respectively with different substrates (Lopes et al., 2007).

Table 3: Lag phase for different substrates under different COD/sulfate ratios in two tested reactors, R_1 and R_2 (Lopes et al., 2007)

Substrate	Sludge (COD/SO_4^{-2})	pH	Lag phase (days)
Glucose	Inoculum(9)[a]	7	0.83
	R1(4)	6	9.7
	R2 (1)	6	2.8
Hydrogen	Inoculum(9)[a]	7	0.4
	R_1 (4)	7	0.9
	R_2 (1)	7	0.7
Lactate	R_1 (4)	6	11
	R_2 (1)	6	5
Ethanol	R_1 (4)	6	11
	R_2 (1)	6	2
Acetate	R_1 (4)	7	11
	R_2 (1)	7	11
Propionate	R_1 (4)	7	19
	R_2 (1)	7	9
Butyrate	R_1 (4)	6	5
	R_2 (1)	6	4

a) Inoculums was tested for methangenic activity without any sulfate present in the reactor

3.2COD/sulfate ratio theoretical value

The theoretical value for COD/sulfate ratio is the stoichiometric value inwhich enough sulfate is available to convert all substrate to carbon dioxide, and the amount of sulfate removal is optimized for that certain substrate. A decrease or increase in the ratio beyond that optimal point causes decline in the removal efficiency. Different values of the ratio have been reported in the literature. Lens and Vissre, (1998) reported that a ratio of 0.67 or higher contains enough COD for SRB to remove all sulfate. This is a theoretical ratio based on stoichiometry and assumes that all the COD is in a form that can be utilized by SRB (Lens and Vissre, 1998, Piña-Salazar et al., 2011).

The COD/sulfate ratio affects the redox reaction, COD and sulfate concentrations and intermediate and final products. Antonio et al. (2008) reported that increasing the COD/Sulfate ratio to 2.5 resulted in a higher sulfate removal, (95%) and the ratio of 0.67appeared to have insufficient food to convert the entire sulfate in the reactor, which resulted in a lower sulfate removal (37%). In the case of the ethanol the change in the ratio had no affect until reaching a ratio of 2.0 and then increases of the ethanol concentration in the effluent were observed. This ethanol increase was thought to be linked to insufficient sulfate concentration in the reactor to oxidize all the ethanol (Antonio et al., 2008). Similarly increasing the sulfate reduction generates more hydrogen sulfide and converted more ethanol to acetate.

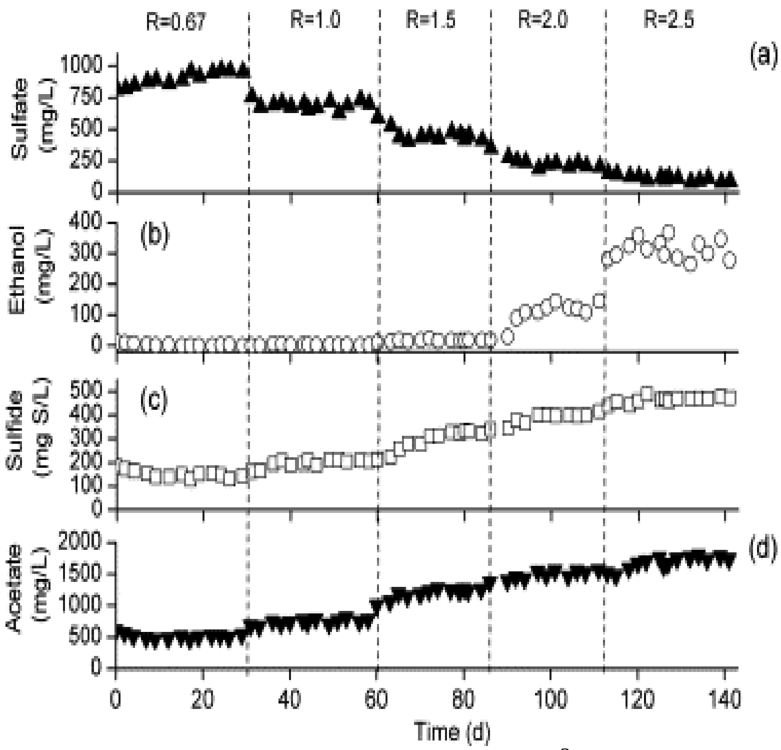

Figure 3: Effect of different feed COD/SO_4^{2-} ratios on: (a) effluent sulfate, (b) effluent ethanol, (c) sulfide production and (d) acetate production in the UASB during 142 days, where R = feed COD/SO_4^{2-} ratio (Antonio et al., 2008)

Different factors control the COD/sulfate ratio such as the concentration of sulfate and type of electron donor. In thermophilic batch reactors, increasing sulfate concentration without changing the ratio would resulted in increasing sulfate removal (Lopes et al., 2007). In this situation, the high availability of sulfate for bacteria subsequently increases the electron flow, which was the cause of the high removal rate. Lopes et al. (2007) used different loading rates to obtain the same ratio when different substrates were in use to improve the removal rate.

3.3Inhibition of SRB and the effects of iron

Sulfate reducing bacteria are very sensitive species and highly responsive to environmental conditions as well as any change in the dissolved inorganic content in wastewater. One group of highly inhibiting elements to SRB are metals, especially heavy metals. Heavy metals can be found in high concentrations in industrial and municipal runoff, and in surface and underground water. Of those, Fe^{+2}, Fe^{+3} and Ni^{+2} are considered as elements of interest with respect to sulfate reduction in environmental waters and wastewaters.

Based on the covalent states of the abundant iron ions in the environment, its effects on SRB differ under anaerobic conditions. Rao et al. (2000) reported that Fe^{2+} has a positive effect on sulfate reducing bacteria, and the effect was temperature dependent. Increasing the temperature enhanced sulfate reduction further, while in colder temperatures less effect was observed, which was attributed to the generation of the sulfide and FeS precipitation (Berner, 1969). It could be suggested that the formation of FeS would result in less H_2S, reducing its toxic effects on SRB. Thermodynamic analysis indicates that, under anaerobic conditions, ferrous iron is the favored redox state of iron. Thus, it is well accepted that high iron concentrations in many anaerobic ground water systems reflect the reduction of highly insoluble Fe^{3+}oxyhydroxides to the more soluble ferrous state Fe^{2+} (Hem, 1985).

Unlike Fe^{2+}, the presence of Fe^{3+} in the sulfate reduction systems has a non-toxic negative effect. The competition between the iron reducing bacteria and the sulfate reducing bacteria is the controlling factor in this iron inhibition. According to Lovley and Phillips, (1987) the presence of Fe^{3+} in the sulfate reduction system redirects the electron flow from sulfate to Fe^{3+}, resulting in sulfate reduction inhibition. They reported 86% to 100% inhibition which was a clear indication that iron reducing bacteria outcompeted sulfate reducing bacteria under the tested conditions. Iron reducing bacteria can consume substrate such as dissolved hydrogen, formate, and acetate at very low concentrations, lower than thresholds required by sulfate reducing bacteria (Francis and Derek, 1992). The conditions in which the inhibition occurs are dependent on the concentration of the substrate, type of substrate, and the form and concentration of the iron ion involved. Lovley and Phillips (1987) reported complete SRB inhibition by Fe^{3+} when substrate was limited. However if excess substrate was present, Fe^{3+} had no inhibition on sulfate reduction and both bacteria were propagated. Francis and Derek (1992) also reported that iron ion concentrations had different effects on SRB inhibition, when they observed complete inhibition by Fe^{+3} concentration that exceeding 1 mg/L in an aquifer and increasing in SRB present when the concentration was 0.05 mg/L. Lovley and Phillips (1987) suggested that Fe^{3+} involved in the reduction of matters in that the presence of Fe^{3+} in the Potomac River estuary showed minor redirection in electron flow, while addition of amorphous ferric oxyhydroxide resulted in sulfate reduction inhibition. One of the important parameters defining the right condition for iron or sulfate reduction is mass ratio between Fe^{2+}/H_2S. Chapelle et al. (2009) reported that a Fe^{2+}/H_2S ratio of 10 or above was in favor of Fe^{2+} while the ratio of 0.30was in favor of H_2S.

In addition to non-enzymatic reduction of Fe^{3+} by sulfate reducing bacteria, the sulfur element produced from the reduction of the sulfate could subsequently react with Fe^{+3} (Pyzik and Sommer, 1981). Enzymatic reduction was reported by several SRB species such as *Desulfovibrio* species including *D. desulfuricans*, *D. vulgaris*, and *D. baculatus*, as well as *Desulfobacterium autotrophicum* and *Desulfobulbus propionicu* (lovley et al,. 1993). They all were reported to reduce Fe^{3+} in the presence of suitable electron donors such as H_2 after depleting the entire sulfate available (lovley et al., 1993). There was not enough energy produced from this redox reaction to support SRB growth which was considered as a clear indication that reduction of the Fe^{3+} by SRB cannot inhibit SO_4^{2-} reduction. However the possibility of Fe^{3+} to be reduced under very low concentration of the substrate makes it favored by SRB in this condition, and that leads to SO_4^{2-} inhibition (lovley et al., 1993).

CHAPTER 4: Reactors Configurations

There are various types of reactors that have been used to maintain favorable conditions for SRB to achieve sulfate reduction and organics removal. The history of anaerobic reactors dates back to the 19^{th} century when a Frenchman, Mouras invented aseptic tank to treat wastewater anaerobically in 1881 (McCarty, 1982). This was followed by an Englishman, Cameron, who in 1895 designed similar reactors with a better treatment output (McCarty, 1982). After all, the treatment efficiency of those reactors has not exceeded 40% removal of organic matters due to the misconception that settling particles is the most important component in wastewater needed to be removed. This ignores the main biological treatment point, that organic material dissolved in water can be degraded by the bacteria present in that environment, if enough contact time and good mixing is provided between bacteria and organic matters(Van Haandel et al., 2006).

Anaerobic treatment eliminates the use of air in degrading organic matters when the chain of reactions takes place inside the reactor, as the fermentation reactions by AB start to convert organic matters such as short chain organic acids to acetate and hydrogen. Subsequently SRB and MA oxidize hydrogen and acetate in the presence of sulfate or CO_2 to obtain H_2S or CH_4. The biological treatment provided a clean elimination of pollution with small energy involved, making it a favored process in waste treatment. Another advantage of anaerobic treatment is that less biomass is produced from the same amount of COD removal (Tchobanoglous and Burton, 1991). However to make this chain of reactions reliable and appropriate, a certain conditions have to be met. For example, Young and McCarty (1969) operated up flow anaerobic filters successfully as the first anaerobic reactor taking into consideration the role of microorganisms in degrading organic matter by offering enough contact time when water passes through the filter material. In general, the biological sulfate reduction includes passive and active treatment where each one of them has its advantages and disadvantages. Passive treatment depends completely on natural process for treatment and utilizes gravity for fluid movement. There is no need for chemical or pumping in that situation. It treats low concentration influents only, and takes long periods of time for the treatment. For example wetland or permeable reactive barriers used to treat polluted underground waters. Active treatment uses physical, chemical, and biological methods for faster treatment of both low and high concentration wastewater. Economic factors and environmental effect are important in determining which system is right for any particular situation (Jeff et al., 2005)

Various configurations of reactors have been used for anaerobic treatment such as fluidized bed reactor, packed-bed downflow reactor, packed-bed upflow reactor, upflow anaerobic sludge blanket (UASB), expanded granular sludge bed (EGSB), gas lift reactor, anaerobic hybrid reactors, and membrane bioreactors. All those different reactors for SRB with their definitions and properties are treated in a greater detail in the following sections.

4.1 Up-flow anaerobic sludge blanket reactors (UASBs)

Upflow anaerobic sludge blanket reactors (UASB) are widely used anaerobic reactors in wastewater treatment Figure 4. In a UASB reactor, the influent comes forth uniformly from the bottom passing through sludge beds into a digestion zone (Lettinga et al., 1980). Enough contact between the water and the biomass present as granular particles in the sludge bed increases the efficiency of SRB in degrading organic matter. Harada et al. (1994) reported 75% of COD removal by SRB alone in a mixed culture inside a UASB using propionate or hydrogen as substrate while lower SRB activity was observed after the addition of acetate. The same removal rate was observed by the SRB and MA from the addition of glucose, denoting the importance of substrate type and the bacterial species involved. In a USAB, the formation of anaerobic granular sludge is considered as a major sign of successful reactor (Hulshoff Pol et al., 2004). The formation of the granular sludge is favored by the combination of high upward velocity and short hydraulic retention time (Alphenaar et al., 1993). However, decreasing the hydraulic retention time is desirable economically if high removal rates are to be obtained. Subsequently, the water is moving up through a phase separator into the settling zone where the sludge or biomass has settled into the sludge bed as illustrated in Figure 4. The gas chamber in the settling zone collects biogas produced in the digestion zone. The UASB reactor provides two important conditions for any biological system to succeed (Hulshoff Pol et al., 1983):

1. Good contact between the incoming water and the biomass, and
2. A large sludge mass in the system.

UASB reactors have shorter detention time and consume less energy compared to other anaerobic treatment technologies. The biogas generated can be used to offset operational expenses and the moving gas bubbles cause turbulence inside the digestion zone to help distribute the biomass around the sludge bed equally. The reactor provides many more advantages such as: high COD removal rate, no need for support media, and simple reactor constructions (Weiland et al., 1991). However, there were some reported disadvantages such as: high area demand, difficulty in controlling granulation process, granular formation inhibited by some wastewater content such as NH_4^+. The reactor has a long start-up period (Weiland and Rozzi, 1991). Overall, UASB reactors are highly recommended in the warmer climate due to its high removal rates (Azimi and Zamanzadeh, 2004).

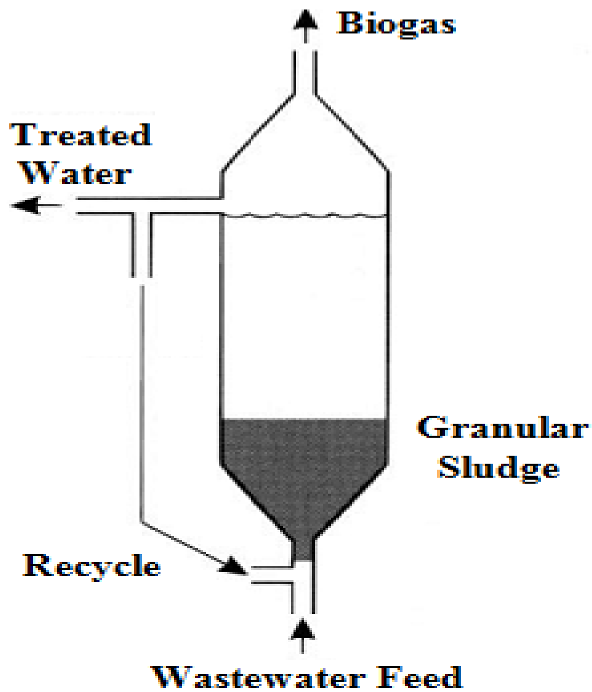

Figure 4: Schematic of UASB reactor **(EPA 2011)**

4.2 Anaerobic filters (AF)

Anaerobic filters were developed in the early 20th century when Young and McCarty operated these types of filters to improve the septic tanks low removal rates (Young and McCarty., 1969). An anaerobic filter is a fixed bed reactor containing solid media where the biomass is retained and biofilms are formed (Figure 5). The attached biofilms consist of different microbial species, such as sulfate reducing bacteria, methanogenes, and many others (Takahiro and Ryoko, 2005). The conditions that favor SRB over other species need to be maintained to allow SRB to compete with other species for sulfate removal. Those conditions may include high sulfate concentrations, appropriate hydraulic retention time and temperature (Penaud et al., 1997, Cha and Noike, 1997). Different media have been used such as gravels, crushed rocks, cinders, or specially formed plastic (Young and Yang, 1989). The solid media provide a large surface area for biomass to attach, and enough contact time between bacteria and wastewater to achieve treatment (Young and Yang, 1989). Flow direction also affects the treatment performance with up-flow patterns more favorable to prevent biomass from being washed out while down-flow gives the advantage of gravity utilization and lower overall operating cost (Young and Yang, 1989). An anaerobic filter can have many advantages such as little electricity usage, ability to use local media in construction, high BOD removal rates and biogas production. Disadvantages include only low COD concentration influent limiting the filters performance and clogging of the reactors due to increase in biofilm thickness (Saleh and Mahmood, 2004).

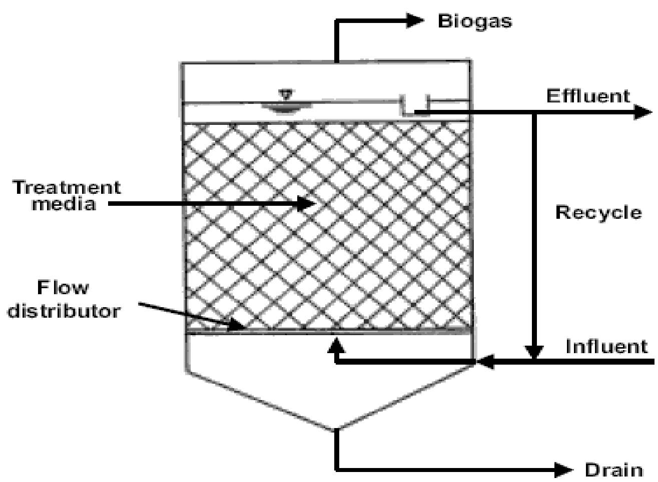

Figure 5: Schematic of an Anaerobic Filter (EPA 2011)

4.3 Anaerobic hybrid reactors (AHRs)

The anaerobic hybrid reactors or the hybrid up-flow anaerobic reactors combine two reactors, UASB and AF, to aggregate the advantages and reduce the disadvantages of the two reactors. An anaerobic hybrid reactor typically consists of an up-flow sludge blanket in the lower part and an anaerobic filter reactor in the upper part (Figure 6). All the media usages and the conditions applied to each of the two mentioned reactors are applicable to AHRs. It was first developed by Maxham and Wakamiya in 1981 (Elmitwalli et al., 1999). Also higher COD removal rates occurred due to the dual operating conditions compared to UASB or AF individually or any other anaerobic reactors (Elmitwalli et al., 1999). It has been widely used in different industries including but not limited to wastewater treatments, distillery spentwash water, fiberboard manufacturing wastewaters, pharmaceutical wastewater, Penicillin-G wastewater, and phenolic wastewater (Shivayogimath and Ramanujam, 1999, Fernandez et al., 2001, Ramakrishnan and Gupta, 2006, Mullai et al., 2011). To improve removal rates in an AHR, a procedure such as air stripping to prevent bacterial inhibition was introduced to remove sulfide from the reactor (O'Flaherty and Colleran, 1999). The authors reported that 95% removal rate of COD dropped to 60% due to accumulation of sulfide inside the AHR.

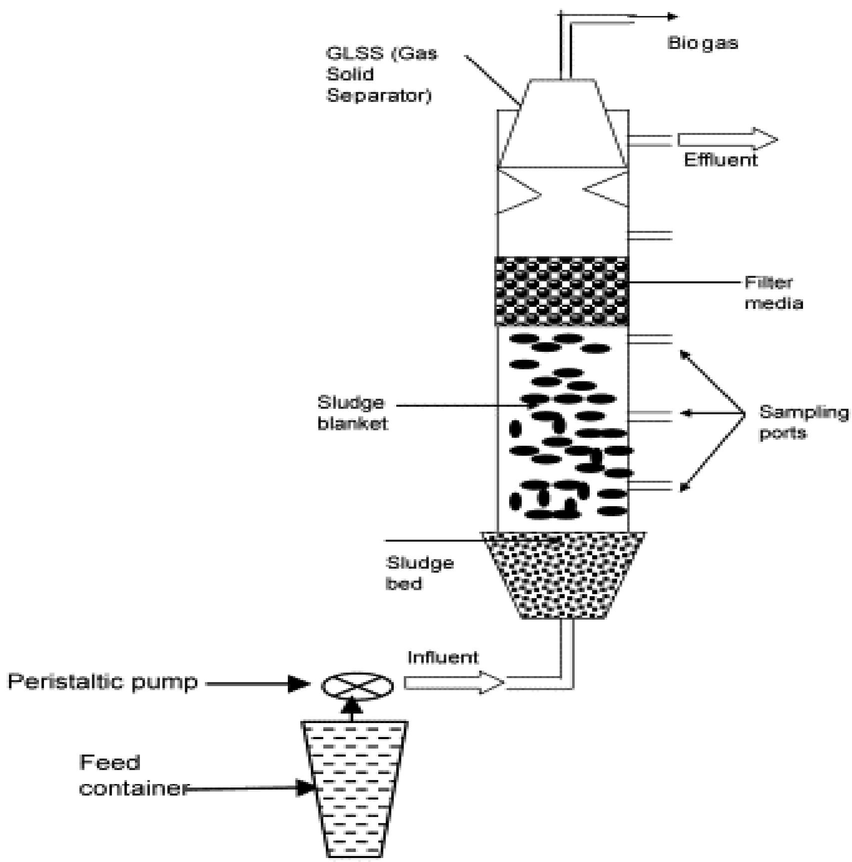

Figure 6: Schematic of an anaerobic hybrid reactor (Ramakrishnan and Gupta, 2006)

4.4 Expanded granular sludge beds (EGSBs)

Expanded granular sludge bed reactors (Figure 7) are a modification of the UASB reactor to reduce the disadvantages by treating the dead zone and reducing hydraulic short circuiting inside the reactor (Richard et al., 2000). The modification provides better hydraulic mixing such that the granules are partially fluidized by recycling the effluentat liquid up-flow velocities of 5–6 m h^{-1} or above, which could be achieved by adequate height/diameter ratio (Richard et al., 2000, Pereira et al., 2002). In opposing findings, an up-flow velocity higher than 5.5 m h^{-1}may result in sludge washout due to the buoyancy forces from the gas production, specially with loading rates higher than 7 mg COD/L·d (Kato et al., 1994). However different conditions and reactor sizes or the type of the settler used could be the reason behind the differences. A toxic biodegradable organics, such as formaldehyde with high concentration, was reported to be removed by an EGSB reactor as well as short chain organic acids such as acetic, propionic, butyric, maleic, glyoxylic and benzoic acids (Richard et al., 2000). EGSB is suitable for high and low organic loading rates in comparison to conventional UASB reactors. Additionally high biogas production in the reactor increases the mixing rate (Seghezzo et al., 1998). EGSB reactors are suitable for sulfate reduction (Weijma et al., 2000). Sulfate reducing bacteria can outcompete methanogenic archaea in EGSB reactors at a temperature 65 C^{o} and pH 7.5, at methanol limiting or excessive conditions and hydraulic retention times 14 and 3.5 h (Weijma et al., 2000).

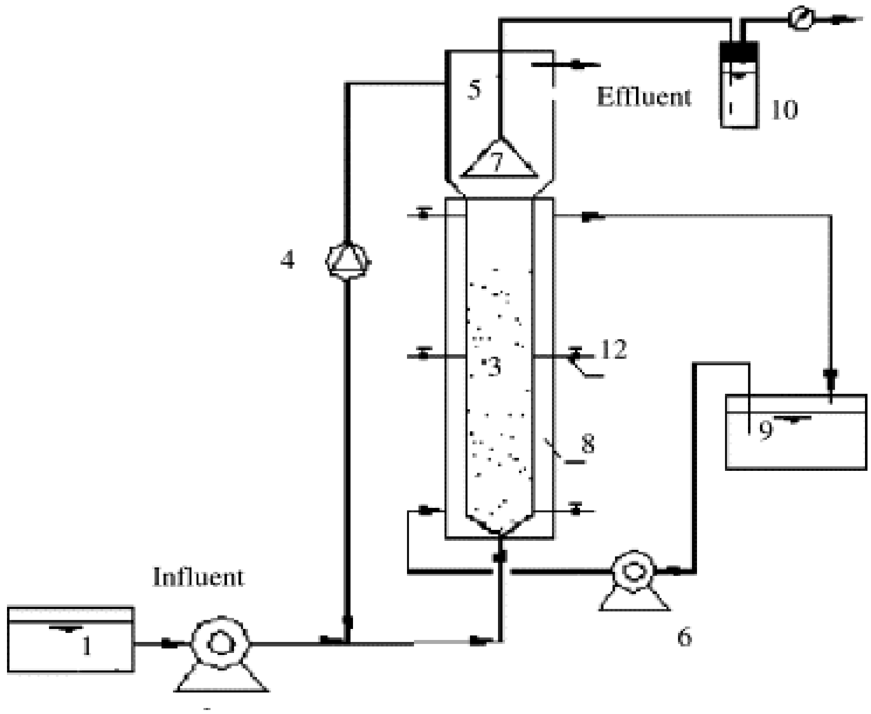

Figure 7: Schematic of an EGSB reactor (Wang and Kang, 2005)

4.5 Anaerobic fluidized bed reactors (AFRs)

Anaerobic fluidized bed reactors (Figure 8) contain fine carrier particles used for microbial film development. Development modifies the particles density, size and shape, and subsequently their hydrodynamic behavior. These particles with the attached biofilm fluidized by the up-flow velocity are generated from the influent and the recycled effluent (Saravanane and Murthy, 2000). By its definition, a fluidized bed always expands over 30%. If the expansion is less than30%, the reactor would be named an expanded bed (Grasius et al., 1997). Different carrier particles were reported to be used efficiently. Sand was used with starch based food waste, chemical waste, bakery waste, and brewery waste. Zeolite, sand and activated carbon were used for the treatment of sewage (Hickey et al., 1991). Many parameters affect the biomass development and therefore affect the start-up period, such as liquid flux rate, scale of the reactor, gas flux and organic loading rate (Hickey et al., 1991). Furthermore, particle size can also have influences on the start-up period (Heijhen et al., 1989). Sand particles with a diameter of 0.35 mm were much faster in the startup period than sand particles with a diameter of 0.75 mm. The reason behind this was thought to be the lower liquid shear with smaller particles (Heijhen et al., 1989). AFR has many advantages over other anaerobic reactors;including high treatment efficiency, no clogging of the reactor, no problem of sludge retention, least chance of organic shock load, high sludge activity and small area requirement (Saravanane and Murthy, 2000). Disadvantages of the reactor design include long startup periods due to the formation of the film in the carrier, high energy consumption, and difficulties due to control of the biofilm thickness (Heijhen et al.,1989).

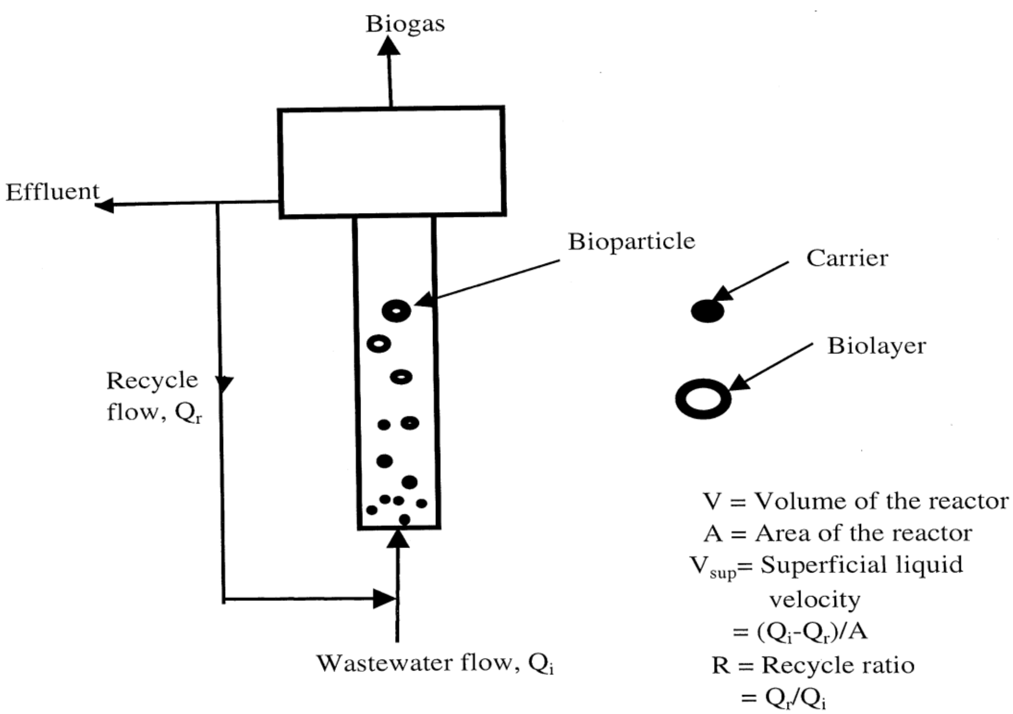

Figure 8: Schematic of a fluidized bed reactor (Saravanane and Murthy, 2000)

4.6 Aerobic baffled reactors (ABRs)

Aerobic baffled reactors (Figure 9) are another modification of the up-flow anaerobic sludge blanket (UASB). They are staged reactors where biomass retention is enhanced by forcing the water flow through several compartments (Barber and Stuckey, 1999, Sponza and Isik, 2002, Kaksonen, and Puhakka, 2007) to allow increased protection against toxic materials and higher resistance to changes in environmental parameters such as pH and temperature (Barber and Stuckey, 1999). It was used to treat low and high strength wastewater (Witthauer and Stuckey, 1982). Low mass transfer was observed in the case of low strength wastewater, resulting in a low removal rate and less gas production, which means less mixing and in some cases biomass starvation occurs in the letter compartment in a high hydraulic retention time(Orozco, 1988). In the case of high strength wastewater a high mass transfer occurs resulting in high gas production and subsequently increasing biomass removal.

The main advantage of ABRs is their ability to allow different conditions favored by several kinds of bacteria at the same time due to the presence of several compartments. Other advantages are listed in the Table 4.

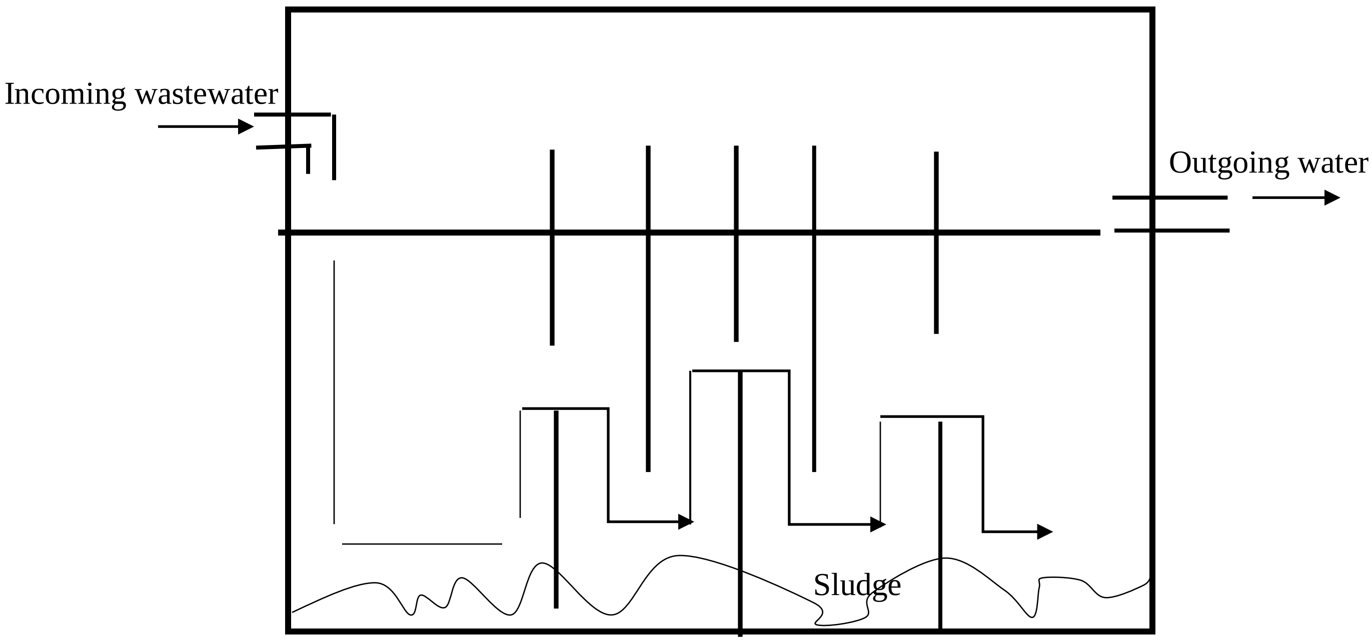

Figure 9: Schematic of anaerobic baffled reactors (ABRs)

Table 4: Advantages associated with the anaerobic baffled reactor (Barber and Stuckey, 1999)

Advantage
Construction
1 Simple design
2 No moving parts
3 No mechanical mixing
4 Inexpensive to construct
5 High void volume
6 Reduced clogging
7 Reduced sludge bed expansion
8 Low capital and operating costs
Biomass
1 No requirement for biomass with unusual settling properties
2 Low sludge generation
3 High solids retention times
4 Retention of biomass without fixed media or a solid-settling chamber
5 No special gas or sludge separation required
Operation
1 Low HRT
2 Intermittent operation possible
3 Extremely stable to hydraulic shock loads
4 Protection from toxic materials in influent
5 Long operation times without sludge wasting
6 High stability to organic shocks

CHAPTER5: Conclusions

Anaerobic biological treatment of wastewaters containing sulfate using (SRB) can have significant energy, environmental, and economic advantages over aerobic biological treatment. Although the later can treat high amount of waste in short time the amount of energy used and the sludge produced offset the benefit. Anaerobic treatment requires less energy with very little sludge produced.Wastewater contains different organic and inorganic compounds that suitable for SRB degradation, make it all a good alternative to remove the waste and precipitate metals in that water in sulfide form, deficient in substrate would be fulfill by adding external electron donors. A wide range of inorganic and organic compounds could be applied as electron donors for SRB, such as hydrogen, short chain of fatty acids, alcohols, organic wastes. Various factors affecting the choice of the electron donors include energy yield from the sulfate reduction reaction, competition among different microorganisms for limited sources of food, COD/sulfate ratio, and the surrounding conditions (pH, temperature). Also some electron donors might be the best choice but the electron donor itself has a downside such as its high cost. For example hydrogen is a favored electron donor for most of the SRB but the high cost of producing hydrogen offset all its benefit.

The COD/sulfate ratio is an important factor for anaerobic treatment system employing SRB. It can be used to optimize treatment performing for a given wastewater. High ratio value (>2) have been reported to resulted in high sulfate removal rate. However, increasing the ratio higher could decrease the sulfate removal rate and select methanogenic archaea as the predominant microorganisms at value of 4. On the other hand lowering the ratio value could result in SRB inhibition due to food limitation. Inhibition of SRB by heavy metal has been observed, iron have different effect on SRB depending on the ion covalence. The present of ferrous (Fe^{+2}) ion in the engineering treatment system may improve sulfate removal efficiency due to FeS formation and by lowering H_2S in the system.This decreases hydrogen sulfide toxicity to SRB, and the effect is temperature dependent. Ferric ion (Fe^{3+}) may result in high iron reducing bacteria activities and outcompete SRB for substrate

Reactors can be designed to bring the best situation suitable for SRB to improve removal of sulfate and organic wastes. Anaerobic filters and upflow anaerobic sludge blanket reactors were the early reactors used in anaerobic treatment and most of the latter reactors were modifications to those two reactors to aggregate the positives and overcome the negatives. Most of the anaerobic reactors used domestic material in their construction such as gravel and small rocks and that make them less expensive and easy to construct in the remote area.

There is no optimum electron donor to serve in all conditions but there is an optimum condition that will help produce the best outcome in the wastewater treatment system. For a given wastewater research is needed to optimize those conditions and to identify electron donors from different organic materials available in wastewater that are suitable for SRB. The SRB anaerobic treatment method is promising as many researchers have shown> 80% sulfate removal for a wide range of wastewaters.

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