ANAEROBIC TREATMENT OF HIGH SULFATE WASTES

by

Barry L. Hilton

A thesis submitted to the
Faculty of Graduate Studies
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Anaerobic treatment of high sulfate wastes presents a challenge to the design engineer due to the generation of hydrogen sulfide which results from the reduction of oxidized sulfur compounds. The generation of H_2S has long been associated with a loss of methane production. The research presented here investigated the limits, capabilities, nature of the process and the effects of sulfide toxicity as pertaining to engineered systems for the anaerobic treatment of high sulfate wastes. Sulfide toxicity to lactose utilization, methanogenesis and sulfate reduction was investigated.

Continuous flow laboratory anaerobic reactors were fed a synthetic waste, whey, or spent sulfite liquor for the carbon source and $CaSO_4$ or Na_2SO_4 as the source of oxidized sulfur. Three reactors were operated in the methanogenic mode, two were in the unstripped sulfidogenic mode, and nine were in the stripped sulfidogenic mode. Sulfide stripping was by gas recycle (Biosulfix® process) and explored the use of a variety of agents for sulfide absorption.

Sulfide toxicity batch tests were performed in 100 mL glass syringes incubated in a 35°C water bath.

Carbon removal (>90 %) in methanogenic and stripped sulfidogenic reactors was comparable although methane production in the sulfidogenic reactors was significantly less than in the methanogenic reactors. The optimal $S_r/C_o = 0.3$ for stripped sulfidogenic reactors. A "steady-state" sulfur reduction rate of

1.3 g/L·d at B_V = 2.2 g/L·d TOC was achieved. Maximum carbon removal required maintenance of a syntrophic population of methanogens and sulfate reducing bacteria. Sulfate reduction was performed by the incompletely oxidizing sulfate reducing bacteria. Total inhibition of methanogenesis occurred when CO_2 was removed from the reactors. Optimal sulfur reduction occurred above pH 6.0.

Carbon flow was diverted from sulfate reduction to methanogenesis at total sulfide concentrations of 1000 mg/L and at pH 8.0. Sulfate reduction was inhibited by increased concentrations of total sulfides, regardless of pH whereas methanogenesis and lactose utilization were inhibited by the presence of unionized H_2S.

To Ann, Joy, and David

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1. INTRODUCTION

1.1 History

The high rate of industrial sulfate accumulation constitutes a growing environmental concern. Large quantities of sulfates are generated by the electric power industry from flue gas desulfurization sludges. The pulp and paper industry, the pharmaceutical industry, and segments of the food industry also produce effluents which are high in sulfates and other species of oxidized sulfur compounds. The application of methanogenic treatment processes to these wastes maybe hindered by the resulting reduction of sulfur species to sulfides by sulfate reducing bacteria (SRB).

Most of the data on sulfide toxicity and sulfide stimulation come from low-carbon natural ecosystems (King 1984; Oremland and Polcin 1982; Winfrey and Zeikus 1977) or from pure-culture laboratory experiments, both of which are quite unlike the industrial waste treatment reactor.

At low concentrations, both the oxidized and reduced forms of sulfur are required by the methanogenic bacteria to function at optimal levels (Wellinger and Wuhrmann 1977; Mountfort and Asher 1979). At high concentrations of oxidized sulfur, the methanogenic pathways appear to be blocked by the combined effects of sulfide inhibition (or even toxicity) and the shortage of immediate methane precursors: hydrogen, methanol, and acetate. This threshold of inhibition has been assumed to be

200-300 mg S^{2-}/L (Lawrence et al. 1964; Kroiss and Plahl-Wabnegg 1983). This concentration may be regarded as a case-specific value, depending upon the age of the culture, its history of sulfide exposure, the type of organic matter in the wastewater, and the sulfide species as related to pH (Kroiss and Plahl-Wabnegg 1983; Koster et al. 1986).

The selectivity of various SRB and MPB for energy substrates is quite varied. As shown in several recent studies, the spectrum of organics utilized by the SRB is much wider than in the case of methanogens (Imhoff-Stuckle and Pfening 1983; Laanbroek and Pfening 1981; Postgate 1984). This explains early successes in utilizing SRB to accelerate anaerobic stabilization of sewage sludges (Burgess and Wood 1961; Sadana and Morey 1962; Pipes 1960).

More recently, the mixed-culture work of Middleton and Lawrence (1977), DLA, Inc. (1982b) and Olthof et al. (1986) have indicated the substantial advantages of sulfidogenic pathways in application to high sulfate complex industrial waste streams. In sulfidogenic pathways, organic matter is oxidized using sulfate (or other oxidized sulfur compounds) as an electron acceptor. This is in contrast to the conventional methanogenic pathway where volatile fatty acids are broken down to methane and CO_2 and where CO_2 is autotrophically assimilated into cell carbon (Daniels et al. 1984).

Middleton and Lawrence (1977), using seed cultures obtained from anaerobic digesters and acetate as the carbon source, grew sulfate reducing bacteria to the exclusion of methanogens.

Schonheit et al. (1982) showed that at 300 mg/L acetate, MPB and SRB utilized acetate at equal rates. However, at 6 mg/L, the acetate consumption rate was 15-fold higher for the SRB than the MPB. Lovley et al. (1982) found that the SRB usually outcompete MPB due to a lowering of the partial pressure of hydrogen below levels that could effectively be utilized by MPB. Isa et al. (1986a, b) noted that $S_r/C_o = 0.08$ in reactors fed acetate and $S_r/C_o = 0.17$ in reactors fed acetate and ethanol. They concluded that SRB could not outcompete MPB for acetate. The presence of a hydrogen donor such as ethanol enhanced sulfate reduction. MPB were more easily retained than were the SRB in their reactors.

1.2 Anaerobic metabolism

Substrate utilization in anaerobic reactors can be broken into two major categories: assimilation and dissimilation. Assimilation is the incorporation of compounds into the carbon structure of the cell. Examples of assimilation are the incorporation of sulfide in the synthesis of cysteine or the formation of acetate from CO_2.

Dissimilation is the breakdown of complex compounds into simpler forms. Examples are the decarboxylation of acetate to methane and CO_2, the reduction of sulfate to sulfide, or the mixed acid fermentation of complex carbohydrates to simpler compounds such as acetate, ethanol, CO_2, H_2, and lactate.

1.2.1 Energy metabolism of sulfate reducing bacteria

The SRB generate ATP by the dissimilatory reduction of oxidized sulfur compounds such as sulfate, sulfite, bisulfite, and thiosulfate. In this process, oxidized sulfur is the terminal

electron acceptor and the electron donor is either hydrogen or a carbon compound. The reduction of oxidized sulfur is limited by the availability of a suitable electron donor. Typical reactions are (Postgate 1984):

[1.1] $4\ H_2 + SO_4^{2-} \text{-----} > S^{2-} + 4\ H_2O$

[1.2] $CH_3COO^- + SO_4^{2-} \text{-----} > H_2O + CO_2 + HCO_3^- + S^{2-}$

acetate

[1.3] $4\ HCOO^- + SO_4^{2-} \text{-----} > 4\ HCO_3^- + S^{2-}$

formate

[1.4] $4\ CH_3COCOO^- + SO_4^{2-} \text{-----} > 4\ CH_3COO^- + 4\ CO_2 + S^{2-}$

pyruvate acetate

[1.5] $2\ CH3COHCOO^- + SO_4^{2-} \text{-----} > 2\ CH_3COO^- + 2\ CO_2 + S^{2-}$

lactate acetate

Equations 1.2-1.5 can be restated in terms of the ratio of sulfur reduced (S_r) to Total Organic Carbon (TOC) fed (C_o), S_r/C_o, and in terms of the ratio of sulfur reduced to TOC removed (C_r), S_r/C_r are shown in Table 1.1.

Hydrogen can be used by incompletely oxidizing sulfate reducers such as Desulfovibrio and Desulfotomaculum as well as the complete oxidizers such as Desulfobacter (Postgate 1984). Acetate can be used by Desulfovibrio baarsii, Desulfotomaculum acetoxidans and Desulfococcus multivorans. Formate has been shown to be utilized by Desulfovibrio baarsii, Desulfococcus

multivorans, Desulfonema limicola, Desulfosarcina variabilis, and Desulfotomaculum nigrificans. Only D. baarsii and D. sapovorans have been shown to utilize long (up to C_{18}) chain fatty acids. The remaining species (majority) of Desulfovibrio such as D. sulfuricans, D. vulgaris, D. gigas, and D. saporovans require lactate or pyruvate as carbon sources to reduce sulfate in accordance with equation 1.4 or 1.5 (Postgate 1984).

Table 1.1 Theoretical S_r/C_o and S_r/C_r for selected fatty acids.

Carbon Source	S_r/C_o (g/g)	S_r/C_r (g/g)
Formate	0.67	0.67
Acetate	1.33	1.33
Pyruvate	0.22	0.67
Lactate	0.44	1.33

The growth rate of the incompletely oxidizing sulfate reducing bacteria is up to four times that of the complete oxidizers (Nanninga 1985, Middleton and Lawrence 1977). This suggests that the incompletely oxidizing SRB would predominate in anaerobic reactors. However, the presence of complete oxidizers also suggests that it should be possible to utilize both groups of SRB in the treatment of high sulfate wastes.

1.2.2 Anaerobic fermentation

Complex wastes must undergo transformation to less complex compounds such as lactate, pyruvate, ethanol, or acetate prior to metabolism by either sulfate reducing bacteria or methanogenic bacteria.

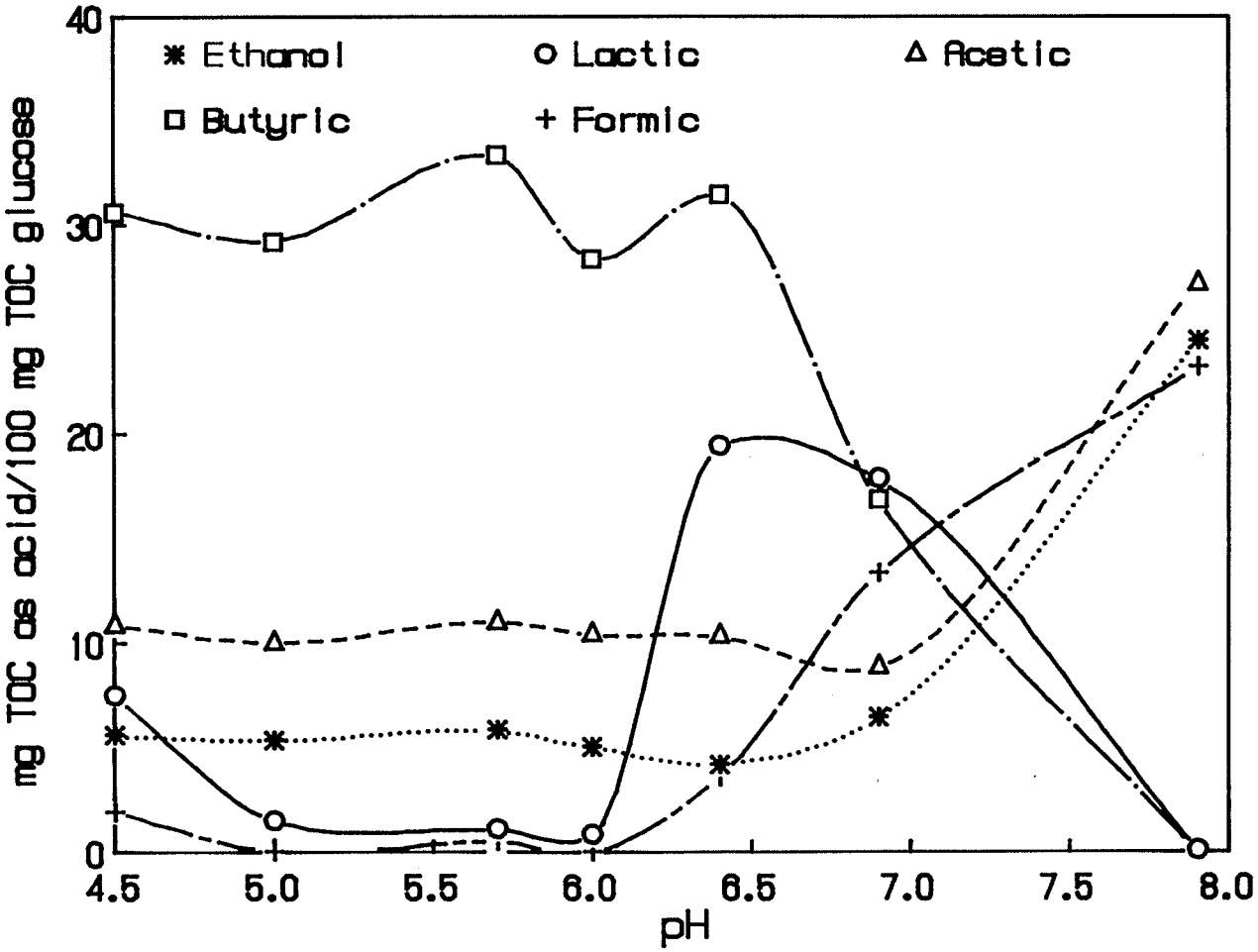
Anaerobic reactors treating high sulfate wastes include: citric acid plant (Kroiss 1987), edible oil wastes (Saw et al. 1987), acetate (McKinney 1986; Isa et al. 1986a, b), molasses (Maree and Strydom 1987), municipal sewage (Pipes 1960; Lawrence and McCarty 1965; Olthof et al. 1986), industrial sludge (Olthof et al. 1986), distillery plant effluent (Szendry 1982), pulp and paper wastes (Eis et al. 1983).

1.2.2.1 Glycolysis

Glycolysis is the specific series of reactions whereby energy is generated by the dissimilation of glucose to simpler compounds such as pyruvate, lactate, and acetate. The end products of glycolysis provide the carbon and energy source (e.g., lactate, pyruvate, propionate, acetate, ethanol) for sulfate reducing bacteria and methanogens.

Zoetemeyer et al. (1982) studied changes in the end products of glycolysis as a function of pH and hydraulic retention time. Figure 1.1 is a plot of the published data for $\mu = 0.9\ \mu_{max}$. It can be seen that the production of lactic acid is optimal between pH 6.1-7.6. Below pH 6.0, and above pH 7.9, the production of lactic acid is minimal. The production of butyric acid was constant up to pH 6.4; thereafter it decreased to zero at pH 8.0. Above pH 6.5,

Figure 1.1 The percentage yield ~~(g TOC acid/100 g TOC glucose)~~ of products of fermentation of glucose as a function of reactor pH (after Zoetemeyer et al. 1984).



acetate, formate, and ethanol increased until the maximum value was attained at pH 7.9. Chartrain and Zeikus (1986), using powdered whey, found that at pH 7.0, lactose was metabolized primarily into lactate, ethanol, acetate, formate, and carbon dioxide.

Based upon the studies of Zoetemeyer et al. (1982) and Chartrain and Zeikus (1986), one would expect that changes in the pH and the HRT of the reactor would determine the products of fermentation. In the case of incompletely oxidizing sulfate reducing bacteria, increases in the production of lactate would result in increases in sulfate reduction and vice versa.

1.3 Sulfur toxicity

The formation of hydrogen sulfide in anaerobic reactors is the result of the reduction of oxidized sulfur compounds and of the dissimilation of sulfur amino acids such as cysteine. In anaerobic reactors, sulfur reduction is performed by two major groups of sulfate reducing bacteria: a) incomplete oxidizers which incompletely oxidize compounds such as lactate to acetate and CO_2 (equations 1.4, 1.5), and b) complete oxidizers (acetoclastic SRB) which completely oxidize acetate to CO_2 and HCO_3^- (equations 1.2 and 1.3). Both groups utilize hydrogen for sulfate reduction.

The dissolution of H_2S in water forms an equilibrium system as follows:

[1.6] $$H_2S <-----> H^+ + HS^- <-----> 2\ H^+ + S^{2-}$$

The equilibrium between H_2S and HS^- is pH dependent in accordance with the following reaction:

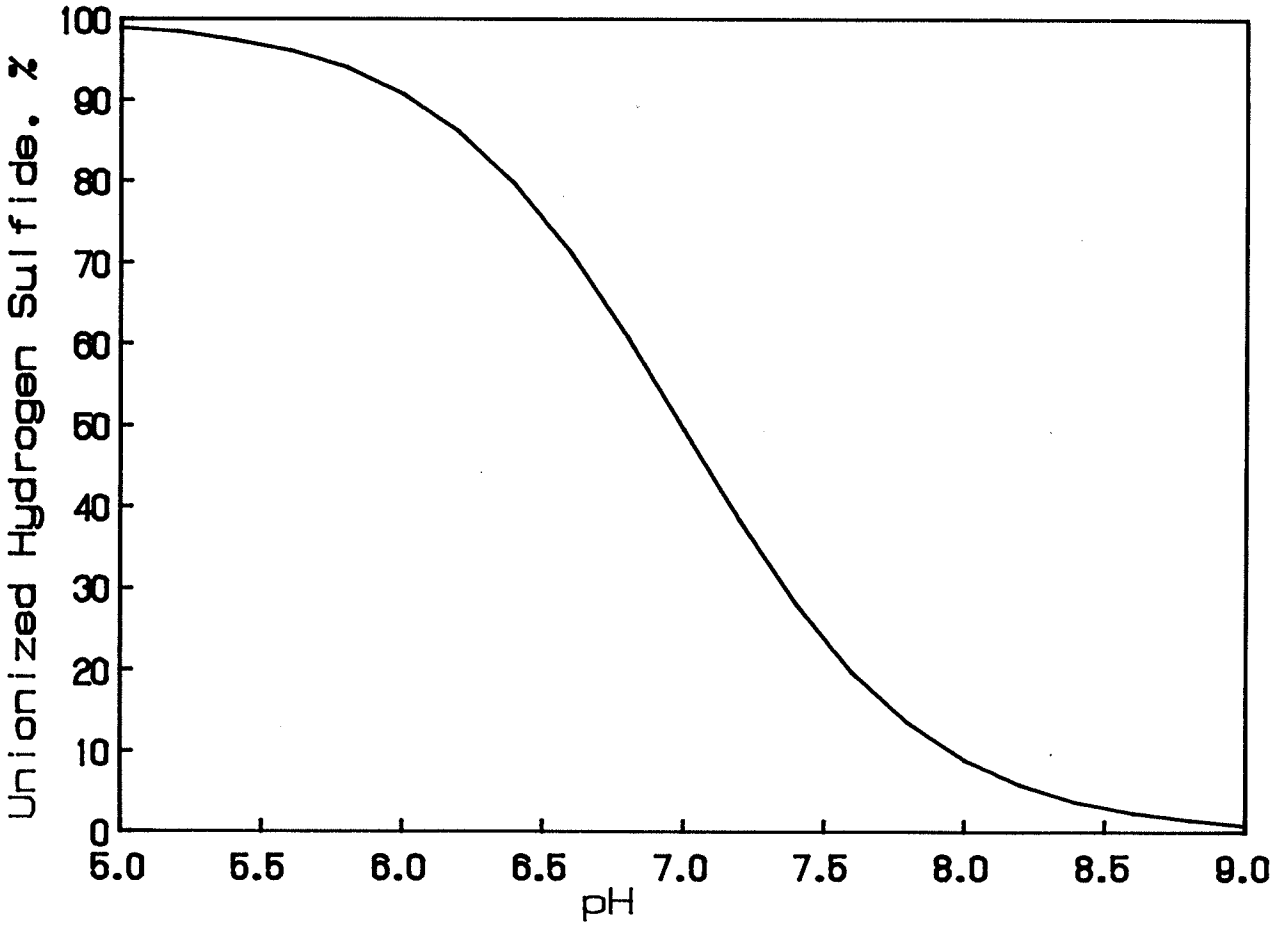
[1.7] $$H_2S = [1 + 1.02 * 10^{(pH-7)}]^{-1}$$

The percentage of un-ionized H_2S drops from 90% at pH 6.0 to 50% at pH 7.0 to 10% at pH 8.0. This variation is most significant in anaerobic treatment since the pH range of anaerobic reactors is maintained between pH 6.0 and 8.0 with the generally accepted optimal pH for methane production being between pH 6.8 and 7.5. Furthermore, for experimental purposes, by adjustments in pH, it is possible to study the relationship between different concentrations of un-ionized H_2S and different aspects of anaerobic treatment such as lactose uptake, sulfate reduction, and methanogenesis.

Studies of sulfide toxicity to anaerobic treatment have shown that the generally accepted sulfide concentration for complete inhibition is 200 mg/L S^{2-} (Aulenbach and Heukelekian 1955; Lawrence et al. 1964). Kroiss and Plahl-Wabnegg (1983), in a study of flocculant sludge found that a decrease in methane production was associated with un-ionized H_2S concentrations as low as 50 mg/L with a complete loss of methane production at 200 mg/L. In that study, the decrease in methane production coincided with a decrease in carbon removal and an increase in the concentration of volatile fatty acids (VFA). When the un-ionized H_2S concentration increased above 200 mg/L, the formation of VFA decreased. In another study using granular sludge, Koster et al. (1986) found inhibition of methanogenesis at un-ionized H_2S concentrations as low as 50 mg/L. Other studies have shown that methanogenic bacteria can tolerate sulfide concentrations up to 1000 mg/L S^{2-} (Mountfort and Asher 1979; McKinney 1986). Other studies have shown that the addition of 8-22 mg/L S^{2-} stimulated

the production of methane (Wellinger and Wuhrmann 1977; Ronnow and Gunnarson 1982), reflecting the need for the biosynthesis of sulfur based amino acids.

Figure 1.2 The percentage of unionized H_2S in relationship to pH.



2. PURPOSE AND SCOPE

Anaerobic treatment of high sulfate wastes presents a challenge to the design engineer due to the generation of hydrogen sulfide which results from the reduction of sulfur compounds. The generation of H_2S has been associated with a loss in methane production (Rudolfs and Amberg 1952; Lawrence et al. 1964). The addition of gypsum ($CaSO_4$) to sludge has been shown to be a feasible means of desulfurizing gypsum and promoting the generation of sulfides which could be scrubbed to provide elemental sulfur (Sadana and Morey 1962; Burgess and Wood 1961; Pipes 1960; DLA, Inc. 1982b.

Recently, DLA, Inc. (1982b), demonstrated the applicability of sulfidogenic pathways in the treatment of high-sulfate complex industrial waste streams. That work, using pig manure, acetate, and gypsum, demonstrated control of reactor total sulfide concentrations below 50 mg S^{2-}/L, by the use of a gas recycle system as shown in Figure 3.1 in which H_2S was absorbed by a solution of zinc acetate. In that study, COD removals were 95-98% and there was a substantial production of methane.

The success of DLA, Inc. in the removal of soluble sulfates with anaerobic reactors (their patented Biosulfix® process) suggested a need for further research to gain further understanding for the limits, capabilities, and nature of the process.

The purpose of the studies presented here was to gain a greater understanding of the sulfate reduction process as they