ANAEROBIC TREATMENT OF HIGH SULFATE WASTES

by

Barry L. Hilton

A thesis submitted to the
Faculty of Graduate Studies
of the
University of Manitoba
in partial fulfillment of the degree

Doctor of Philosophy

Winnipeg, Manitoba

November, 1987 

ISBN 0-315-37248-6

ANAEROBIC TREATMENT OF HIGH SULFATE WASTES

BY

BARRY L. HILTON

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

ANAEROBIC TREATMENT OF HIGH SULFATE WASTES

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

ACKNOWLEDGMENTS

Sincere gratitude and appreciation are extended to my thesis advisor, Dr. Jan A. Oleszkiewicz, Department of Civil Engineering at the University of Manitoba for his patience and guidance during the investigative and analytical phases of this research.

Special thanks go to Dr. Arthur B. Sparling, Department of Civil Engineering, the University of Manitoba, for his assistance, guidance, and wisdom. Without his presence, I would have never ventured down this path.

Thanks go to Dr. Gregory Blank, Department of Food Science, the University of Manitoba, for his commentary regarding questions of microbiology and whose advice helped steady the course of research.

Gratitude goes to Dr. Nathan C. Burbank, Tucson, Arizona, without whose encouragement I would have been unable to commence this journey.

Appreciation is extended to Mr. John Clark and Mr. Steven Meyerhoff, Department of Civil Engineering, the University of Manitoba, for their invaluable assistance in the manufacture of specialized equipment utilized in this research.

Gratitude, love and appreciation go to Dad and Diane, Dr. and Mrs. Harold A. Hilton for their confidence that I could make it and for the considerable financial assistance which made it possible.

Very special thanks go to Mrs. Judy Tingley, Department of Civil Engineering, the University of Manitoba, for her daily assistance in the maintenance and operation of the delicate

research equipment and for her invaluable assistance in the intensive testing program which was an integral part of this research. Her skills and dedication immeasurably lightened the burden of testing and guaranteed the quality of the analytical results.

Thanks go to Zdislaw J. Oziemblo and Stan Mateja, for their assistance in the operation and construction of the reactors and assistance in analyses.

This research was funded by grants from the Natural Sciences and Engineering Research Council of Canada.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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.

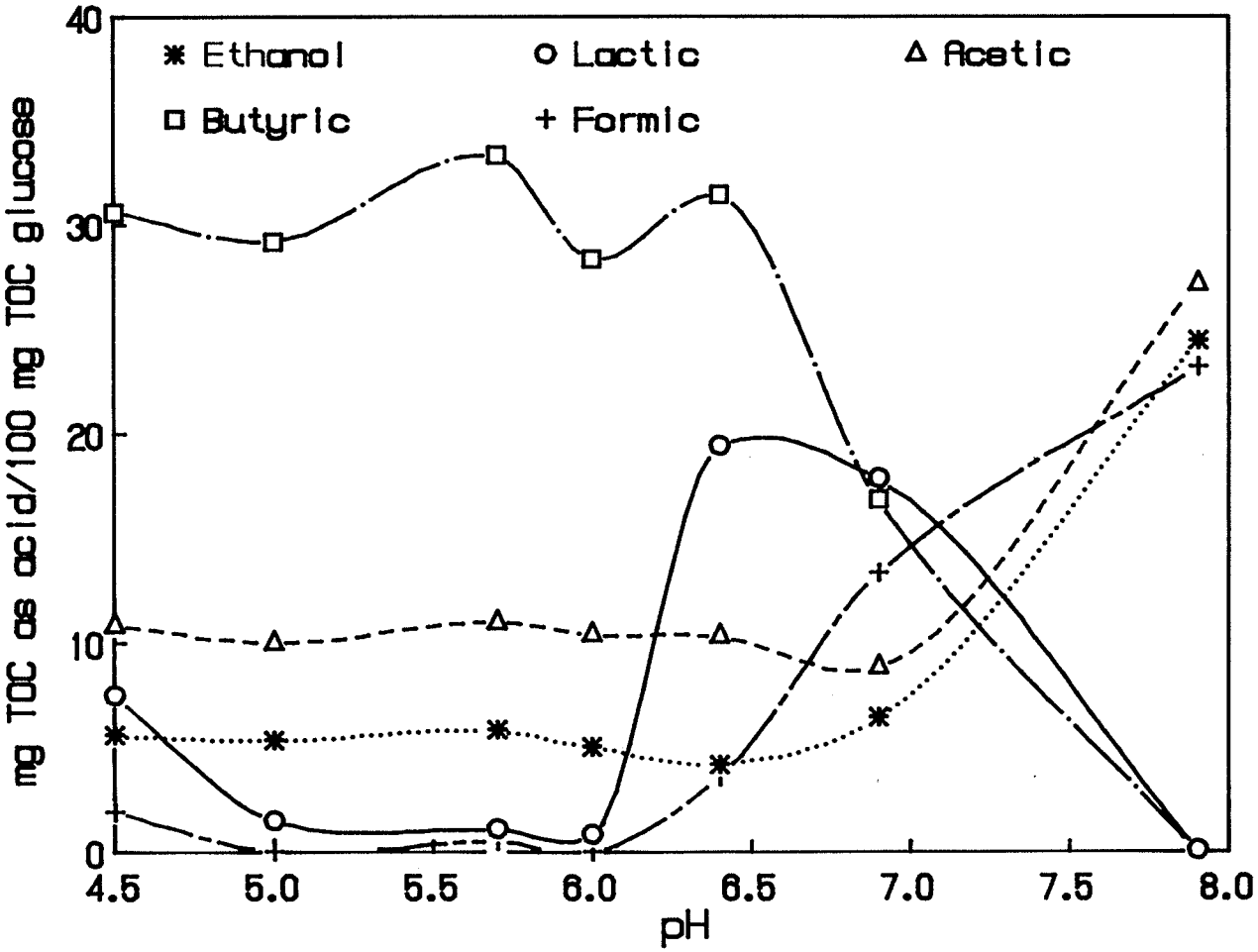
Anaerobic reactors treating high sulfate wastes include: citric acid plant (Kroiss 1987), edible oil wastes (Saw et al. 1987), acetate (McKinney 1986; Isa et al. 1986a, b), molasses (Maree and Strydom 1987), municipal sewage (Pipes 1960; Lawrence and McCarty 1965; Olthof et al. 1986), industrial sludge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

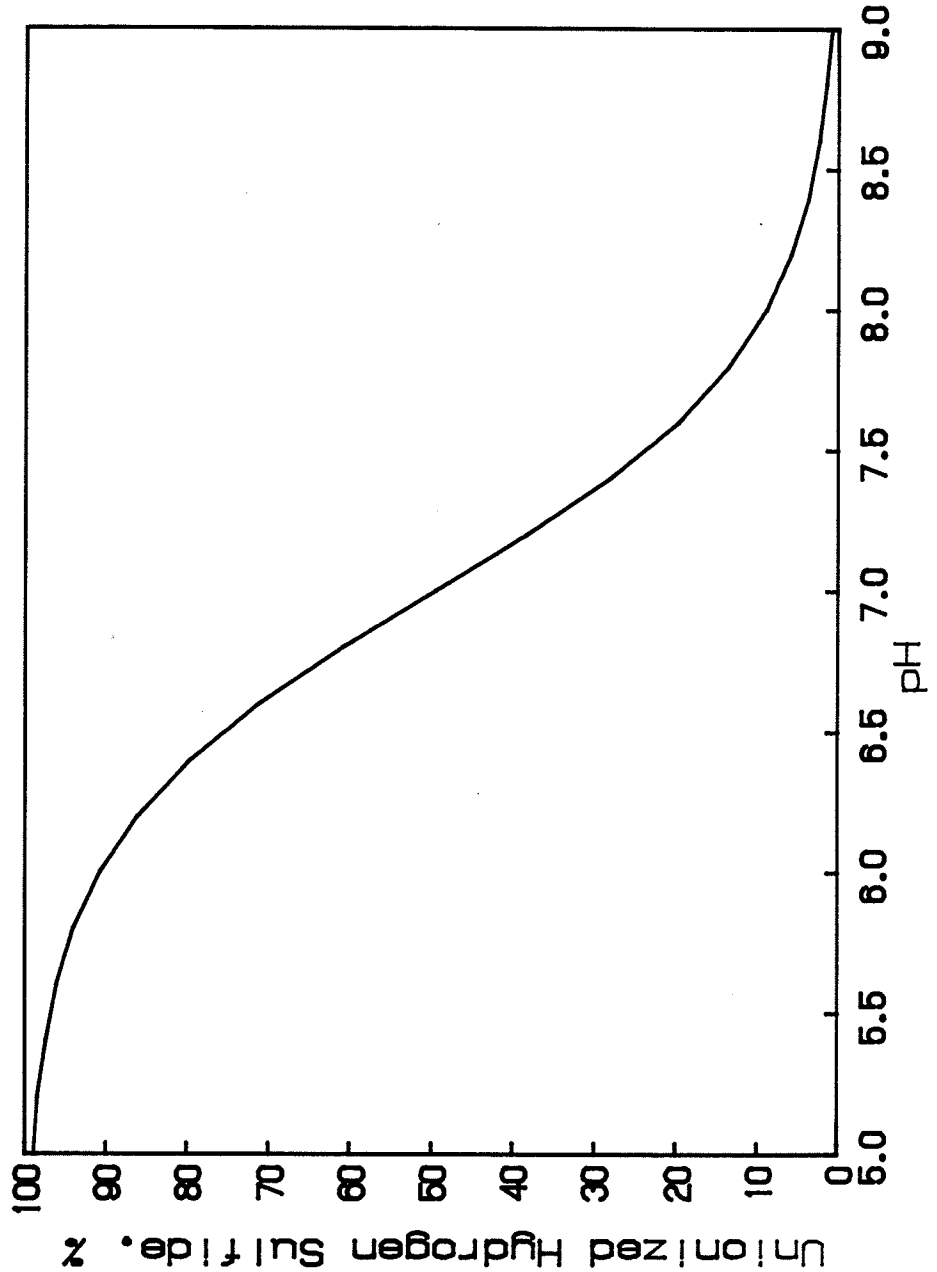


Figure 1.2 The percentage of unionized H_2S in relationship to pH.

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

pertain to engineered systems for the anaerobic treatment of high sulfate wastes.

Objectives:

a) determine the efficiency of anaerobic processes operating under similar loads with different incoming sulfate compositions and different residual sulfide concentrations as affected by the lack of or presence of gas stripping;

b) determine the extent of conversion of sulfates to sulfides;

c) establish the diversion of carbon flow from methanogenesis towards sulfidogenesis in the presence of increasing concentrations of sulfates;

d) compare the treatment of different carbon sources in the presence of high concentrations of sulfates;

e) divert the entire carbon flow towards sulfate reduction;

f) study the effects of a wide range of sulfide concentrations upon aspects of the anaerobic treatment of high sulfate wastes: lactose utilization, sulfate reduction, methanogenesis, and the carbon flow under conditions of high sulfates and varying concentrations of un-ionized H_2S.

Hypotheses:

a) carbon removal in a stripped sulfidogenic reactor would be comparable to carbon removal in a methanogenic reactor receiving the same carbon substrate;

b) carbon removal in acclimated unstripped sulfidogenic reactors would be comparable to carbon removal in stripped sulfidogenic reactors;

c) sulfate reduction in an anaerobic reactor would be independent of the form of carbon in the feed;

d) the entire flow of carbon in a stripped sulfidogenic reactor could be diverted in support of sulfate reduction and would result in greater than 85% carbon removal:

   i) a population of incompletely oxidizing sulfate reducing bacteria would be established;

   ii) acetoclastic sulfate reducing bacteria would outcompete methanogens for carbon resulting in a syntrophic culture of incompletely oxidizing and completely oxidizing sulfate reducing bacteria;

e) sulfide toxicity to the lactose utilizing, acetate utilizing, and sulfate reducing bacteria would be due to the concentration of un-ionized H_2S, not the concentration of total sulfides.

3. EQUIPMENT AND PROCEDURES

3.1 Continuous Flow Reactors

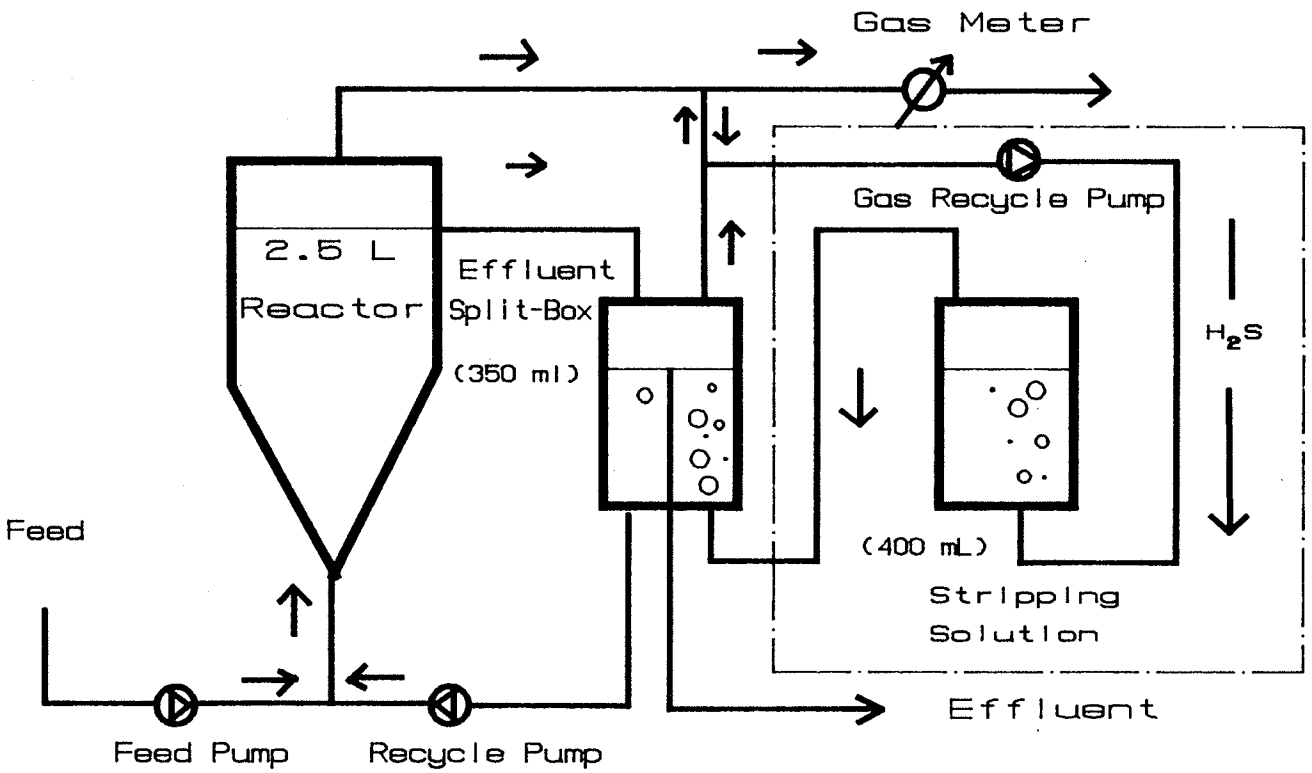
Figure 3.1 is a schematic diagram of 2.5 L anaerobic upflow sludge bed reactors maintained in a walk-in environmental chamber at 35°C. The bottom of the reactors consisted of 1 L plastic Imhoff cones; the tops consisted of 100 mm i.d. plexiglass tubes with bolted on caps. The assembly was manufactured in the machine shop of the Department of Civil Engineering. Recycle and gas lines were 3/8 in (9.5 mm) Tygon™ tubing.

Masterflex™ tubing pumps with 7015-21 stainless steel heads were used for recycle. Feed pumps were Masterflex™ tubing pumps with 7014-21 stainless steel heads. Tygon™ pump tubing failed frequently, therefore it was replaced with more durable silicon tubing. For reactors R9-R14, Norprene™ tubing was used. The Norprene™ tubing provided desired reliability and required minimal changes. Recycle pumps were adjusted to maintain an upflow velocity of 0.5 m/h in the reactors.

A gas recycle system (to strip H_2S from the recycle stream) was composed of Air-Cadet™ diaphragm pumps connected in series with an effluent split-box located in the liquid recycle line and with a container of H_2S absorbing solution (Figure 3.1).

The reactors were loaded with a flocculant sludge screened from an active anaerobic digester at the City of Winnipeg, North End Treatment Plant.

Figure 3.1 Schematic diagram of laboratory scale continuous-flow reactors, R1 - R14. Reactors R2, R4, R7, R9 - R14 included a gas stripping system as enclosed by the dashed line.



The sulfide absorbing solutions were zinc acetate, ferric chloride, NaOH, and ARI-311C™. ARI-311C™, a proprietary chelated iron compound which may be regenerated by aeration, was obtained from ARI Technologies, Inc., Palatine, IL USA. ARI-311C™ was developed as an efficient, cost-effective method of scrubbing "sour" gas from the petroleum industry. Zinc acetate and ferric chloride were used for R2, R4, and R7. ARI-311C™ was used in R9-R14 because of the capability for recharge, the efficiency of H_2S absorption, and ease of handling in comparison to ferric chloride.

Initially, reactors R1 (unstripped) and R2 (with stripping) initially were fed acetate, milk solids, and glucose. Cheese whey (whey contains 68 % lactose, galactose-β-1,4-glucose) was 50% of the COD from day 34 to day 119. After day 120 whey was the sole carbon and nutrient source. Increasing concentrations of sodium sulfate were added as the source of oxidized sulfur.

Initially, reactors R3 (unstripped) and R4 (stripped) were fed acetate, milk solids, and glucose. After day 120, cheese whey and spent sulfite liquor provided all the carbon to the reactors. Increasing concentrations of sodium sulfate were added as the source of oxidized sulfur. Spent sulfite liquor (SSL) was obtained from the Abitibi-Price paper mill at Pine Falls, Manitoba.

Reactors R5 and R6 were operated as methanogenic reactors and were fed the same substrate as R3 and R4 except that no sodium sulfate was added. Data from R5 were not used due to problems with the equipment which resulted in unstable operation.

Reactors R7 and R8 were fed 30% milk solids, 30% sodium acetate, 20% SSL, and 10% beef extract (COD basis). The stripped, sulfidogenic R7 was loaded with calcium sulfate in lieu of sodium sulfate at 0.5 - 0.7 g/L·d. The methanogenic R8 was fed an equivalent amount of calcium in the form of $Ca(OH)_2$.

Feed for all reactors was made fresh weekly and stored in 20L containers at 4°C.

Initially, gas production was measured with volumetric wet storage tanks with an acidified brine solution. Triton WRC Model 181 gas meters were used for the remainder of the research.

3.2 Batch Studies

Batch culture experiments were conducted in 100 mL Perfectum™ glass syringes with fitted plungers. The technique was originally presented by Sobkowicz and Klemm (1986). Specially designed stainless steel tips were machined such that one end fitted the Luer-Loc™ tips of the syringes and the other end accepted rubber gas stop septa. The plungers moved easily permitting maintenance of atmospheric pressure. The syringes were maintained in a 35°C water bath.

A stock solution of whey (2000 mg/L; 800 mg/L TOC) was prepared using tap water. Whey (50 mL), sludge (5 mL) and sodium sulfide were adjusted to a final volume (100 mL) after pH adjustment to 6.0, 7.0, and 8.0 (HCl, NaOH). Syringes were filled to contain 60 mL.

The stock basal medium (Tables 3.1 and 3.3) for methane toxicity (50 mL), sludge (5 mL), and sodium sulfide were adjusted

to a final volume (100 mL) after pH adjustment to 6.0, 7.0, and 8.0 (HCl, NaOH). Syringes were filled to contain 60 mL.

The stock basal medium (Tables 3.2 and 3.3) for sulfide toxicity to sulfate reduction (50 mL), sludge (5 mL), and sodium sulfide were adjusted to a final volume (100 mL) after pH adjustment to 6.0, 7.0, and 8.0 (HCl, NaOH). Syringes were filled to contain 60 mL.

Sodium sulfide was added from a stock solution to make 0, 50, 100, 250, and 1000 mg S^{2-}/L in the final 100 mL solution in all three experiments.

3.3 Analytical Procedures

Measurements for pH were made with a Radiometer pH meter. Calibration was checked daily. Gas analyses were performed on a Gow-Mac 550 gas chromatograph equipped with a thermal conductivity detector, stainless steel column, and Poropak Q media. Hamilton gas-tight syringes were used for injection. Helium was the carrier gas. A molecular sieve was used with nitrogen as the carrier gas for analyses of hydrogen. The VFA were analyzed on a Gow-Mac 750 gas chromatograph equipped with a borosilicate glass column filled with 80/100 mesh Chromasorb 101 media and equipped with a flame ionization detector.

Chemical oxygen demand (R1-R8) was performed by the closed reflux colorimetric method in accordance with APHA (1985).

Total organic carbon (R9-R14, batch studies) was performed using a Dohrmann DC-80 Total Organic Carbon Analyzer equipped with an ultraviolet detector, automatic sampler and integrator.

Table 3.1 Basal medium for methanogenic bacteria.

Compound	g/L
KH_2PO_4	0.4
NH_4Cl	0.6
$CaCl_2 \cdot 2H_2O$	0.3
$MgCl_2 \cdot 6H_2O$	0.8
KCl	0.6
NaCl	0.4
Yeast Extract	0.4
Acetic Acid	2.0
Trace Elements*	2.0 mL

Adjust pH to 7.0 with NaOH, fill to 1L with distilled water.

* See Table 3.3

(After Medium G, Postgate 1984)

Table 3.2 Basal medium for sulfate reducing bacteria.

Compound	g/L
KH_2PO_4	0.40
NH_4Cl	0.60
$CaCl_2 \cdot 2H_2O$	0.30
$MgCl_2 \cdot 6H_2O$	0.80
KCl	0.60
NaCl	0.40
Yeast Extract	0.40
Lactic Acid	2.20
Na_2SO_4	3.60
Trace Elements*	2.0 mL

(After Medium G, Postgate 1984)

* See Table 3.3

Table 3.3 Trace Element Solution

Compound	g/L
$FeCl_2 \cdot 4H_2O$	1.5
H_3BO_3	0.06
$MnCl_2 \cdot 4H_20$	0.10
$CoCl_2 \cdot 6H_2O$	0.12
$ZnCl_2$	0.07
$NiCl_2 \cdot 6H_2O$	0.025
$CuCl_2 \cdot 2H_2O$	0.015
$NaMoO_4 \cdot 2H_2O$	0.025

(After Postgate 1984)

Total and volatile non-filterable residue were performed in accordance with APHA (1985).

Sulfides from reactors R1-R8 were performed by iodometric titration in accordance with APHA (1985). For R9-R14, after day 40, sulfides analyses were performed using an Orion™ specific ion probe. Calibrations were made daily and continuously during a test run. Sulfates were analyzed on a Technicon Autoanalyzer in accordance with the automated procedure outlined in APHA (1985).

Lactose was analyzed on a Waters high pressure liquid chromatograph (HPLC) equipped with a Biorad HPX-85H column and refractive index detector.

4. RESULTS AND DISCUSSION

4.1 CONTINUOUS FLOW REACTORS (R1-R8)

4.1.1 Introduction

A series of eight reactors was used to study carbon removal, methane production, and sulfate reduction under conditions of differing carbon sources and differing sulfide stripping systems. Two reactors (R1 and R3) were operated without stripping, three were stripped (R2, R4, R7), and three (R5, R6, and R8) were operated in a purely methanogenic mode (no addition of sulfates). Unstripped reactors (R1, R3) were included to compare the performance of stripped and unstripped reactors with respect to sulfur reduction, carbon removal and methane production. Methanogenic reactors were included to compare sulfidogenic and methanogenic reactors with respect to carbon utilization and methane production.

Details of the equipment and procedures for this set of experiments are outlined in Section 3, Equipment and Procedures.

4.1.2 Reactors R1 and R2

Reactors R1 and R2 were fed sodium sulfate and a variety of carbon sources including cheese whey. During the first 50 days of the experiment, the carbon source included glucose, acetate, methanol, and milk solids. From day 50 to day 75, the carbon source was in the form of gradually increasing percentages of diluted cheese whey. After day 75, only diluted cheese whey was used as the carbon source.

The performance of reactors R1 and R2 fed diluted cheese whey and sodium sulfate is illustrated in Figure 4.1. The data are marred by variability in the feed composition. Selected average results for reactors R1 and R2 are presented in Tables 4.1-4.4.

At COD concentrations around 3 g COD/L and sulfate concentrations below 1 g S^{6+}/L, the performance of the stripped (R2) and unstripped reactor (R1) was comparable. Nevertheless, the stripped reactor still outperformed the unstripped by an average of 10%. Due to the testing procedures employed at the time, this 10% may have been due to unaccounted for COD demand by sulfides in the effluent.

Higher sulfate concentrations, applied after day 140, above 1.5-2 g S^{6+}/L, did not affect the stripped R2 but did induce a drop in COD removal from 80% down to 20-30% for the unstripped R1. The removal efficiency of the stripped reactor (R2) was more consistent than the unstripped reactor (R1) and stayed above 70% regardless of the variability in the incoming load. It began to drop from 80-90% COD removals at 6-7 g COD/L·d to 70+% at loads exceeding 8 g COD/L·d and sulfate loads above 2 g S^{6+}/L·d.

The difference in COD removal between R1 (56%) and R2 (88%) was concomitant with a decrease in the quantity of methane produced and an increase in the VFA of R1. Reductions in COD removal efficiency in R2 were accompanied by reductions in

Figure 4.1 COD percent removal, COD load and sulfur load for unstripped (R1) and stripped (R2) reactors.

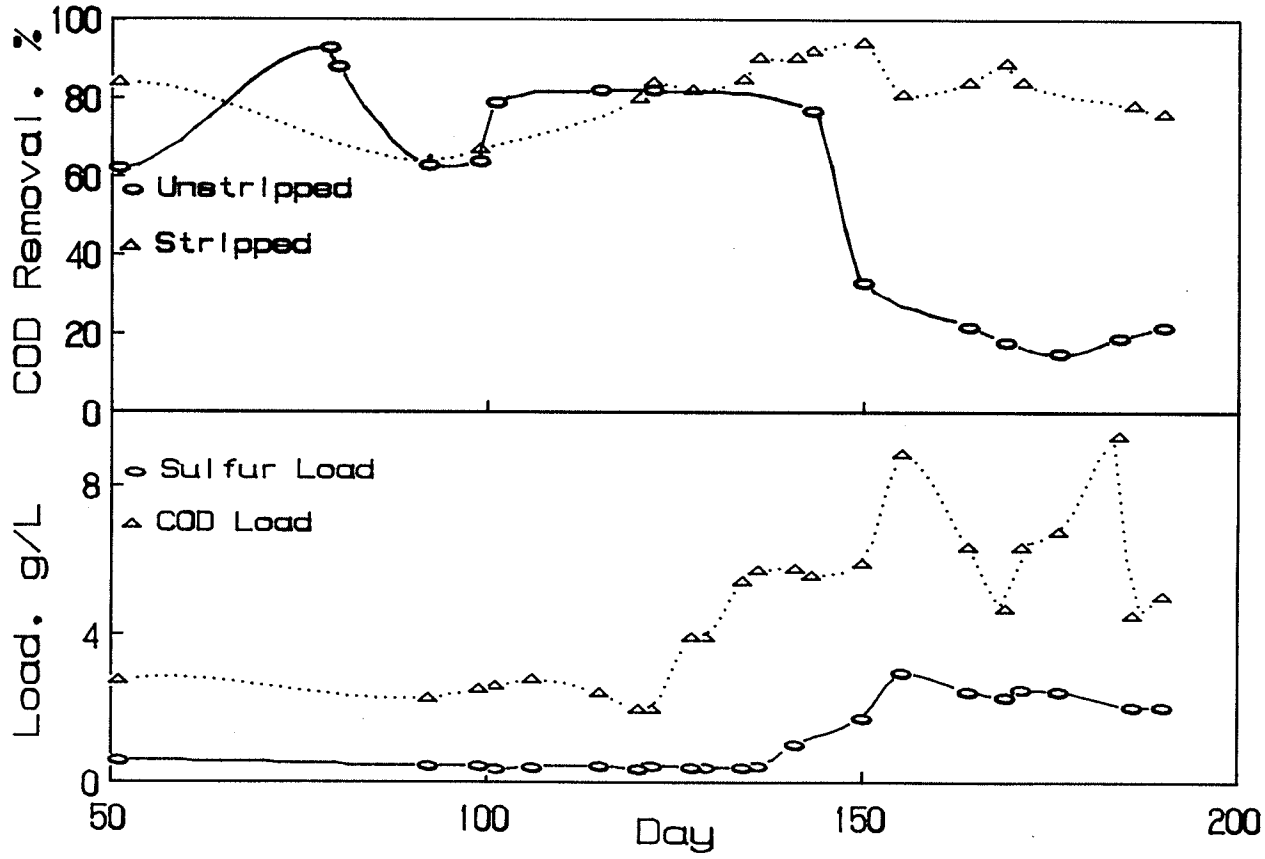


Table 4.1 Comparison of reactors R1 and R2 loaded with whey

Day since startup	Load (g/L·d)		Methane production (L/g COD removed)	Percent removal	
	COD	$S-SO_4$		COD	$S-SO_4$
			Reactor 1 (unstripped)		
115	2.0	0.48	0.09	82	63
120	2.1	0.4	0.02	80	95
122-129	4.1	0.4	0.02	68	95
143-144	6.6	0.8	0	75	93
176	7.0	2.7	0	16	
184	5.5	1.0	0	19	
			Reactor 2 (stripped)		
115	2.24	0.48	0.33	57	40
120	2.0	0.4	0.12	80	92
127	4.0	0.4	0.24	82	75
143	5.6	0.72	0.17	92	95
84	5.28	1.8	0.10	73	67

Table 4.2 COD percent removal comparisons between reactors for experimental days 122-171

	Reactor				
Parameter	1	2	3	4	6
Mean Removal (%)	56.2	88.4	33.9	47.8	51
Standard Deviation	21.9	4.7	13.4	20.7	15.4
Number of observations	11.	11.	10.	10.	11.

Table 4.3 Comparison by t-test of mean COD removals of experimental reactor pairs

Pair	t	P > t
R1, R2	4.756	0.001
R1, R3	2.389	0.04
R2, R4	6.064	0.001
R3, R4	1.783	0.21
R4, R6	0.399	0.7

Note: R1 = unstripped fed whey and sodium sultate; R2 = stripped fed whey and sodium sulfate; R3 = unstripped fed whey, sodium sulfate, and SSL; R4 = stripped fed whey, sodium sulfate, and SSL; R6 = methanogenic fed whey and SSL.

Table 4.4 Sulfides in the effluent (mg S^{2-}/L)

Day since startup	Reactor 1	2		3	4		5	6
6	39	55		66	31		10	19
8	86	124		94	10		10	0
11	247	180	(0.16)	128	80	(0.12)	24	9
16	156	72		160	97		28	16
51	177	0		112	0			
141	150	11	(0.56)	88	31	(0.56)	25	35
169	89	28	(1.03)	214	66		12	8
174	488	324		196	95			
181	60	261		143	73			
183	42	90		197	52		85	
184		224		120	48		28	36
186	28	180		40	156		96	
Average	142	146		120	74		32	26

Note: Sulfur recovered in gas (g/L·d) is shown in parentheses.

the quantity of methane produced by the reactor as shown in Table 4.2. A t-test of the means indicated a significant difference in the COD removal from the reactors at the 0.001 level of significance (Table 4.3).

Analyses of gas production data indicated increased methane generation as sulfide production became less efficient. A relatively high methane generation by R2, when compared with that of the unstripped R1 is illustrated in Table 4.1. That suggests an increasing inhibition of methanogens in R1 with time as both the COD and sulfate load increased.

Long-term sulfide generation data (Table 4.4) for these two reactors shows that the sulfide production gradually increased to reach 1.2-1.36 g S^{2-}/L·d for R2 which coincided with a COD removal rate of 5.36 g COD/L·d removed at 88% COD removal efficiency. Sulfide production in R1 was unsteady and decreased gradually to less than 0.5 g/L·d.

The deterioration of performance of R1 in comparison with R2 was accompanied by an accumulation of volatile fatty acids (VFA) which in R1 increased gradually from 300 to 2500 mg/L. Reactor R2 exhibited a steady VFA concentration below 300 mg/L and a methane production in excess of that observed in R1, which would indicate the dependence of appreciable COD removal efficiency upon the symbiotic relationship between the SRB and MPB. Consequently, one might conclude that the inhibition due to sulfide accumulation primarily affected the VFA conversion phase rather

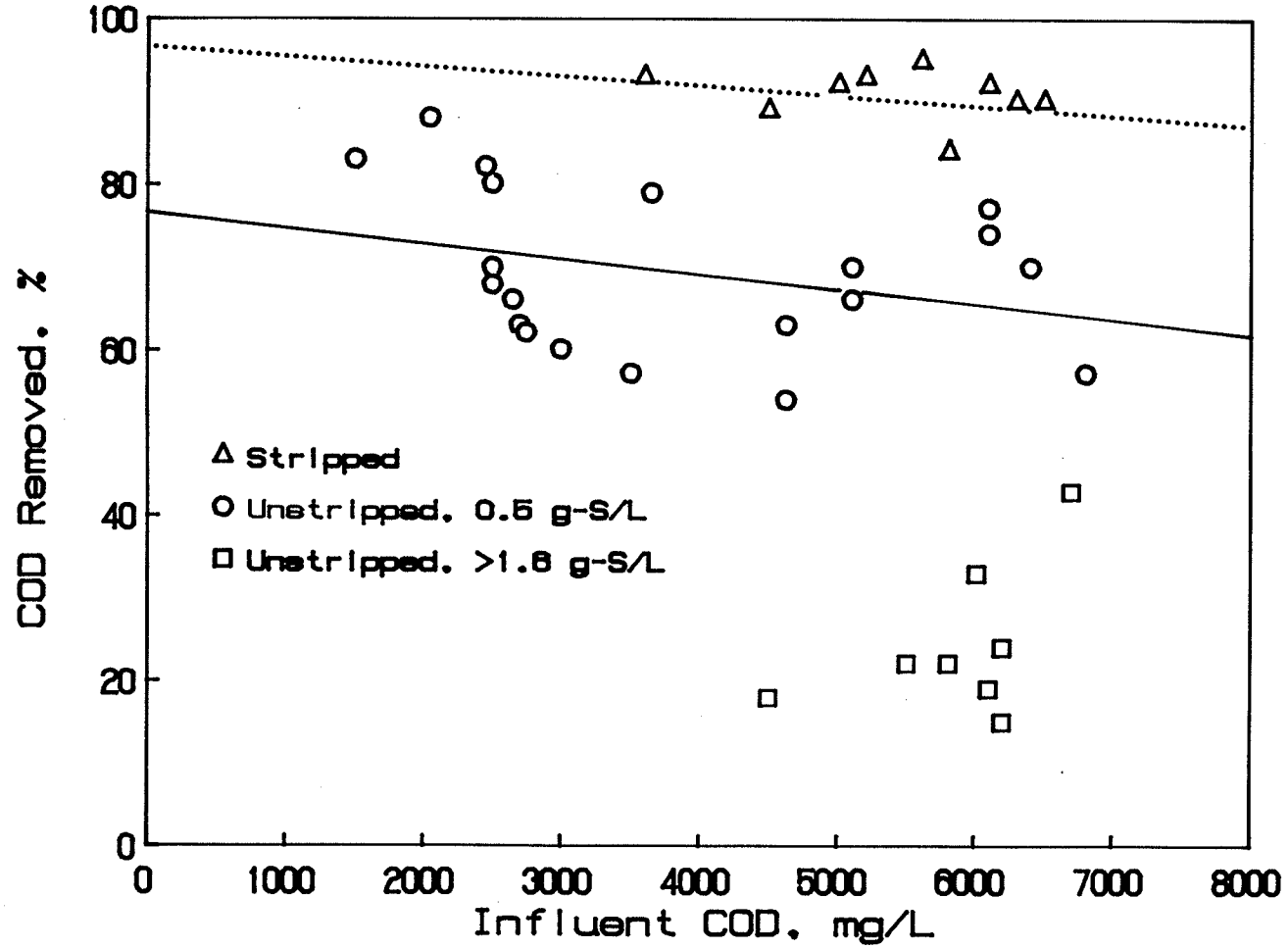
than the acid-generating or acidogenic phase of the anaerobic biodegradation pathway.

The efficiency of COD removal for the stripped reactor was relatively constant, regardless of COD or sulfate concentration (Figure 4.1). Although less than 33 % of the applied sulfur was recovered from the gas which would indicate that only a portion of the applied sulfates were reduced, less methane was produced when more sulfur was recovered from the gas which shows that organic carbon removal was diverted from the production of methane to sulfate reduction.

A further relationship between COD % removal, carbon source, and sulfur load is shown in Figure 4.2. An increase in COD concentration, concomitant with the increase in sulfur load resulted in a very significant decrease in COD removal in the unstripped R2 (squares in Figure 4.2). At lower influent S_o/COD_o ratios (circles), the COD removal in R2 trailed the stripped R1 by about 15 %. In the absence of whey, the performance was similar to that of the stripped reactor. When the carbon source was changed to spent whey, and when the sulfur concentration was increased (high S_o/COD_o ratio), the COD % removal for the unstripped reactor (with whey) decreased to 20 %. Effluent total sulfides in R1 were 39-488 mg S^{2-}/L (equivalent COD = 78-976 mg/L) while in R2 the sulfides were 11-324 mg/L (equivalent COD = 22-648 mg/L).

In the stripped reactor, poor COD removal and negligible gas production were observed during periods of high concentrations of S^{2-}. The pH in both reactors was at 7.8-8.2 (self-established) such

Figure 4.2 COD removal in stripped (R2) and unstripped (R1) reactors in relationship to the influent COD. The unstripped reactor is shown for two different sets of ranges of influent sulfur concentrations.



that the fraction of free H_2S in the reactors normally was no more than 5-14% of the total sulfides. Maximum conversion of sulfates to sulfides was 0.5 g S^{2-}/L.d for R1 and 1-1.2 g S^{2-}/L.d for R2 equipped with stripping.

The relationship between mL methane produced per gram of COD removed and the ratio of influent oxidized sulfur to influent COD (S_o/COD_o) in the stripped reactor is illustrated in Figure 4.3. This shows that, up to a S_o/COD_o = 0.1, the specific production of methane per gram of COD removed decreased with increases in S_o which illustrates a diversion of electron flow from methane production to sulfur reduction. Above an S_o/COD_o = 0.1, the specific production of methane was constant at 0.16 L/g COD_r. This shows that the diversion of carbon flow from methane production to sulfur reduction proceeded only as long as there was a sufficient quantity of electron donor. Above S_o/COD_o = 0.1, the electron flow was no longer diverted to sulfate reduction. This means that in a stripped sulfidogenic reactor, when the $S_o/COD_o > 0.1$, any further addition of oxidized sulfur will not affect the production of methane.

4.1.2.1 Carbon removal and pH in R1

The COD percent removal for the unstripped R1 was plotted against the corresponding values of pH as shown in Figure 4.4. Increased COD percent removal corresponded to increased pH values. In Figure 4.5, the reactor pH is inversely related to the incoming COD load. Increases in pH were associated with

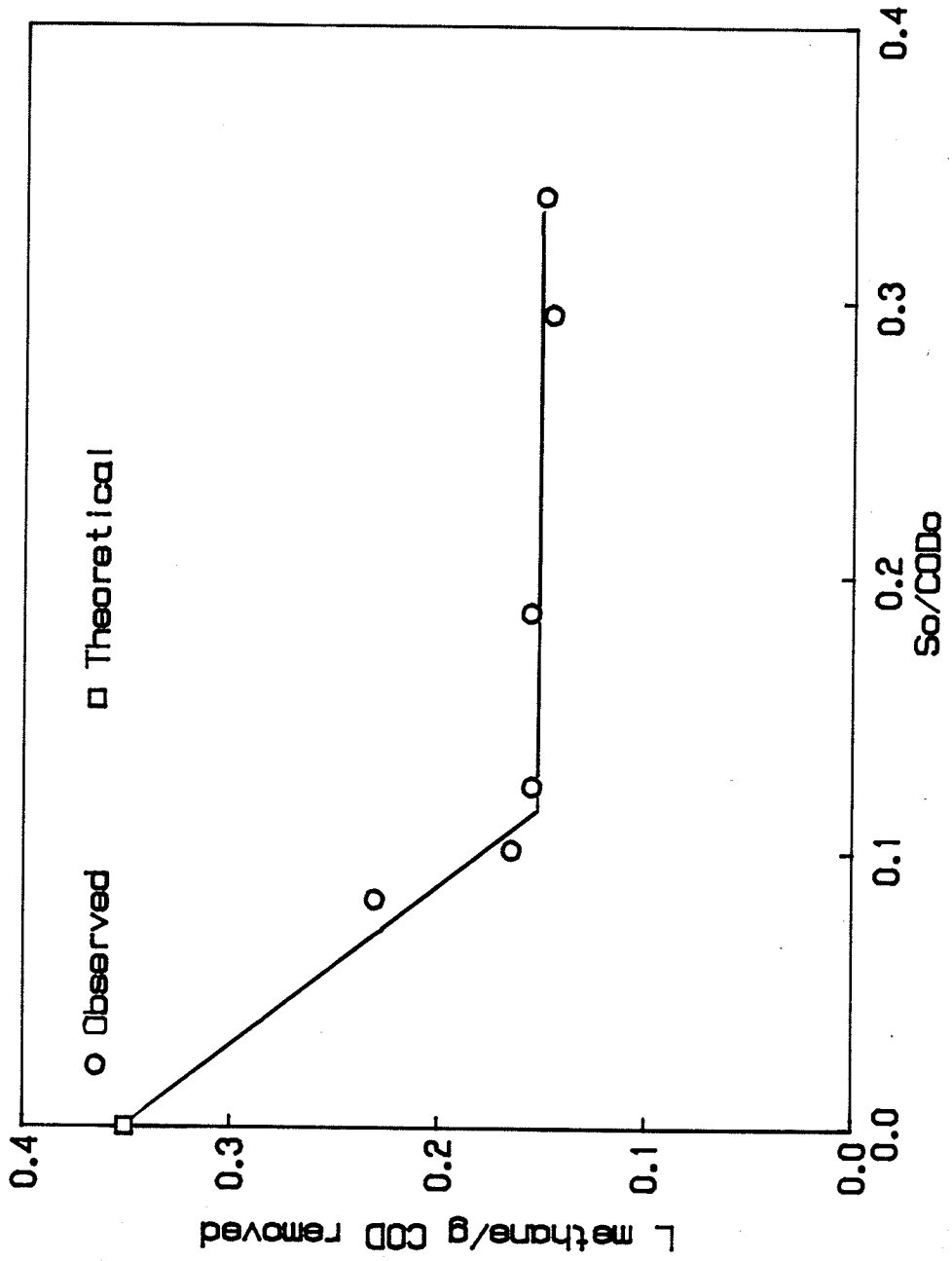


Figure 4.3 Specific production of methane in relationship to the ratio of influent S_o/COD_o.

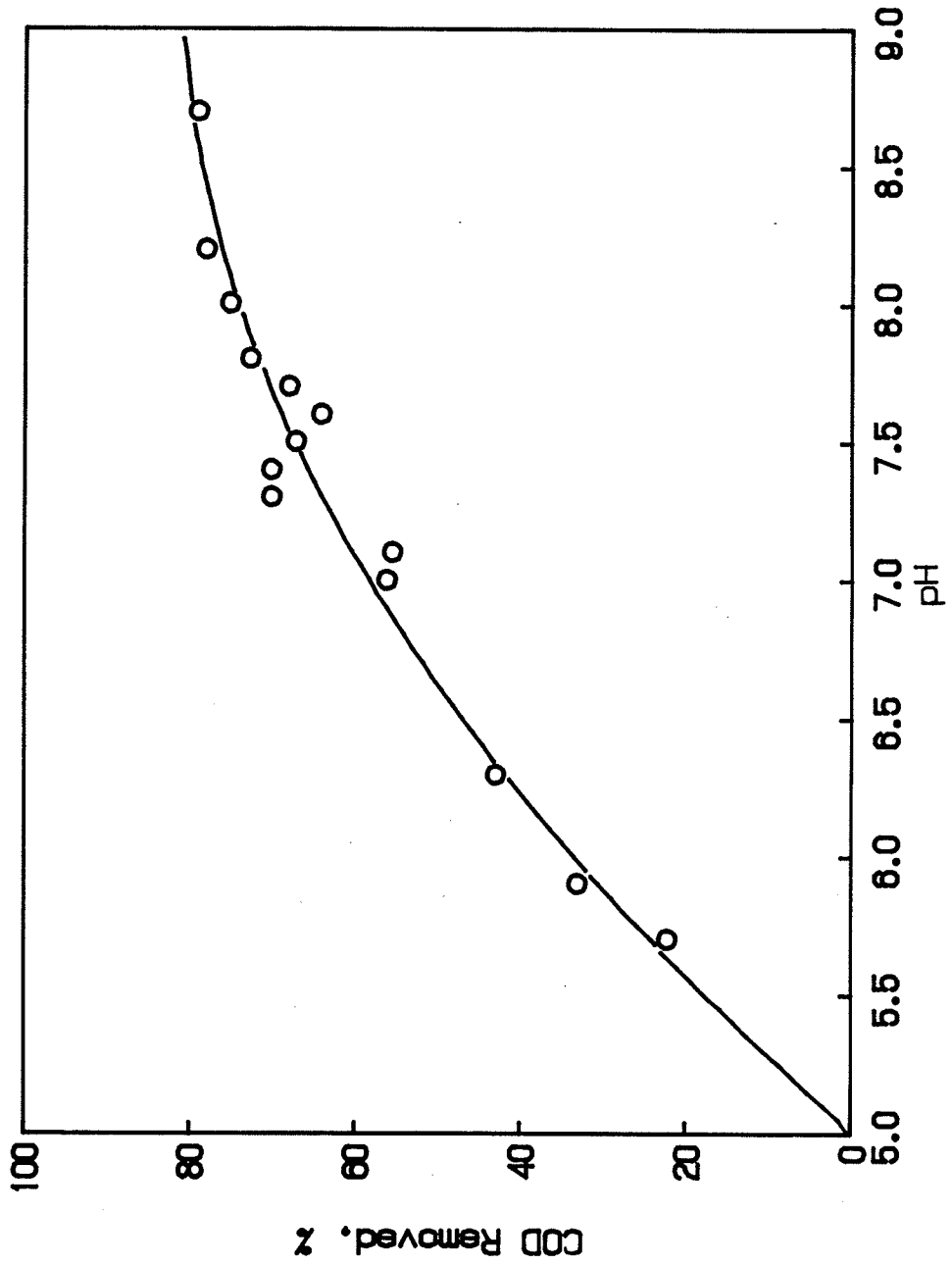


Figure 4.4 The efficiency of COD removal in an unstripped reactor (R1) as a function of pH.

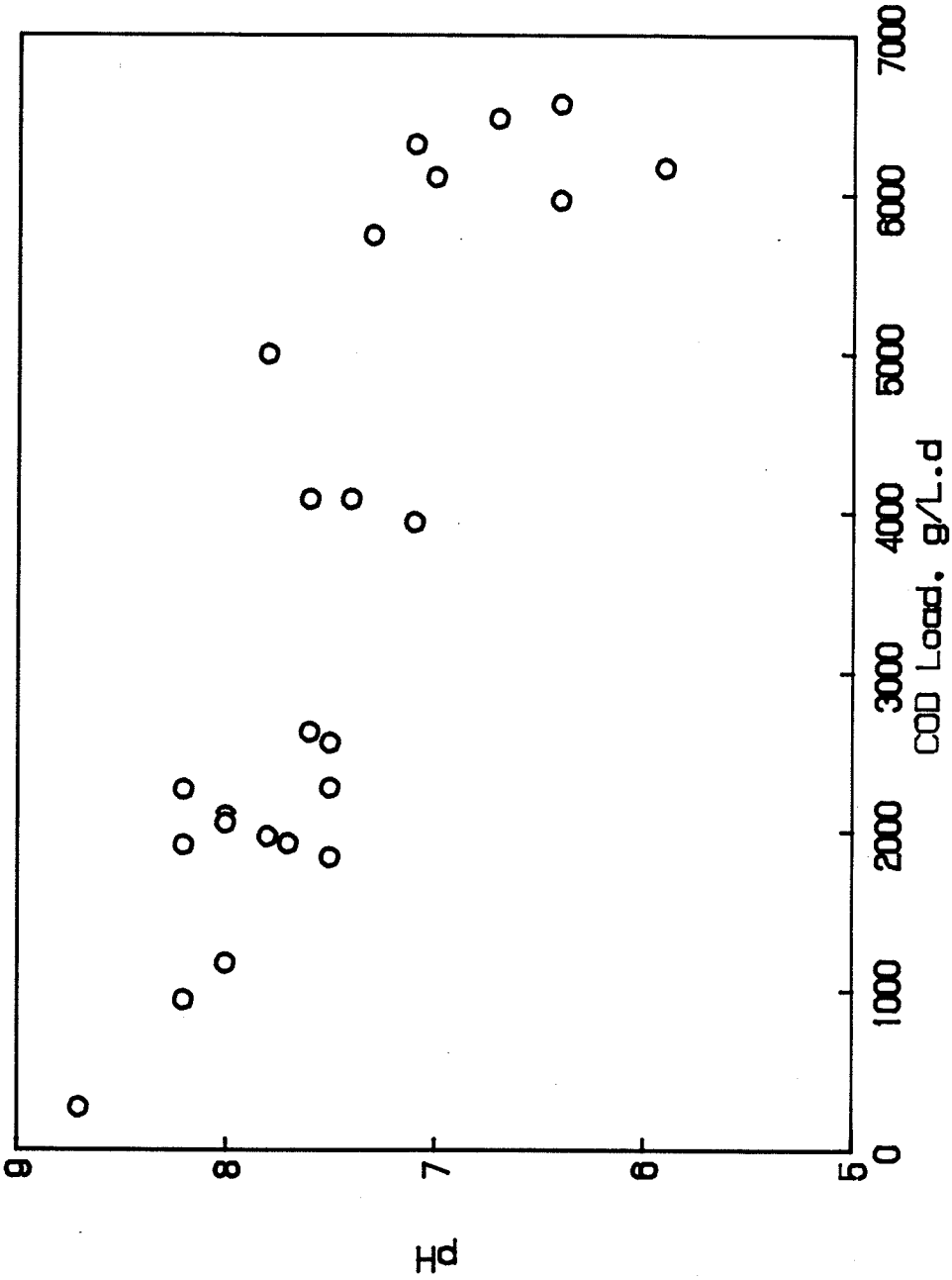


Figure 4.5 The relationship between pH and COD load in an unstripped reactor (R1).

decreases in COD load and vice versa. The decrease in pH with increasing COD load corresponded with a decrease in COD percent removal and an increase in VFA concentration in the effluent.

One possible explanation would be that a decreased influent COD meant that less carbon was available for sulfate reduction thereby resulting in diminished sulfide production. At the minimum influent COD concentration (2000 mg COD/L), if only lactic acid were oxidized, then no more than 330 mg S^{2-}/L would have been generated. At pH 8.0, this would have been equivalent to 33 mg/L un-ionized H_2S. At the maximum COD concentration (6500 mg/L) up to 1081 mg S^{2-}/L could have been generated. At pH 7.0, this would have been 540 mg H_2S/L which is in excess of the concentration of un-ionized H_2S considered to be toxic to the methanogenic process (Lawrence et al. 1965; Kroiss and Plahl-Wabnegg 1983).

A second explanation might be that increased pH values resulted in lower un-ionized H_2S concentrations thereby permitting increased COD removal efficiency due to increased carbon removal by methanogens. Sodium carbonate was added to the feed but was not regularly added to the reactors to maintain control of pH which may explain the range of pH values associated with the COD load as shown in Figure 4.5. Variations in pH due to the addition of sodium carbonate to the feed not withstanding, there was a distinct drop in reactor pH with increases in COD load. This drop in pH was accompanied by a decrease in COD removal efficiency

and an increase in effluent VFA. Methane production was nonexistent below pH 7.5.

Third, increased pH values resulted in diminished fractions of un-ionized H_2S in the reactor thereby resulted in diminished sulfide toxicity to methanogens. Improvements in the efficiency of organic carbon removal were accompanied by increased reactor methane production. Methane production by the unstripped reactor was minimal although it increased with pH (Figure 4.6).

The decrease in COD % removal at increased COD loads was not just a function of decreased reactor pH since decreases in COD % removal occurred above pH 6.5. However, below pH 6.5, pH was also a factor.

When methanogens were not inhibited by the stripping of sulfides from the reactor, acetate was converted to methane. When acetate was removed from the reactors, in the absence of other VFA, the reactor pH was dependent upon the carbonic acid buffering system and generally stabilized between pH = 7.5-8.3. When sulfide concentrations increased, the methanogenic activity decreased resulting in decreased carbon removal and decreased pH due to increased VFA concentrations.

A portion of the carbon flow in a stripped sulfidogenic reactor (R2) was diverted from methanogenesis to sulfate reduction. The diversion of carbon flow was dependent upon the concentration of sulfates in the feed.

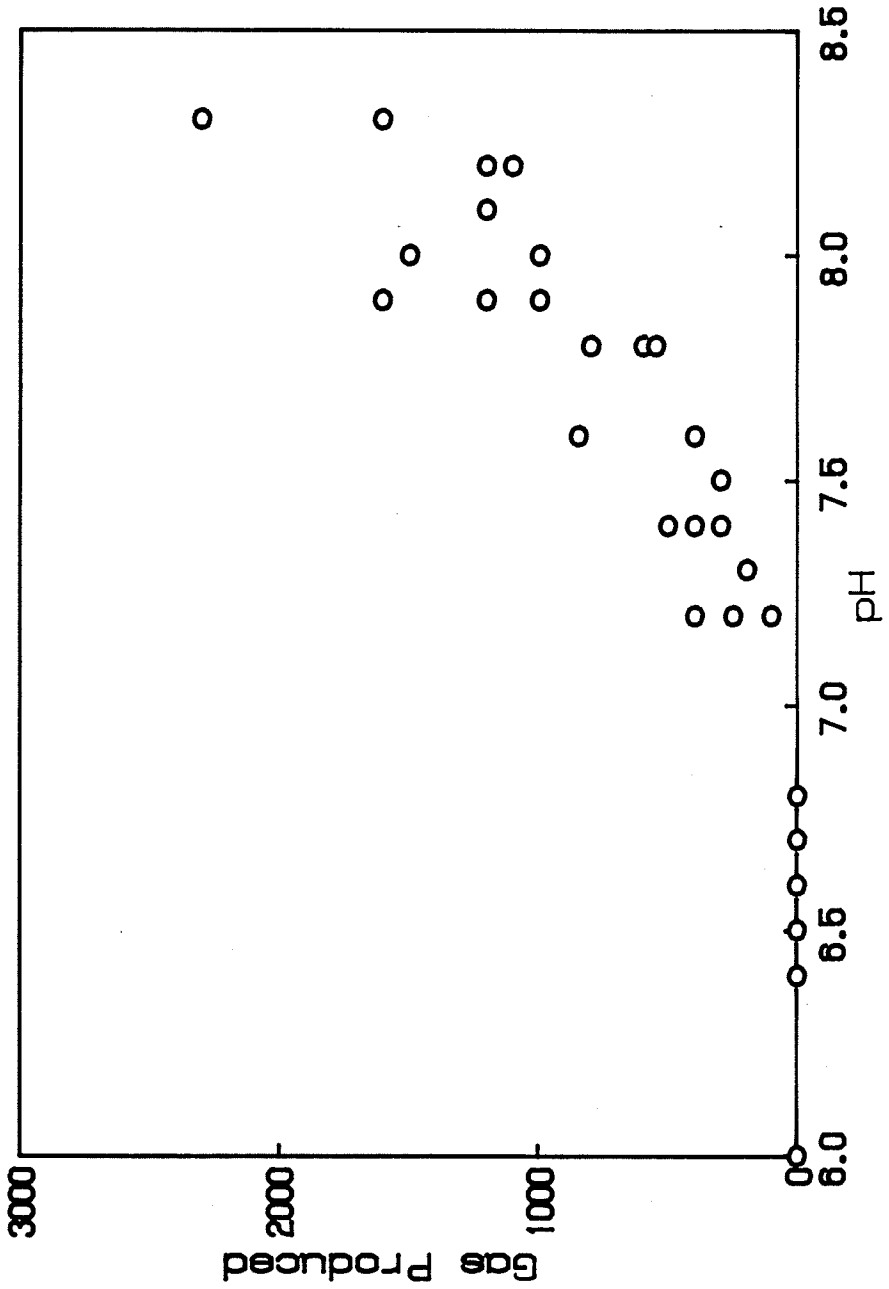


Figure 4.6 Gas production (mL) in an unstripped reactor plotted against pH.

4.1.3 Reactors R3 and R4

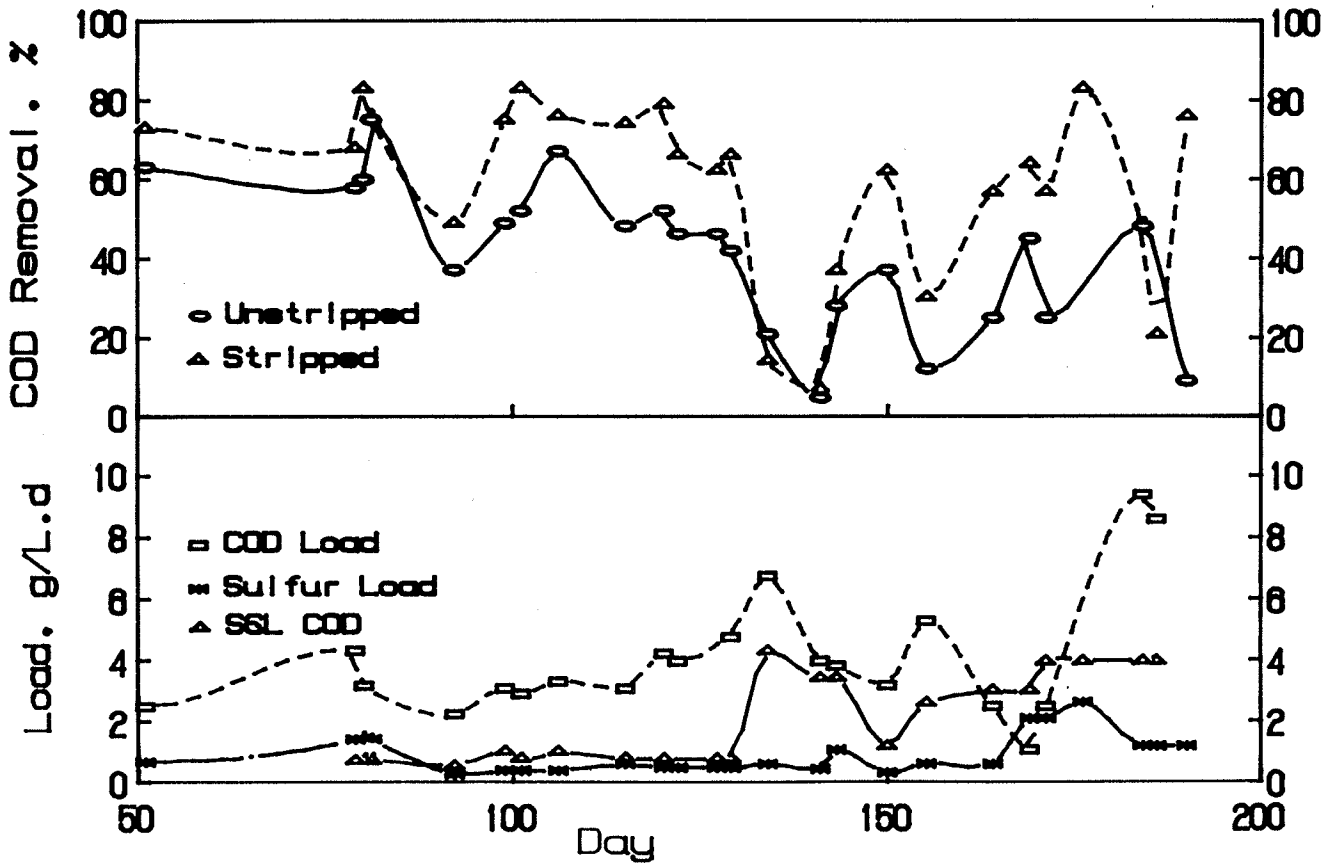
Whey, an increasing concentration of strained SSL, and increasing concentrations of sodium sulfate were fed to R3 and R4 (Figure 4.7). A comparison of performance of the unstripped R3 with the stripped R4 generated results similar to the R1 and R2 comparisons: i) gradual deterioration of the performance of R3 with time as the COD and SO_4 loads increased and ii) relatively steady performance of R4 as the COD and SO_4 loads increased.

The deterioration in R3 versus R4 was not as pronounced as R1 versus R2 where R3 regularly provided a removal 10-25% less than R4. This difference between the two pairs of reactors could be attributed to differences in substrate: R1 and R2 were fed pure whey and sulfate during the latter half of the experiment whereas R3 and R4 were fed increasing portions of SSL with concomitant decreasing portions of whey.

At influent sulfur concentrations below 1200 mg S^{6+}/L, the removal of COD was 10% lower in the unstripped (R3) than in the stripped (R4) reactor. For sulfur concentrations in excess of 1200 mg S^{6+}/L, the difference was greater than 20% even without increased SSL participation in the load. Conceivably, part of this difference may have been due to the presence of sulfides in the effluent although part was due to increased concentrations of measurable VFA in the unstripped reactor, R3.

The total sulfide concentration in the effluent varied from 40-214 mg/L with an average of 120 mg/L in the unstripped reactor and from 0-197 mg/L, with an average of 74 mg/L in the

Figure 4.7 COD removal, COD load, sulfur load, and SSL-COD load for unstripped (R3) and stripped (R4) sulfidogenic reactors fed whey and spent sulfite cooking liquor (SSL).



stripped reactor (R4). At COD loads below 2.4 g/L·d and S_o/COD_o below 0.33, the unstripped reactor (R3) yielded a 60% COD removal. A COD_o = 2.4 g/L and S_o/COD_o = 0.33 no more than 300 mg/L S^{2-} would have been reduced. At pH 7.0, this is 150 mg/L H_2S which explains the success with unstripped reactors at low values of COD_o. This is in apparent contrast with the study of Kroiss and Plahl-Wabnegg (1983), who predicted H_2S toxicity at an influent S_o/COD_o above 0.1.

The unstripped R1 and R3 were successfully run at a S_o/COD_o ratios up to 0.9 with problems beginning above 0.9. For R4 (stripped), the S_o/COD_o ratio essentially had little or no effect on removal as long as the stripping system continued to function. However, when the stripping system failed, total sulfides in the reactor increased with a resulting decrease in COD removal from the reactor. The concentrations of sulfides (Table 4.4) in the reactor effluents were considerably greater than those generally considered inhibitory (200 mg/L) to the biomass. However, in general, the COD removal was inversely proportional to the sulfide concentrations in the effluent.

The VFA concentration in R4 responded quickly to changes in the influent load and the S_o/COD_o. With the normal level between 150-350 mg/L as acetate, the VFA concentration increased to 800 mg/L acetate on day 150 following a sudden doubling of the load on day 138 with a concomitant increase in SSL participation by almost 2 g COD/L·d. The initial difference in the performance of R3 and R4 may be attributed to the fact that the faster growing

Desulfovibrio species (μ_m=0.33d^{-1}) break down lactate to acetate. The acetate degrading microorganisms (MPB, μ_m=0.1d^{-1}; SRB, μ_m=0.13d^{-1}) have lower growth rates thereby requiring a longer acclimation period to develop a sufficiently large population to degrade the acetate.

4.1.4 Comparison of stripped sulfidogenic reactor (R4) methanogenic reactor fed whey and SSL (R6)

Figures 4.8 and 4.9 show the performance with respect to time of a stripped sulfidogenic reactor fed whey, SSL and sodium sulfate (R4) and a methanogenic reactor fed whey and SSL (R6). Performance data from the methanogenic reactor (R5) are not included here as was noted in Section 3, Equipment and Materials. In general, reactors R4 and R6 performed similarly in terms of COD removal and residual levels of VFA although the performance of the sulfate loaded reactor (R4) was less consistent than the methanogenic reactor (R6).

The overall average COD removal was 71% for the methanogenic reactor and 67% (until day 120) for the sulfidogenic reactor at a working load of 2-4 g COD/L.d, when 30% of the COD was contributed by SSL. The difference in performance was attributable to COD demand by sulfides in the effluent of the sulfidogenic reactor (R4). A t-test of the mean COD removal from these reactors (Table 4.3) showed no significant difference between the two reactors.

Figure 4.8 COD removal, COD load, and SSL-COD load for a stripped sulfidogenic reactor (R4) and a methanogenic reactor (R6) fed whey and sulfite cooking liquor.

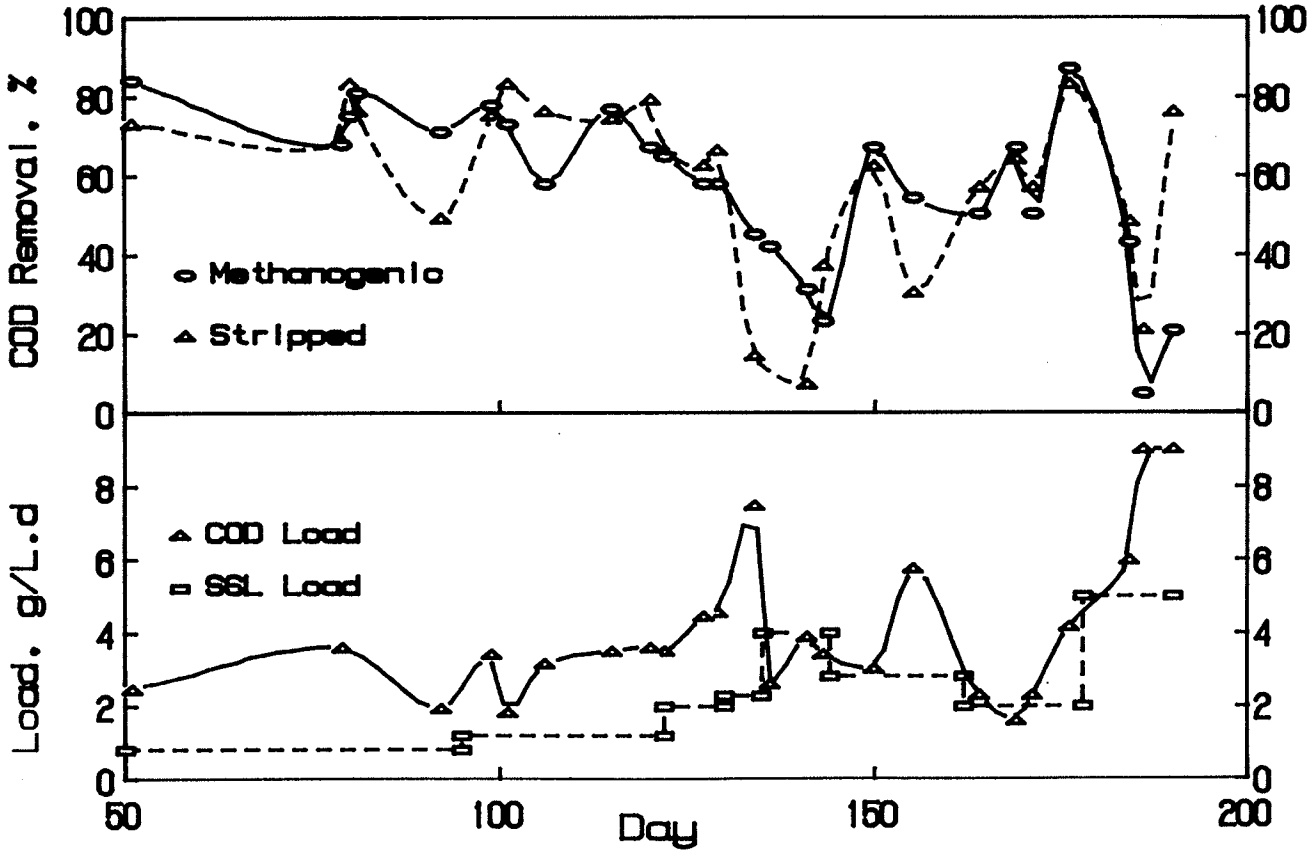
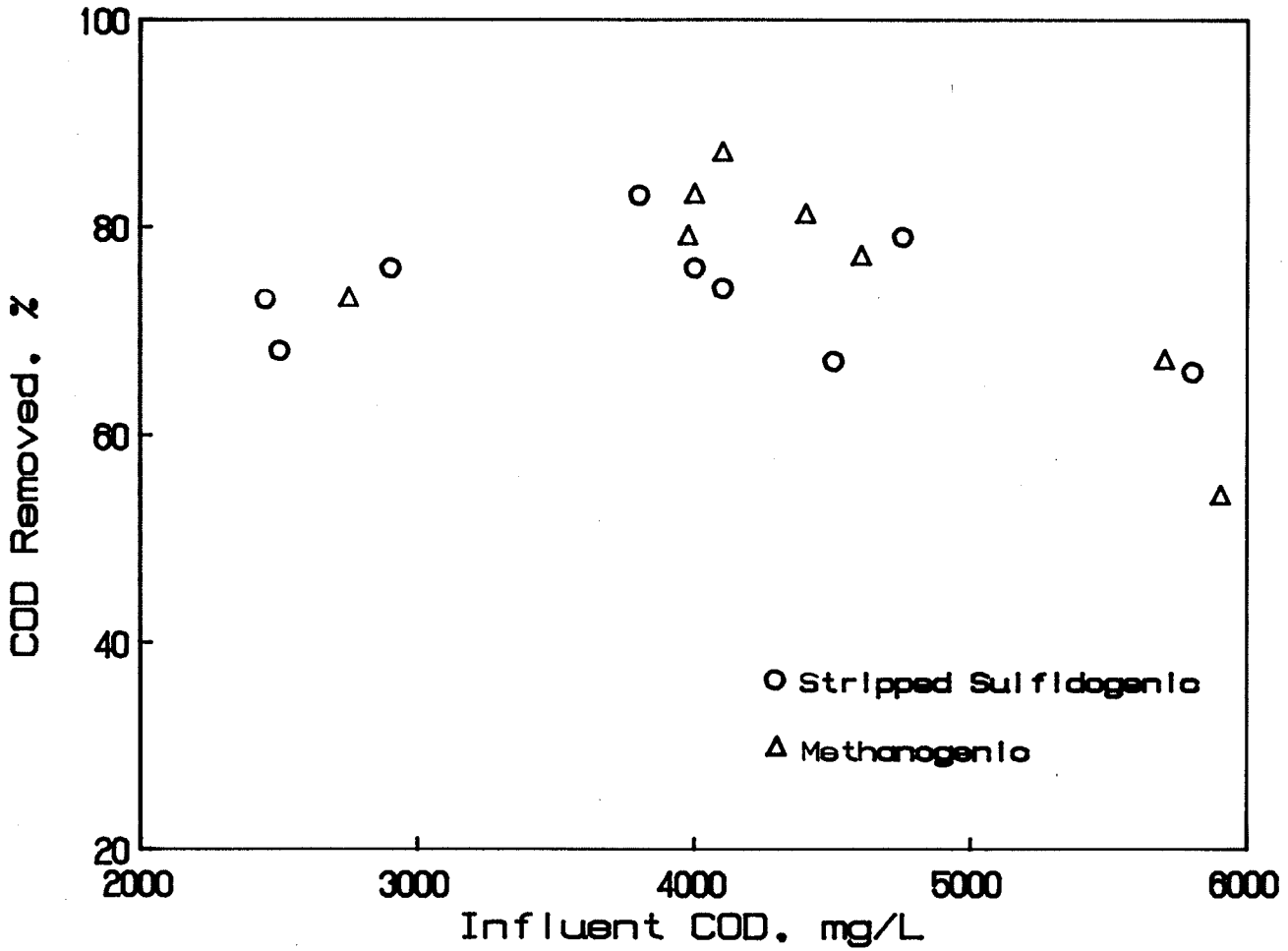


Figure 4.9 COD removal efficiency in relationship to influent COD load for a stripped sulfidogenic reactor (R4) and a methanogenic reactor (R6) fed SSL and spent cheese whey. The sulfidogenic reactor was additionally fed sodium sulfate.



The rapid drop in percent COD removal in both reactors coincided with the increased COD contribution by SSL. The S_o/COD_o ratio in the sulfate loaded reactor (R4) varied from 0.1 to 0.5. The VFA concentration in the stripped sulfidogenic reactor responded quickly to changes in the influent composition. With normal VFA levels between 150-350 mg acetate/L, the VFA increased to 800 mg/L acetate on day 150 following a sudden doubling of the load on day 138 with a concomitant increase in SSL participation by almost 2000 mg-COD/L. The level of VFA in the methanogenic reactor normally was less than 200 mg/L acetate.

The performance of both reactors decreased with increasing influent COD concentrations (Figure 4.9). This decrease in performance was due to the inhibitory nature of SSL in the feed since the decreases in performance occurred concomitantly with increases in the percentage of SSL in the feed.

Following the sudden increase in load and SSL, the VFA concentration increased to 650 mg/L. The level of VFA in the stripped sulfidogenic reactor R4 returned to normal much more rapidly (4-5 days) than in the case of the methanogenic reactor R6 (10 days). This may be an indication of SRB utilizing the VFA syntrophically with the MPB as well as reflecting the much higher growth rates of the SRB.

The average volumes of methane produced were 140 mL CH_4/g COD removed for the stripped sulfidogenic reactor R4 and 243 mL/g for the methanogenic R6. Both values were less than 350 mL/g COD (O°C, 760 mm Hg) removed which would be

expected from a methanogenic reactor fed an easily degradable substrate such as acetate or methanol. These different specific yields of methane proved that the mechanisms responsible for COD removal in the two reactors were different since their COD removals were comparable.

The COD removal efficiency was comparable in the methanogenic and sulfidogenic reactors. However, methane production was different thereby reflecting a diversion of the carbon flow from methanogenesis to sulfidogenesis in the stripped sulfidogenic reactor. The performance of the methanogenic reactor was more stable due to difficulties in maintaining steady-state operation with the sulfide stripping unit in R4.

4.1.5 Comparison of reactors R7 and R8

Figure 4.10 shows the performance of a stripped sulfidogenic reactor (R7) and methanogenic reactor (R8) which were part of the earliest phases of the research. With similar COD loads, the methanogenic reactor (R8) performed better than the stripped sulfidogenic reactor (R7). After day 140, the performance was comparable, suggesting that it took the sulfidogenic reactor longer to acclimate.

The feed for these reactors contained few precursors for lactic acid which could account for the high removals attained after initial acclimation - (over 90% COD removed at 4-6 g/L.d). The $CaSO_4$ fed to R7 was 0.7 g S/L.d, and the S_o/COD_o ratio was 0.14. If all the influent carbon were fermented to lactate, then all or nearly all the available S^{6+} should have been reduced to S^{2-}.

Since this was not the case, it must be concluded that only a portion of the carbon was fermented to lactate and the rest to other compounds such as acetate.

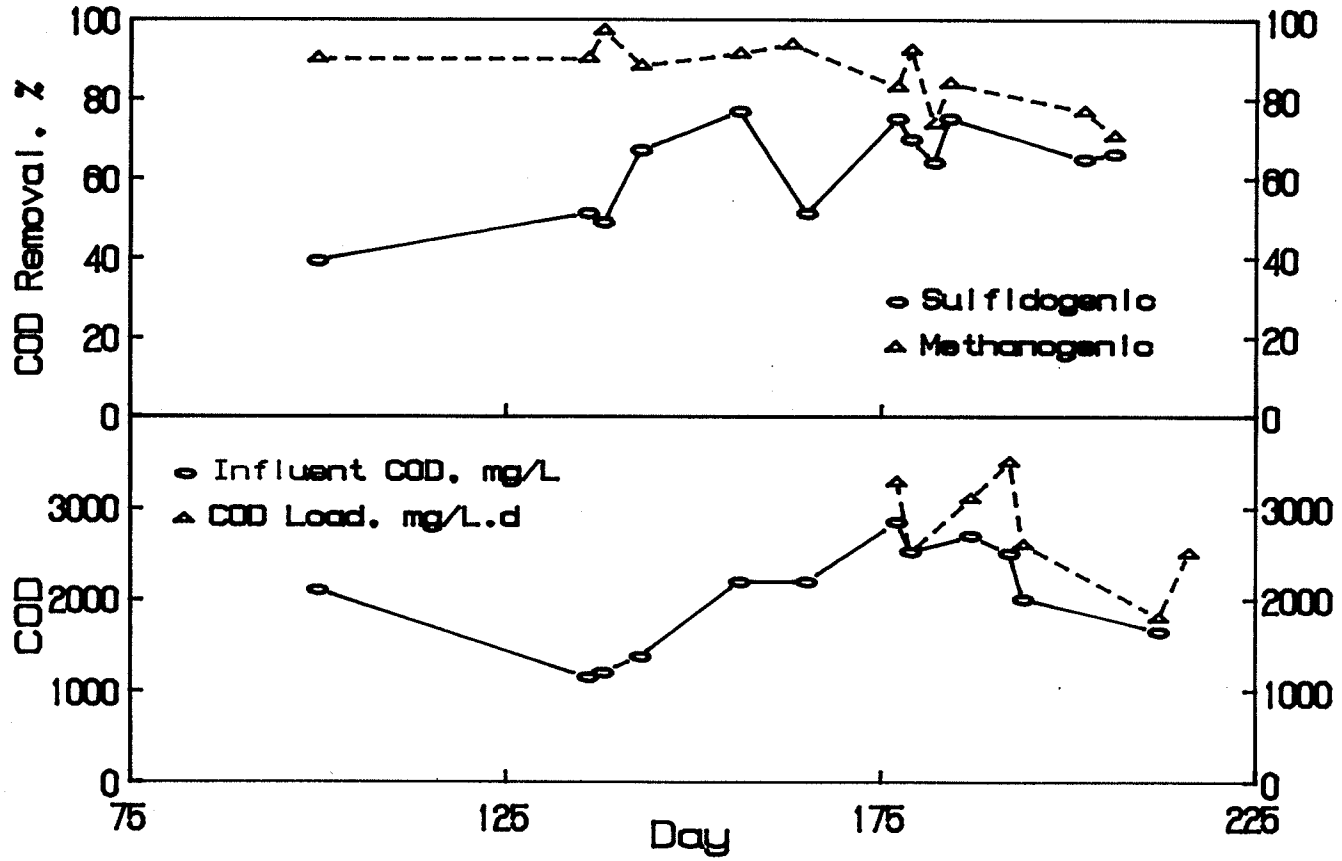
4.1.6 Reactor Stripping

Stripping of the reactor contents was found to be of significant importance to maintain proper conditions for syntrophic generation of methane and hydrogen sulfide in the flow-through reactors. When the stripping system in reactors R2 and R4 failed, there was an accumulation of C2 - C5 volatile acids and a loss of methane production.

It appeared that the microorganisms at the bottom end of the degradation chain (acetoclastic), were the most susceptible to upsets with methane generation being the most sensitive indicator. Methanogenesis gradually returned after the stripping was resumed. This was demonstrated in the initial weeks of the R7 and R8 runs where the difference in COD removal was closely correlated to the excess acetate level in the effluent from R7.

The effects of stripping of sulfides from the reactor contents are visibly demonstrated in Table 4.5 where a sulfate loaded anaerobic sequencing batch reactor was operated without stripping until day 60, then stripping into zinc acetate was turned on (stripping was with gas recycled from the stripper as in Figure 1.3). The data are from a DLA (1982a) suspended growth study in an anaerobic sequencing batch reactor treating piggery wastes enriched with acetate (30% TOC basis) and gypsum sludge residue from a phosphate fertilizer industry.

Figure 4.10 Influent COD concentration and COD removal efficiency for a stripped sulfidogenic reactor (R7) and a methanogenic reactor (R8) fed acetate, milk solids, SSL, and beef extract. Reactor R7 was additionally fed $CaSO_4$.



In the study reported here, conversion of sulfates to sulfides depended upon the overall condition of the system and always coincided with appreciable COD removal. In stripped reactors, it was coincidental with high methane generation. In the DLA (1982a) work reported above, the removal of sulfates was greater than 95%, with organic carbon removal greater than 98% SOC although other DLA (1986) work indicated carbon removal rates as low as 40% removal and low rates of SO_4 reduction.

The unstripped reactor (R3) had appreciable removals (50-60% COD) equal to or trailing the stripped R4 by only 5-10%. In sulfidogenic reactors heavily loaded with carbon and sulfur, however, artificial stripping remains the method of choice, warranting consistent operation since sulfides appear to be detrimental to the acetoclastic microorganisms. This means that a symbiotic coculture of SRB and MPB in R4 was capable of the same job as a methanogenic reactor, in spite of a heavy sulfate load (>1.5 g S/L·d) and a high ratio of influent sulfur to carbon (S_o/C_o, S_o/COD).

The stripped sulfidogenic reactor fed whey (R2) significantly outperformed the stripped sulfidogenic (R4) and methanogenic reactor (R6) fed whey and SSL (Table 4.2). On the other hand, although the unstripped R1 (whey only) outperformed the unstripped R3 (whey + SSL), the performance of R1 was not significantly better than that of R4. The fact that R1 significantly outperformed R3 is evidence that SSL is difficult to degrade anaerobically.

Table 4.5. Sulfate to sulfide conversion with and without gas-stripping in an anaerobic sequencing batch reactor (DLA, 1982).

Day since startup	Influent S^{6+} mg S^{6+}/L	Effluent S^{2-} mg S^{2-}/L	S^{2-} produced S^{6+} infl. %	Removal SO_4(%)	Removal TOC(%)
	without stripping				
60	550	50	15	30	60
	stripping started				
64	560	1	28	29	91
67	600	18	43	67	--
68	570	14	61	78	98
69	530	6	53	78	--
72	340	0	100	94	99
73	660	8	64	95	--

4.1.7 VFA and gas production

The theoretical methane production rate for methanogenic reactors such as R6 should have been 350 mL/g. The observed methane production rates were: 60 mL/g for R1 and R3 (unstripped), 122-146 mL/g for R2 and R4 (stripped), and 243 mL/g for R6. In all reactors, the predominant buildup of VFA was in the form of acetate and occasionally n- and iso-butyrate. Valerate was rarely found. The greatest buildup of acids was in the unstripped reactors (R1 and R3). The stripped reactors (R2 and R4) contained significantly less VFA, which was reflected by their increased COD removal. The difference in COD removal between R1 and R2 as well as between R3 and R4 was numerically equal to the accumulation of VFA, suggesting inhibition of MPB or acetoclastic SRB.

4.1.8 Sulfur loading, sulfides, and toxicity

All work done during these experiments indicated reversible inhibition of acetoclastic SRB and MPB biomass by excess sulfide concentrations. After a period of suppression of the acetoclastic biomass due to accumulated H_2S caused by a stripping failure, the accumulated VFA were metabolized quickly the moment stripping was back in service which was also shown previously by DLA, Inc. (1982a, b).

The conversion of sulfates to sulfides depended upon the overall condition of the system and was always coincidental with appreciable COD removal; in stripped reactors, conversion was coincidental with high methane generation. In the work reported

by DLA, Inc. (1982b), in reactors fed gypsum and piggery wastes, the removal of sulfates was greater than 95%, with soluble organic carbon removal greater than 98%. The removal of sulfates in the study presented here varied from 60 to 95% (S_r/COD_o = 0.12-0.21). Saw et al. (1987) achieved 30-54% (S_r/COD_o = 0.18-0.21), Olthof et al. (1986) achieved up to 95% (S_r/COD_o = 0.09) and Maree and Strydom (1987) achieved 95% sulfur reduction (S_r/COD_o = 0.19).

There is a wide variation in efficiency of removal between the different cited experiments and yet there is a great similarity in the S_r/COD_o of the experiments. This shows that in mixed cultures, as in pure cultures (see equations 1.1-1.5), sulfate reduction is dependent upon the availability of carbon. Sulfate is the terminal electron acceptor. In order to achieve sulfur reduction, there must be a donor, either hydrogen or a reduced form of carbon.

The level of inhibition in the unstripped reactor appeared to be related to the quantity of methane generated and, indirectly, to the biodegradability of organic substrate.

The total sulfides in the effluents from all reactors is illustrated in Table 4.4. Fluctuations in the concentration of sulfides in the stripped reactors reflected (a) frequency of regeneration of the stripping solution and (b) rate of gas sweeping. It is important to note that in spite of fluctuations in the sulfide concentrations in reactor R2 and R4, they were capable of performing at concentrations in excess of 150 mg S^{2-}/L. When the

reactor sulfides were above 200 mg $S2^-$/L, the performance dropped drastically.

4.1.9 Conclusions

1. The reduction of sulfates (60-95%) proceeded best in the gas-stripped reactors.

2. In the reactors fed whey and sodium sulfate, the mean COD removal was 88% in the stripped reactor but only 56% in the unstripped reactor.

3. Stripping of reactor contents of accumulated sulfides increased the removal of COD and increased the production of methane.

4. Inhibition due to sulfide accumulation primarily affected the VFA conversion phase rather than the acid-generating or acetogenic phase of the anaerobic biodegradation pathway.

5. At organic loads below 3 g COD/L·d and sulfate loads above 0.5 g S^{6+}/L·d, the performance of stripped reactors and unstripped reactors was comparable. Above 3 g COD/L·d and 0.5 g S^{6+}/L·d, the stripped reactors outperformed unstripped reactors. This difference was most likely due to sulfide toxicity in the unstripped reactors.

6. COD removal was inversely proportional to the sulfide concentrations in the effluent.

7. The alkalinity generated by sulfate reduction in conjunction with sulfate reduction in the sulfidogenic-methanogenic reactors provided more pH stability than that in purely

methanogenic reactors, thereby reducing the requirement for the addition of buffers to the system.

8. The methanogenic and stripped sulfidogenic reactors receiving SSL provided comparable COD removals. The specific production of methane (L CH_4/g COD removed) was significantly greater in the purely methanogenic reactor (0.25 L CH_4/g COD removed) than in the sulfidogenic reactor (0.15 L CH_4/g COD removed). This difference clearly demonstrated a diversion of organic carbon utilization from methane production to sulfate reduction in the stripped sulfidogenic reactor.

9. The variability in the performance in the stripped sulfidogenic reactor was the result of variations in reactor sulfide concentrations. This was caused by an inability to maintain steady-state conditions with the gas stripping apparatus.

10. The COD removal in the reactors fed SSL plus sulfate was lower than in the reactors fed whey plus sulfate. This was an indication of the relative difficulty of degrading SSL.

11. Minimal sulfate reduction will occur if lactate or lactate precursors are absent.

12. High COD removal (80%) efficiency in the sulfate-loaded gas stripped reactors R2, R4, R7, was always due to syntrophic generation of CH_4 and H_2S.

4.2 REACTORS FED RECONSTITUTED POWDERED WHEY (R9-R12)

4.2.1 Introduction

The experiments conducted with reactors R1 - R8 demonstrated that carbon removal in stripped sulfidogenic reactors was comparable to methanogenic reactors receiving an identical carbon source. A decrease in methane production in the presence of sulfates was not necessarily accompanied by a decrease in removal of organic carbon since the utilization of organic carbon was diverted from the manufacture of methane to the reduction of sulfur.

The data from reactors R1 - R8 did not answer questions regarding the optimal and maximum quantity of sulfur which could be reduced by a stripped sulfidogenic anaerobic reactor nor the operating conditions which would promote maximum sulfur reduction with a minimum of carbon required in the feed.

Improvements in the experimental apparatus, the acquisition of an automated TOC analyzer, and the ability to perform automated sulfate analyses enhanced data quality and quantity. Details regarding the equipment and materials for this phase of the research are outlined in Section 3, Equipment and Procedures.

There are two groups of sulfate reducing bacteria: a) those which incompletely oxidize lactate to acetate and carbon dioxide (incomplete oxidizers such as Desulfovibrio and Desulfobulbus) and b) those which completely oxidize acetate to carbon dioxide (complete oxidizers or acetoclastic SRB such as Desulfobacter).

Calculations of carbon availability and the production of reduced sulfur in the stripped reactors (R2 and R4) suggested that sulfate reduction was only performed by incomplete oxidizing sulfate reducing bacteria. Pure culture studies have shown that incomplete oxidizers such as Desulfovibrio incompletely oxidize lactate or pyruvate to acetate in the process of sulfate reduction (Postgate 1984).

The pure culture work of Kristjansson et al. (1982) and Schonheit et al. (1982) demonstrated the ability of sulfate reducing bacteria to outcompete methanogens for acetate and for molecular hydrogen. Middleton and Lawrence (1977) demonstrated that when acetate was the carbon source, all the carbon was used for sulfate reduction. The experiment with stripped sulfidogenic reactors R9 - R12 was undertaken in anticipation that the entire carbon flow in a stripped sulfidogenic reactor would be diverted towards sulfate reduction.

The reactors (R9-R12) were fed reconstituted powdered whey and sodium sulfate. Reconstituted powdered whey was chosen because of its commercial availability and its relatively nutritionally complete nature which would provide more realistic conditions than would be achievable using a purely synthetic medium. These reactors were operated at B_V = 2-6.5 g TOC/L.d and were continuously stripped as shown in Figure 3.1. The primary stripping solution was ARI-311C™. During this phase of research, there was little methane production which was not a

major concern at the time because the objective was to maximize sulfate reduction.

4.2.2 Operation of R9 - R12

The performance of a stripped sulfidogenic reactor (R11) is shown in Figures 4.11 - 4.12. Reactor R11 was chosen because it exhibited the greatest stability and yielded the least variability of results. From day 30 to day 60 (Figure 4.11), 1.2-1.5 g/L sulfate-sulfur was reduced with an average influent TOC of 4.5 g/L. During this period of time, the ratio of sulfur reduced (S_r) to TOC was S_r/C_o = 0.29. The observed variability in TOC removal was not accompanied by variations in influent TOC nor in influent sulfur. Increases or decreases in TOC removal coincided with similar variations in sulfur reduction although the fluctuations in TOC removal were greater than those for sulfur reduction.

From day 130 to day 146 (Figure 4.12), S_r = 0.9 g/L at an influent TOC of 4.6 g/L for a S_r/C_o = 0.20. At day 148, this increased to 1.3 g/L sulfur reduced at an influent TOC of 4.6 g/L for a S_r/C_o = 0.28. As was noted for day 30 to day 60, there were variations in TOC removed which appeared to be greater than the comparable variations in sulfur reduction.

Figures 4.11 - 4.12, illustrate that some factor other than influent TOC (C_o) or influent sulfur (S_o) influenced the reduction of sulfur and removal of carbon in these reactors. There were difficulties with the operation of the stripping system for these reactors. Since reactor total sulfide concentrations rarely exceeded

Figure 4.11 Influent TOC, TOC removal, influent sulfur, and sulfur reduction in a stripped sulfidogenic reactor (R11) fed reconstituted powdered whey and sodium sulfate for the period of day 30 to day 70 of the experiment.

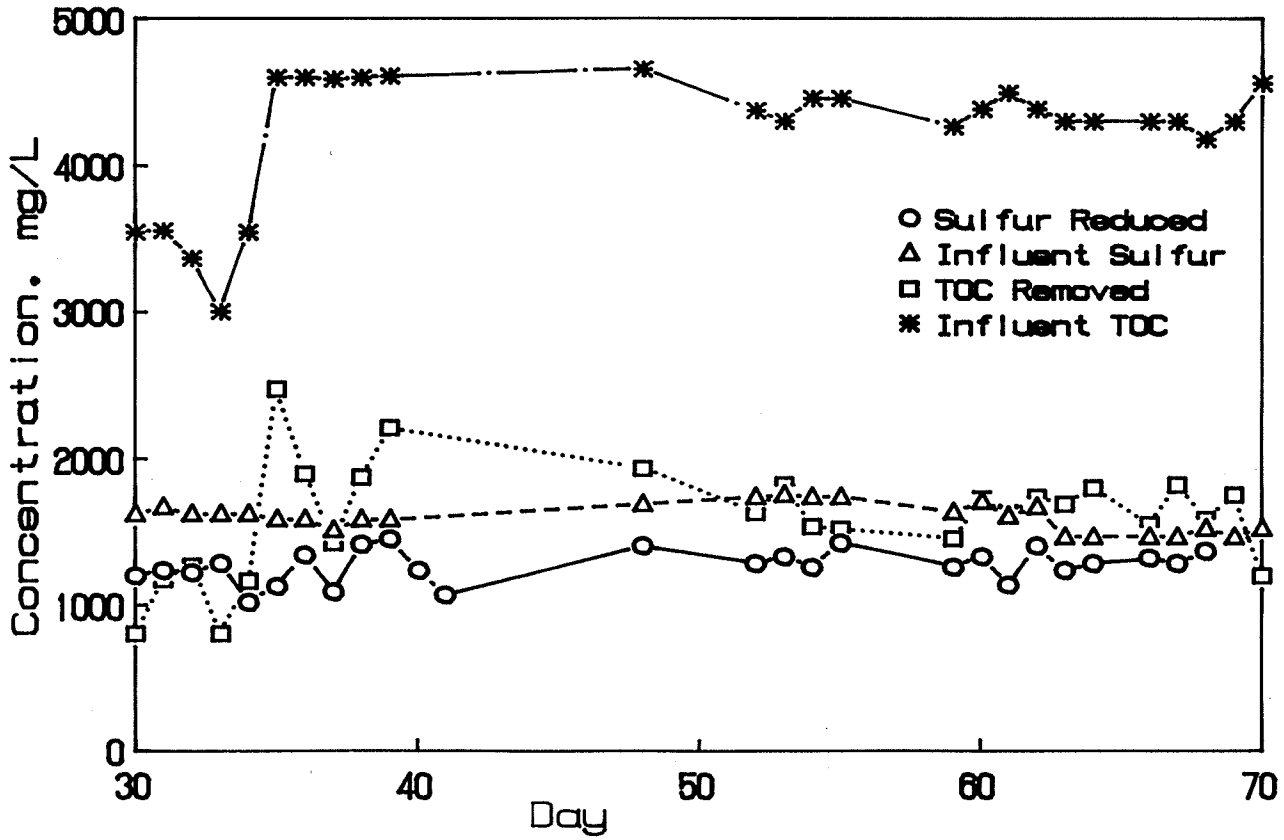
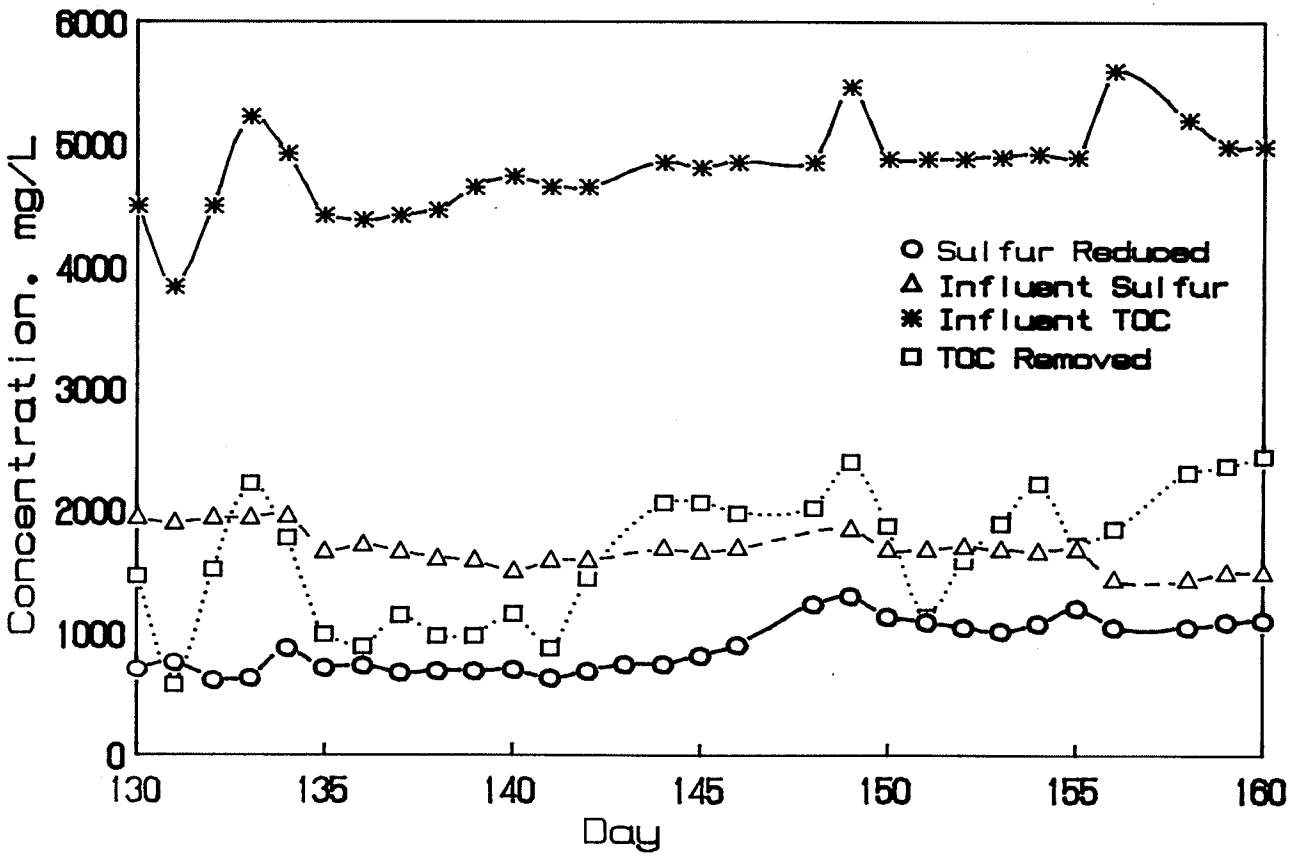


Figure 4.12 Influent TOC, TOC removal, influent sulfur, and sulfur reduction in a stripped sulfidogenic reactor (R11) fed reconstituted powdered whey and sodium sulfate for the period of day 130 to day 160 of the experiment.



50 mg S^{2-}/L, high concentrations of sulfides were not the cause of variations in TOC removal or sulfur reduction.

There was no correlation between TOC removal and F/M (g TOC/g VNFR) (Figure 4.13) at B_V up to 5 g/L·d; therefore, variations in the reactor VNFR were not the cause of the variations in TOC removal efficiency. This was in contrast with the findings of Olthof et al. (1986) where both carbon removal and sulfur reduction were directly correlated to reactor F/M and the form of carbon in the feed.

4.2.3 Relationship between TOC removal and sulfur reduction and variations in reactor pH

One variable, pH, was difficult to control for the duration of this experiment. Reactor R10 was operated in an acidogenic mode (below pH 5.7) for the entire period of operation as an independent reactor (day 0 to day 70).

Figure 4.14 is a plot of TOC removed and sulfur reduced versus pH for reactors R9-R12. All four reactors exhibited steady-state conditions. However, only one, R11, was maintained under steady-state conditions above pH 6.0. Sulfur reduction was closely correlated to the pH: 0.15 g/L·d at pH 4.5 and 1.4 g/L·d at pH 7.2. The values (average S_r for each pH) closely follow the regression line (R^2 = 0.98):

[4.1] $$S_r = -2.4 + 0.525\ pH$$

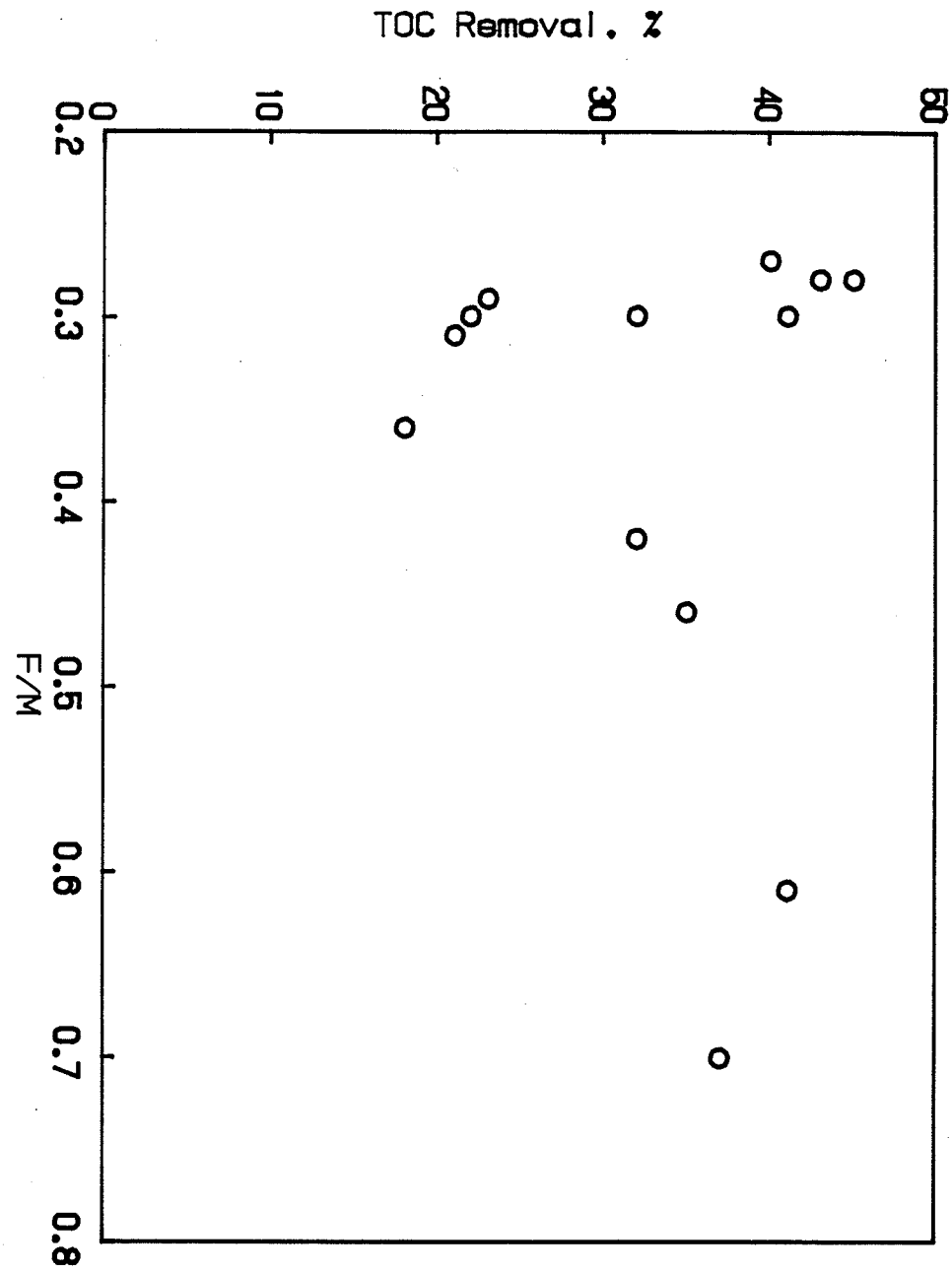


Figure 4.13 TOC removal efficiency versus F/M for R9 and R11.

TOC removal closely followed the increase in pH but the correlation (R^2 = 0.78) was less than between sulfur reduction and pH which would suggest that carbon removal was affected by more than pH or sulfur reduction. The regression line is:

[4.2] $$C_r = -1.99 + 0.535\ pH$$

Overall, there was a high correlation between carbon removal and pH and sulfur reduction and pH. Figure 4.15 is a plot of pH against B_V. The data points are the average B_V for each pH. There was little correlation between pH and TOC load (B_V) for R9 - R12 (Figure 4.15), which is in contrast with the case for the unstripped R1 where pH decreased with increases in B_V.

Figure 4.16 is a plot of S_r/C_o against pH. The data points represent the average S_r/C_o for each pH value. The values for each data point were from R9 - R12 for day 21 - day 50 and day 90 - day 160. Increases in pH were accompanied by increases in S_r/C_o. At pH 5.0, S_r/C_o = 0.09; at pH 7.1, S_r/C_o = 0.25. This can be interpreted to mean that, at pH 6-7.5, 0.20-0.30 grams of sulfur were reduced for every gram of TOC fed to the reactor.

In order to establish the dependence of the sulfate reduction process on the available electron donor (TOC) in the stripped sulfidogenic reactor R11, a plot of the rate of sulfur reduction, $S_r = \Delta S/(\Theta_h \Delta t)$ (g/L.d) versus TOC load applied, B_V (g/L.d) was made. The empirical correlation that was found to fit the collected data pool was plotted in Figure 4.17 (R^2=0.68):

[4.3] $$S_r = 0.29\ B_V$$

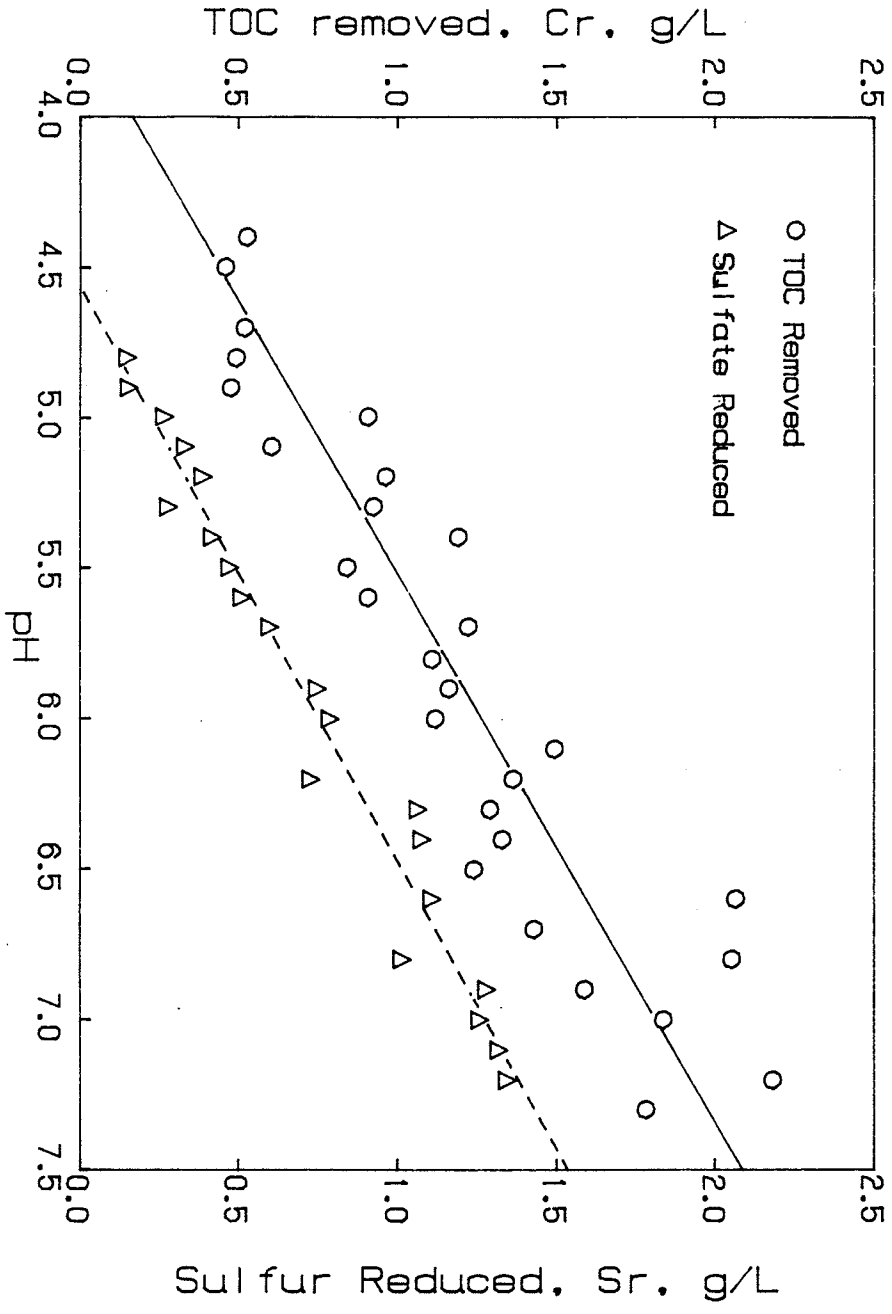


Figure 4.14 TOC removed and sulfur reduced versus pH in reactor R9-R12.

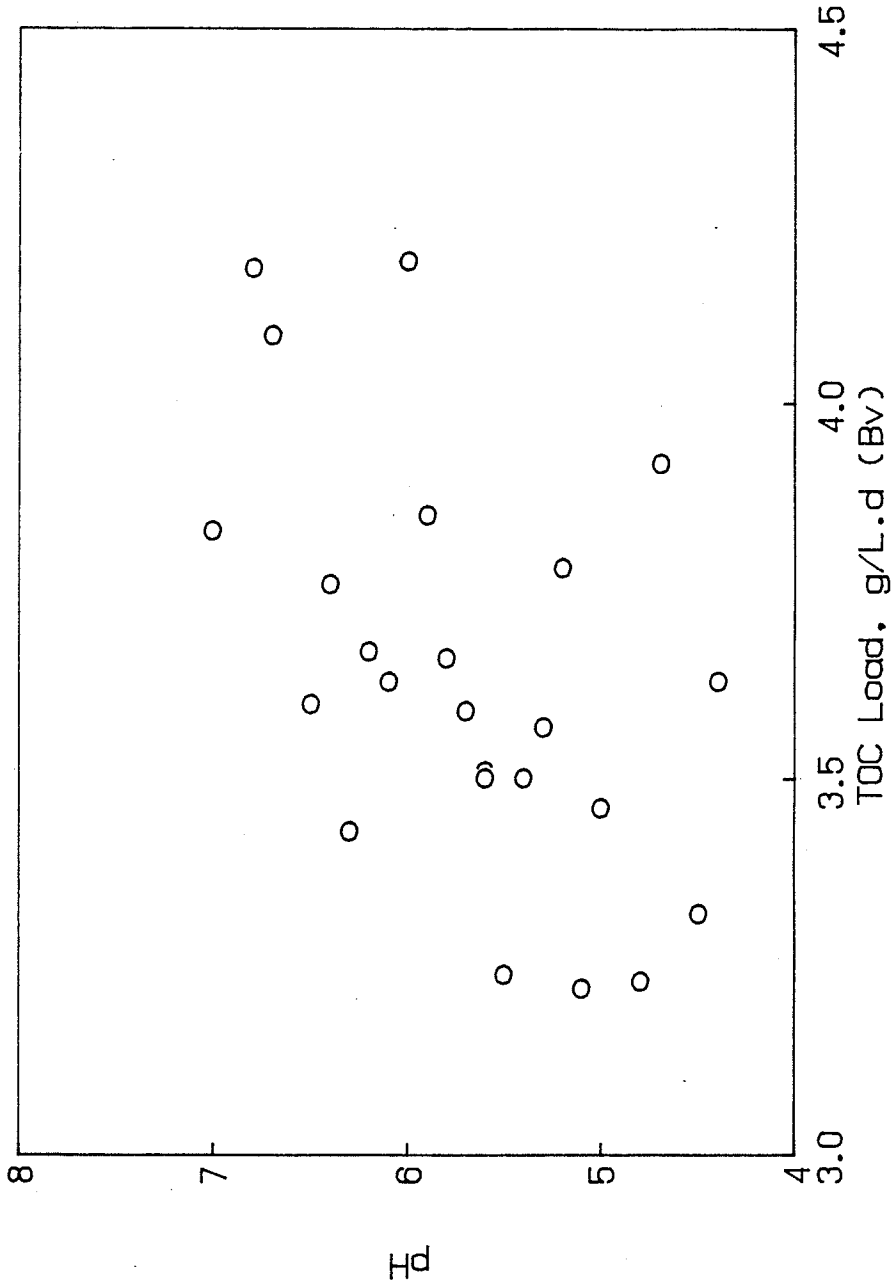


Figure 4.15 Reactor pH versus carbon load (B_V) in heavily loaded stripped sulfidogenic reactors fed reconstituted whey powder and sodium sulfate (R9-R12).

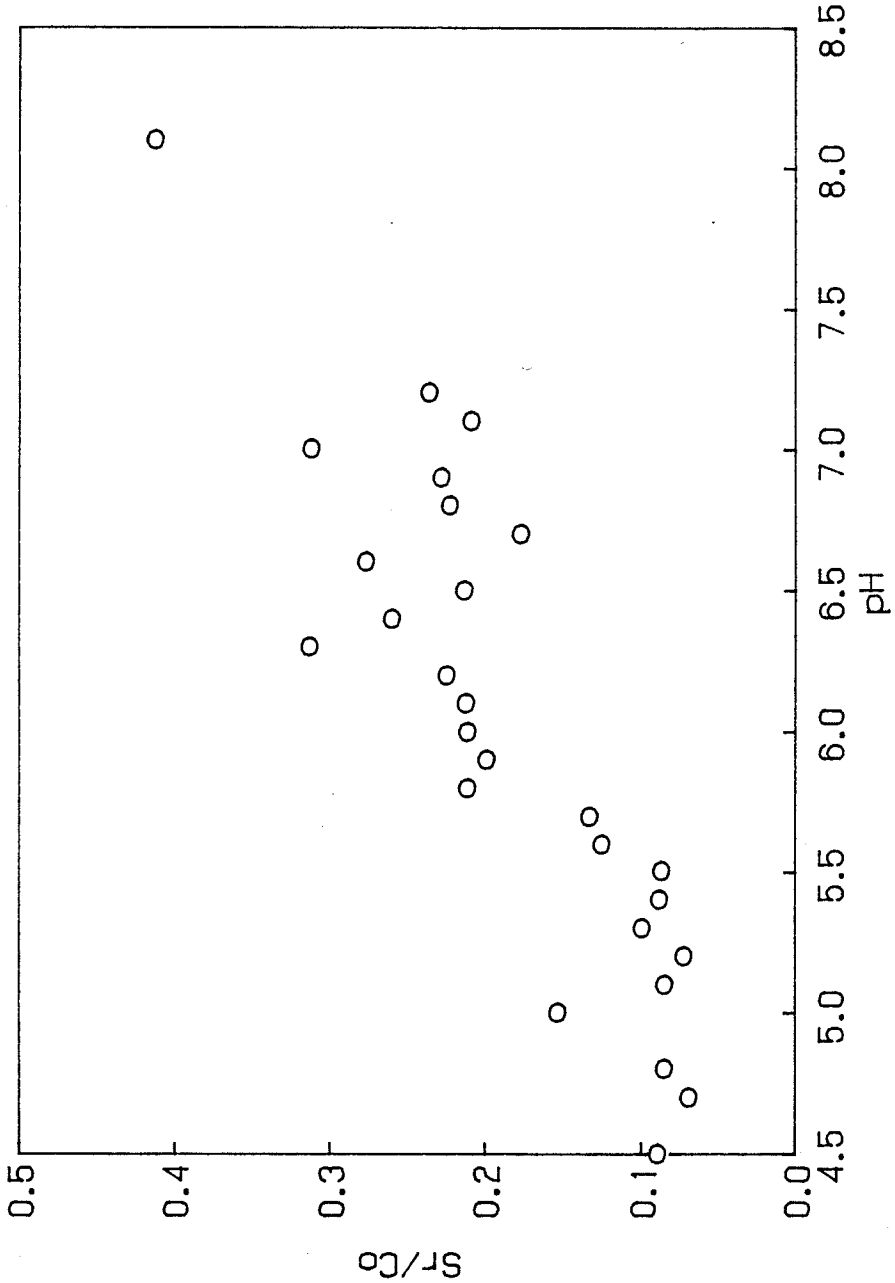


Figure 4.16 The ratio of sulfur reduced/carbon fed (S_r/C_o) plotted against pH for reactors R9-R12.

This corresponds to S_r/C_o = 0.29. The stoichiometric ratio for incompletely oxidizing SRB is S_r/C_o = 0.44 (Table 1.1). Therefore, based upon carbon utilization, the incompletely oxidizing sulfate reducers predominated in this study.

In a continuous-flow, continuously stripped packed bed reactor fed molasses and sulfate, Maree and Strydom (1987) achieved an optimum S_r/C_o = 0.30 which is comparable to the value in this research. The study of Olthof et al. (1986) found S_r/C_o = 0.15-0.5. The value for S_r/C_o = 0.5 is greater than the theoretical value for incomplete oxidizers but was for a treatability study with a low COD_o = 620 mg/L. All the continuous flow reactors had an S_r/C_o = 0.15-0.33 which is characteristic of incomplete oxidizers.

4.2.4 Carbon Removal

4.2.4.1 TOC Load

A plot of the rate of TOC removal, $C_r = \Delta C/(\Theta_h \Delta t)$ (g/L·d) versus TOC load applied (B_V) was made. The empirical correlation that was found to fit the collected data pool was plotted in Figure 4.18 (R_2 = 0.73):

[4.4] $$C_r = 0.06 + 0.35\ B_V$$

The uniform efficiency (35%) is in contrast to the usual response of biological reactors, which show a decrease as the organic load increases but corresponds closely with the work of Olthof et al. (1986) as shown in Figure 4.19. The constant ratio of TOC removed to TOC load in this experiment, 35%, regardless of the sulfate reduced, would suggest that reduction was a linear function of the TOC load applied.

Figure 4.17 Sulfur reduced (S_r) versus carbon load (B_V) for stripped sulfidogenic reactors R9-R12 fed reconstituted whey powder and sodium sulfate.

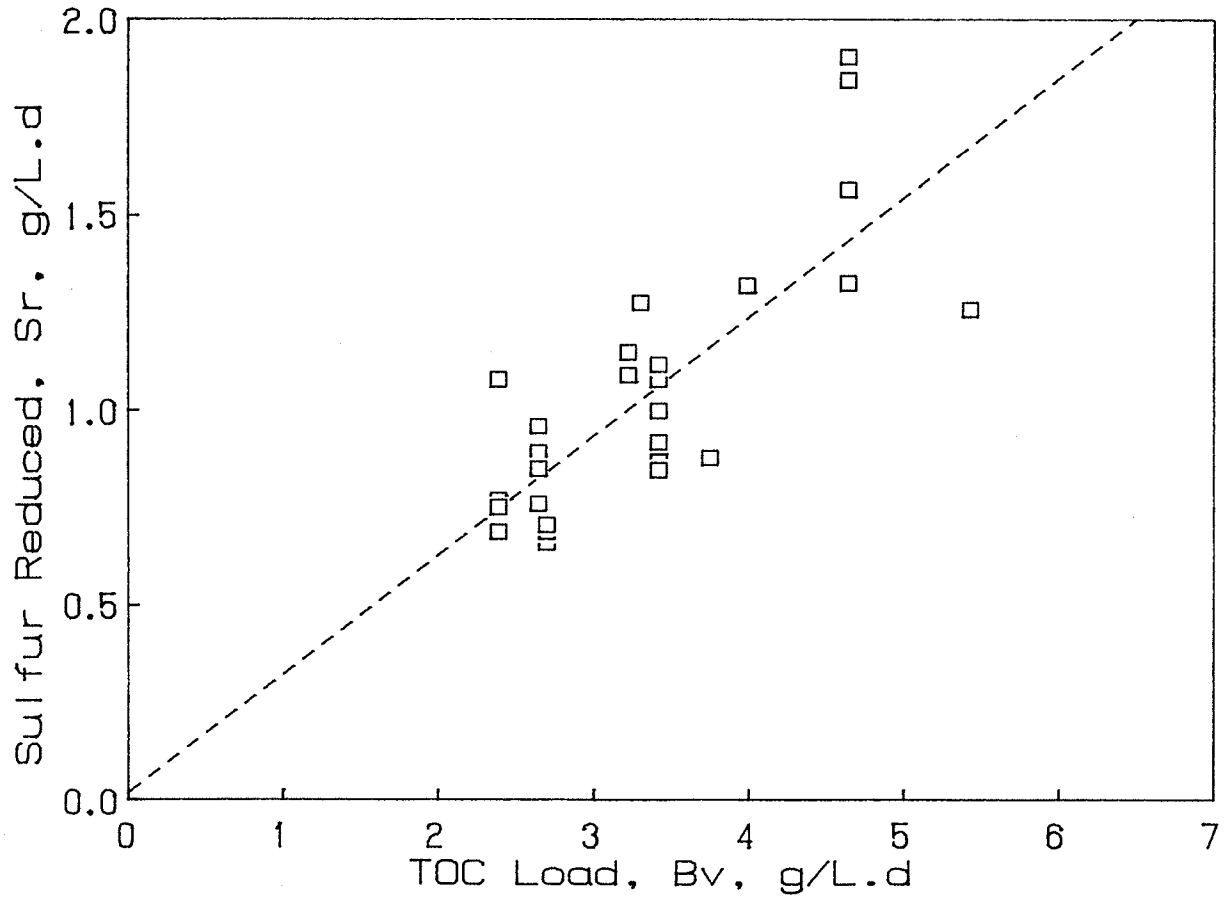
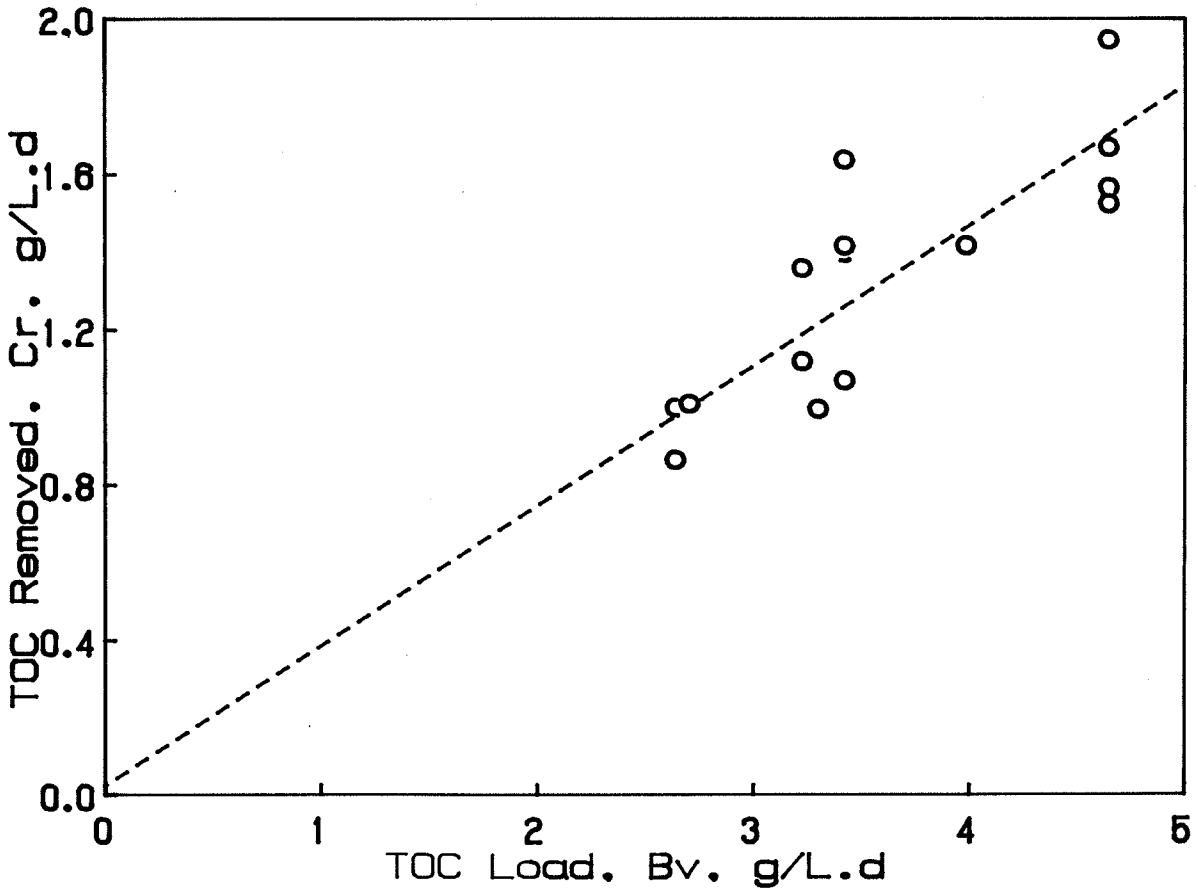


Figure 4.18 TOC removal versus TOC load for stripped sulfidogenic reactors (R9-R12) fed reconstituted whey powder and sodium sulfate.



The data presented here indicated that S_r increased as a constant percentage of increases in the TOC load. The pure culture studies of Domka and Szulczynski (1979, 1981) also indicated a direct correlation between the quantity of sulfate reduced and the quantity of lactate fed. Since the microorganisms in this study appeared to be lactate utilizing, incomplete oxidizers, the maximum value for S_r would appear to be limited by the rate of lactate generation during anaerobic fermentation.

Excess sulfate-sulfur was always present in the reactors (Figures 4.11 - 4.12); only rarely was the reactor sulfate-sulfur concentration below 150 mg/L. During periods of high TOC loading and high sulfate loading, the reactor sulfate-sulfur concentration was greater than 500 mg S^{6+}/L. Therefore, the observed maximum rate of sulfur reduction was not limited by the concentration of sulfur but most likely was limited by lactate availability.

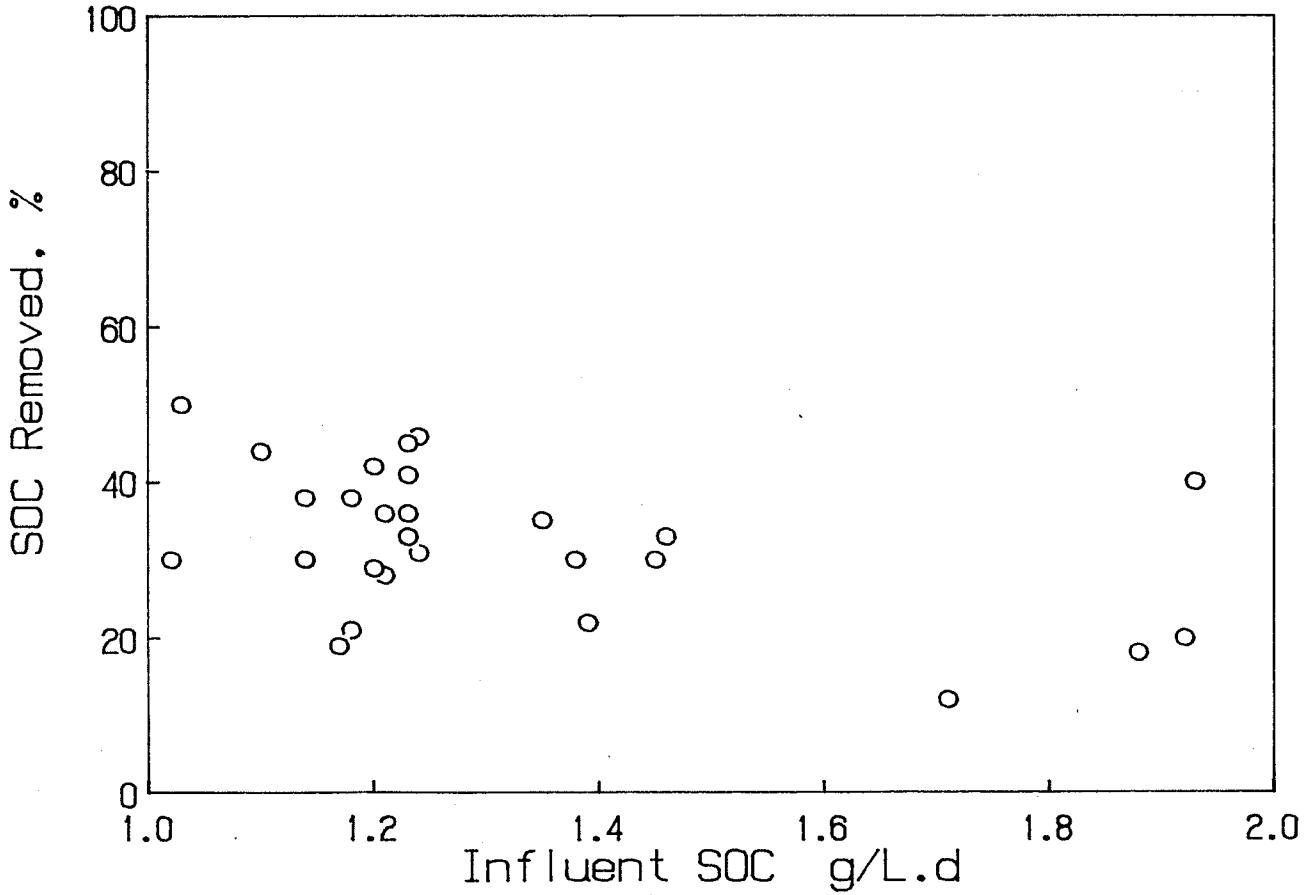
4.2.4.2 Sulfur reduction in relationship to TOC removal

In Figure 4.20, sulfur reduction (S_r) (the values come from R11 only) is (R^2 = 0.78):

[4.5] $$S_r = -0.16 + 0.7\ C_r$$

The production of methane was negligible in this study. Therefore, this means that for C_r = 1.0-2.5, S_r/C_r = 0.5-0.6 for the study presented here. A portion of the carbon removal is due to factors other than sulfate reduction. If sulfur reduction were a strict function of carbon removal, only 1.33 g sulfur would have been reduced per gram of carbon removed (S_r/C_r = 1.33) (see Section 1.2.1). Factors such as assimilation and dissimilation of

Figure 4.19 Efficiency of SOC removal as a function of SOC load in a stripped sulfidogenic reactor receiving industrial sludge and gypsum. (Data from Olthof et al. 1986).



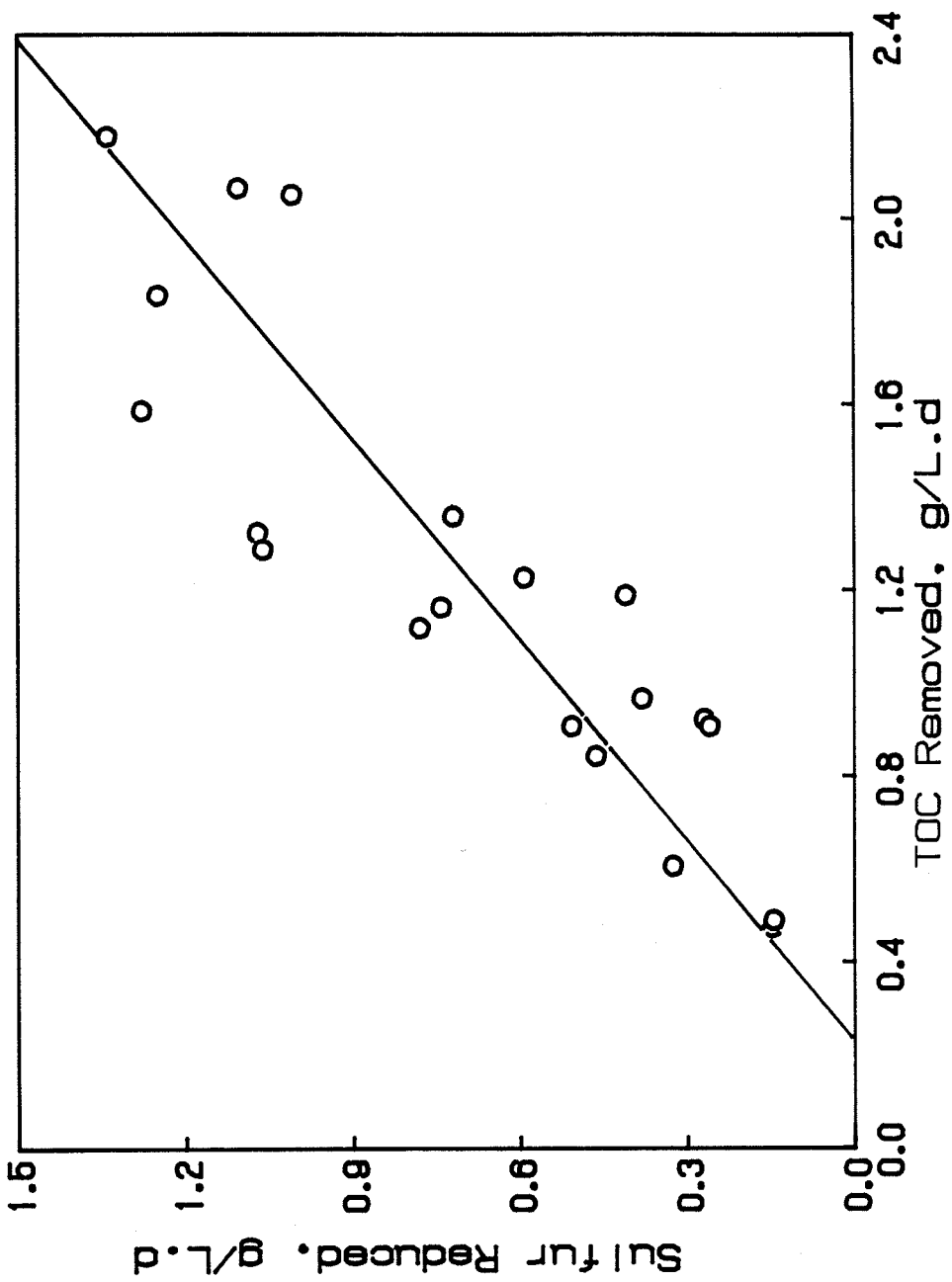


Figure 4.20 Sulfur reduced (S_r) versus TOC removal (C_r) in the stripped sulfidogenic reactor, R11.

carbon by acidogenic microorganisms during glycolysis, assimilation of carbon by SRB, and methane production would account for the difference between the observed S_r/C_r = 0.5-0.6 and the theoretical S_r/C_r = 1.33. The observed S_r/C_r = 0.5-0.6 is similar to that published by others (S_r/C_r = 0.16-0.5, Olthof et al. 1986; S_r/C_r = 0.38, Maree and Strydom 1987; and S_r/C_r = 0.88, Saw et al. 1987). Olthof et al. (1986) and Saw et al. (1987) reported little methane production which is the same as observed for R9-R14.

Saw et al. (1987) reported carbon removal efficiencies as high as 90%. The work of Olthof et al. (1986) and the research presented here employed sludge bed reactors. Maree and Strydom (1987) used packed bed reactors. Isa et al. (1986) reported that acetoclastic SRB demonstrated poor attachment and were easily lost in attached growth reactors. Comparing carbon utilization in flocculant sludge bed reactors (Olthof et al. 1986; this study) with attached growth reactors (Isa et al. 1986a, b; Maree and Strydom 1987; Saw et al. 1987), attached growth reactors appeared to provide a more suitable environment for retention of complete oxidizing SRB.

The studies of Isa et al. (1986), Kroiss (1987), Szendry (1984), and Saw et al. suggest that sulfate reduction is substrate dependent. The work of Isa et al. stated that complete oxidizing SRB do not necessarily compete well with MPB when acetate, ethanol, or formate are the carbon sources. Szendry (1984) reported 0.37 L CH_4/g COD removed for high sulfate reactors receiving distillery waste (COD_o =80 g/L and SO_4 = 7 g/L,

S_o/COD_o = 0.03). Kroiss (1987) reported 0.32 L/g methane production in the operation of full-scale anaerobic reactors treating citric acid plant wastes (COD_o = 17 g/L, SO_4-S = 0.95 g/L, S_o/C_o = 0.15, 50 % SO_4-S reduced, S_r/COD_o = 0.03, S_r/C_o = 0.08).

The results of this study and the results of Olthof et al. (1986), DLA (1982) and Cappenberg (1978) have shown that sulfate reducers and methanogens can be cultured in the same reactor. On the other hand, Saw et al. reported no methane production with COD removals >90 % when treating high sulfate edible oil wastes.

The relationship between sulfate reduction and pH shows that the efficiency of sulfate reduction improves with increasing pH. A batch study (section 4.3.4) showed that, between pH 6.0 and 8.0, there is no difference in sulfate reduction with respect to pH when lactate is the carbon source. More sulfur was reduced at pH 7.2 than at pH 6 (Figure 4.19) in continuous flow stripped sulfidogenic reactors fed reconstituted whey powder. Therefore, one would conclude that sulfate reduction in R9-R11 was by incomplete oxidizing SRB. Furthermore, sulfate reduction was limited by the generation of lactate.

4.2.5 Acetate Utilization and Diauxic Growth in R9-R11

The residual acetate concentration in reactors R9-R11 averaged 3075 mg/L (s.d. = 996) which indicated low acetate uptake in the reactors. Pure culture studies have shown that some sulfate reducing bacteria which prefer lactate as a carbon source also metabolize acetate during the process of sulfate reduction (Postgate, 1984). When microorganisms are able to utilize two

different carbon sources but utilize one carbon source more easily than another, this process is called diauxie.

The following experiment was performed in order to test for the presence or absence of diauxie (in this case, lactate utilization rather than acetate utilization). The feed pumps were turned off and the reactor supernatant was tested for TOC, sulfates, and sulfides. The initial TOC was in excess of 4 g/L, the initial sulfates were in excess of 0.2 g S/L, and initial sulfides were less than 30 mg/L in the reactor supernatant.

During the 14 day period (day 65 - 80) when the feed pumps were turned off, there was no removal of TOC and no sulfate reduction. If diauxie were present, the sulfate reducing bacteria would have switched to acetate oxidation and reduced the remaining sulfur. Since TOC removal and sulfate reduction did not immediately occur, it was concluded that there was no diauxie.

At the end of this phase of the experiment, the feed pumps and gas recycle pumps were turned off but the liquid recycle pumps were left running. After a two month break, the reactor supernatant was tested for TOC, VFA, and sulfates. Analyses showed that 77% of the remaining TOC was removed, 97% of the remaining VFA were removed, and 97% of the remaining sulfates were reduced. It was concluded that the additional TOC removal and sulfate reduction were a result of a shift in the microbial population and were not due to diauxie because of the lag time required to utilize the VFA for sulfate reduction.

4.2.6 Reactor Solids

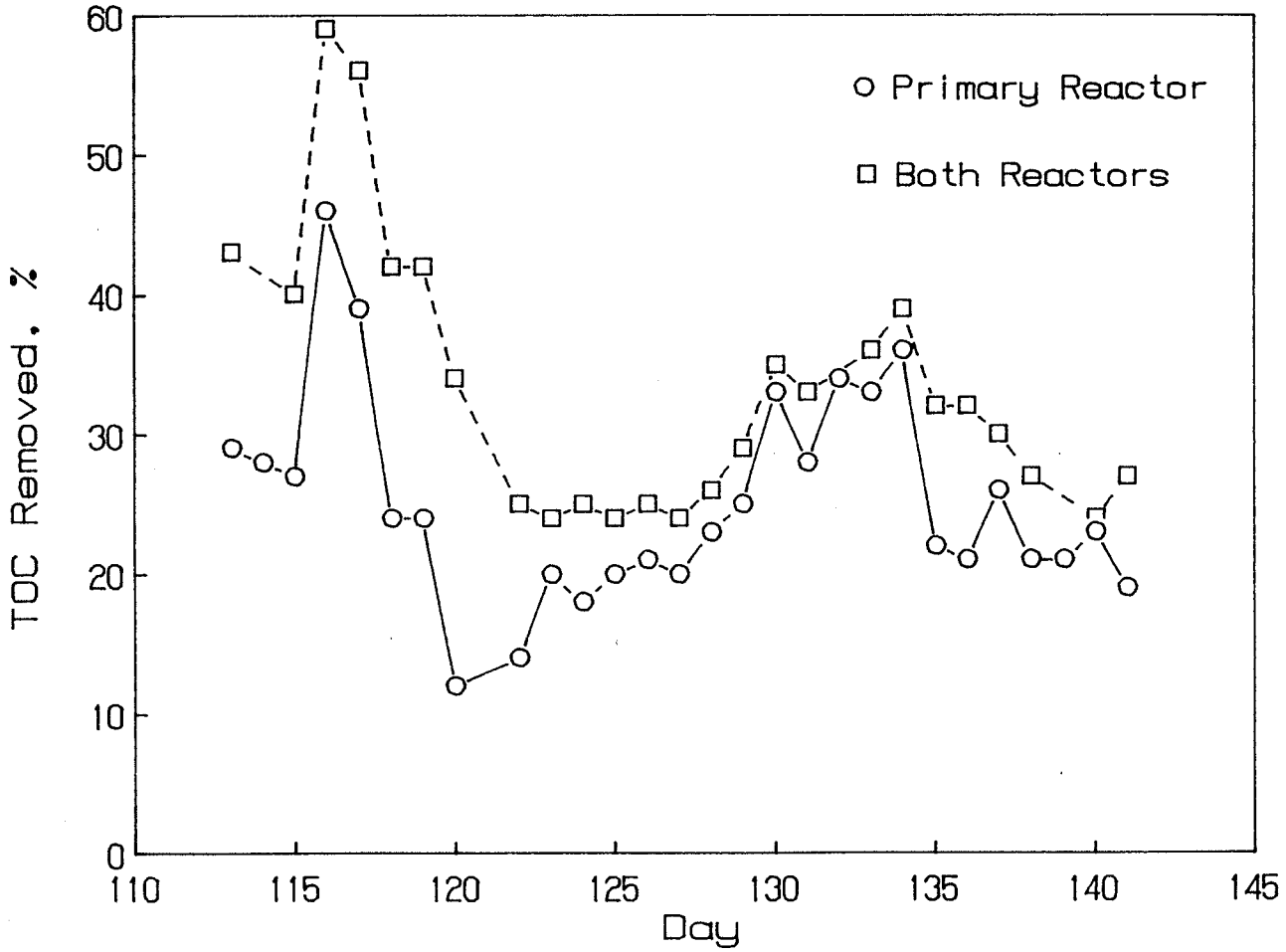
The maximum concentration of volatile solids in the reactors, 23 g/L, coincided with the maximum observed rate of sulfur reduction (U_S) in the reactors and the greatest TOC load to the reactors. The average specific sulfate utilization (reduction) rate was U_S = 0.06-0.08 g S+6/g VSS.d; the average specific substrate utilization rate (for TOC) was U_c = 0.08 g TOC/g VSS·d.

Solids were continuously lost in the effluent due to gas purging of the split-boxes. Isa et al. (1986) showed that acetoclastic SRB were lost more rapidly than MPB in fixed-film continuous flow reactors. Because of these findings, improved solids retention in this study may have increased the sulfate-sulfur reducing capacity of the system by retaining more of the acetoclastic biomass fraction.

4.2.7 Reactors in Series (R13, R14)

Separate secondary 2.5 L continuous flow reactors (R13, R14), connected in series to the two primary 2.5 L units, collected all the solids lost with the effluent from the primary reactors effectively doubling the HRT and SRT of the system. The pH of the secondary reactors remained between pH 6.5 and 8.2. When the primary reactor was operating at a steady-state with pH > 6.5, the second reactor removed an additional 8% of the TOC to raise the total TOC removal from 35% to 40%. Figure 4.21 shows that the overall TOC removal improved with the use of two reactors.

Figure 4.21 TOC removal in a primary stripped sulfidogenic reactor (R9, R11) and a secondary reactor (R13, R14). The primary reactor was fed reconstituted powdered whey and sodium sulfate.



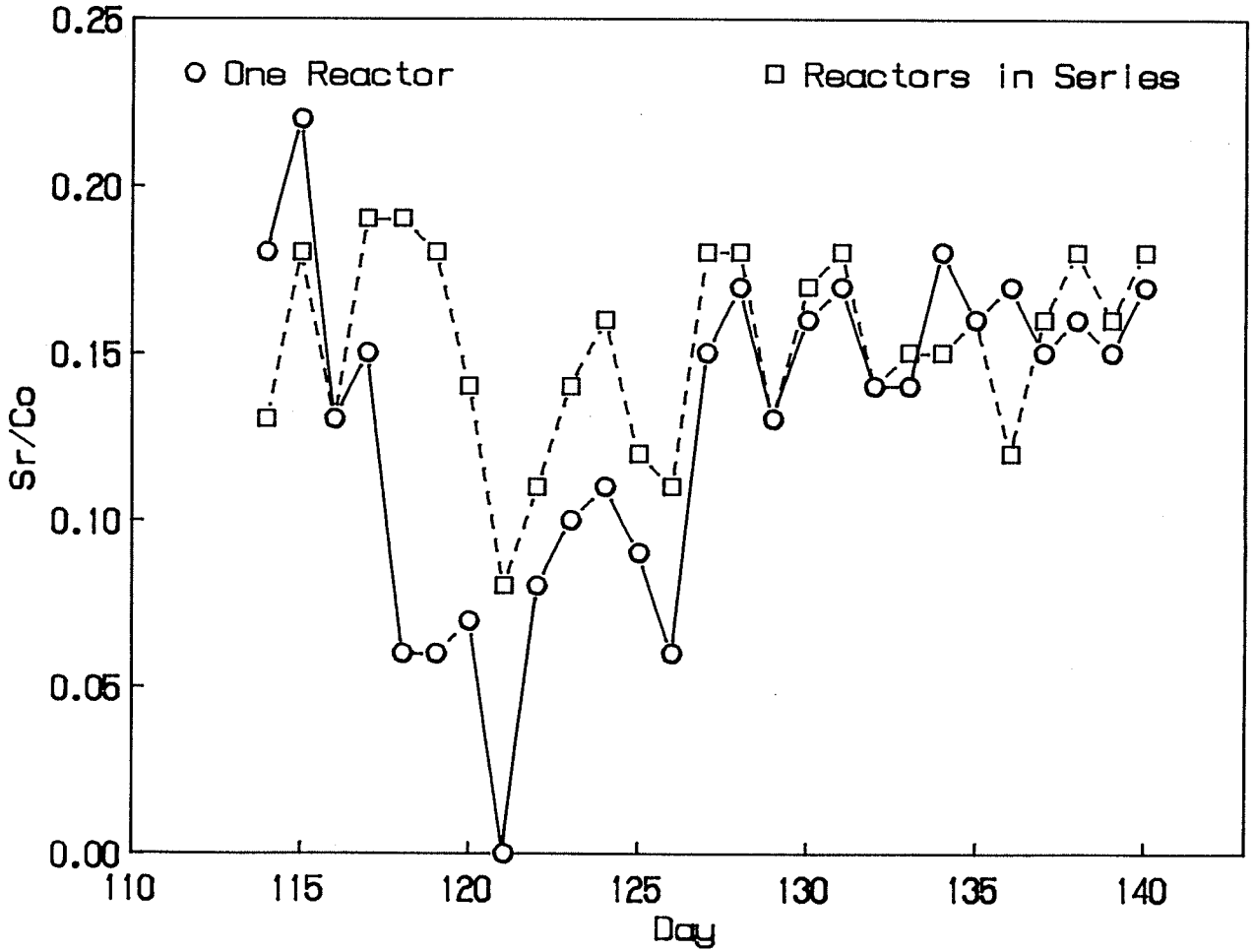
The addition of a second reactor doubled the HRT and SRT of the system yet provided little improvement in the S_r/C_o (Figure 4.22). From day 115-130, the pH of the first reactor was <6.0 whereas the pH of the second reactor was pH 7-7.5. After day 130, there was no difference in the S_r/C_o.

Based upon the latter data, one would conclude that, for this system, a lowered volumetric loading rate would increase the reliability of this system. These results also showed that secondary reactors did not improve TOC removal or sulfate reduction sufficiently to warrant the addition of the secondary units. It also showed that activity by acetoclastic SRB was minimal since sulfur reduction was limited to lactate availability as evidenced by the minimal change in S_r/C_o.

4.2.8 Methane Production

Methane production was a significant source of carbon removal in stripped sulfidogenic reactors R2, R4, and R7 (Section 4.1). In spite of reactor acetate concentrations as high as 3000 mg/L, very little methane was produced during the operation of R9-R12. Several possibilities were considered concerning the suppression of methane: sulfide toxicity, toxicity of metabolic byproducts, competition for hydrogen, CO_2 limitation, and toxicity by un-ionized acetic acid.

Figure 4.22 S_r/C_o in a single stripped sulfidogenic reactor (R11) and two reactors in series (R14). The reactor was fed reconstituted powdered whey and sodium sulfate.



4.2.8.1 Inhibition Due to Metabolite Toxicity

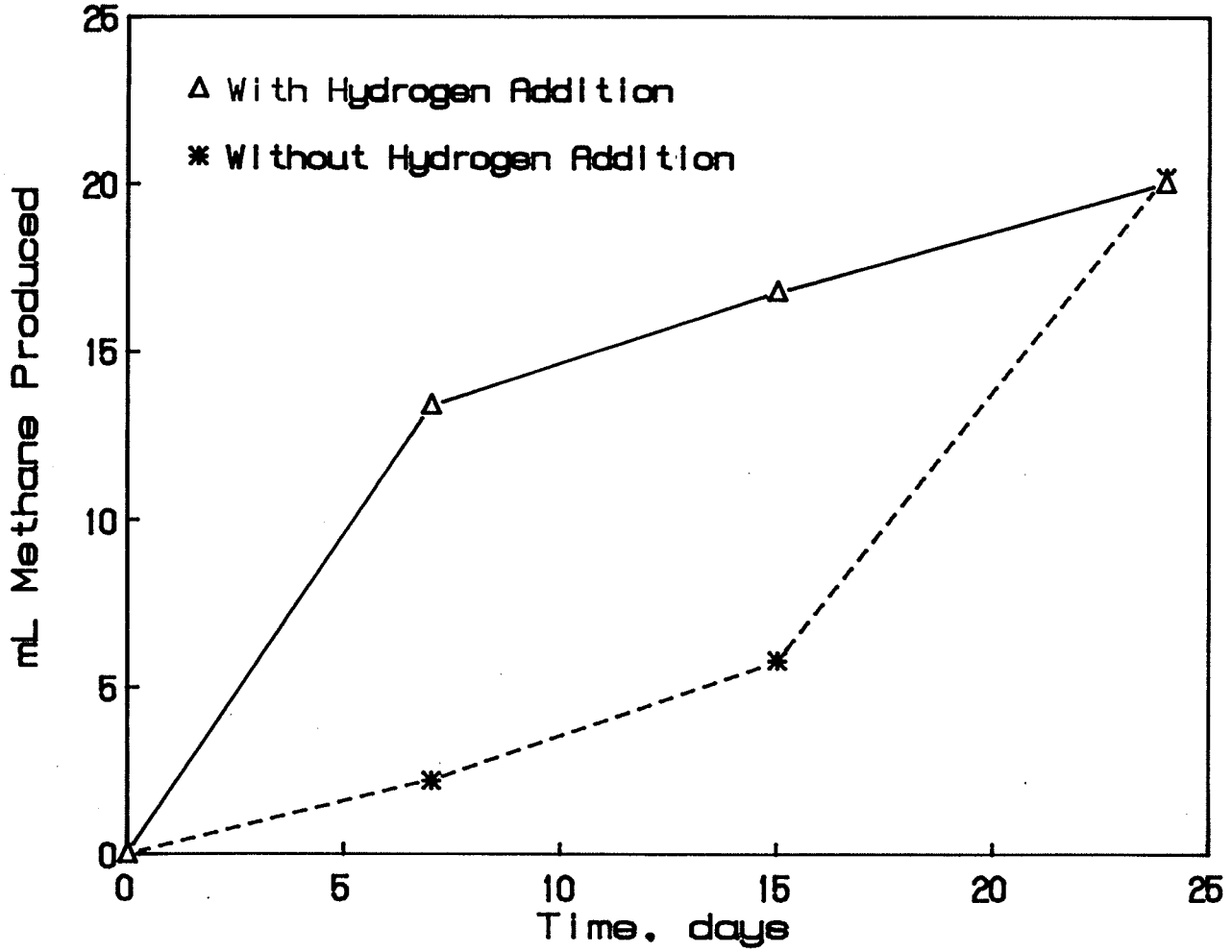
If inhibitory compounds were formed between sulfides and the metabolic products of the SRB, inhibition would have resulted in a buildup of VFA and a gradual decrease in the rate of TOC removal from the system as the TOC load increased. Effluent (50 mL) from one of the reactors was mixed with sludge (5 mL) from a methanogenic reactor was incubated in a syringe in a 35°C water bath. The residual acetate was converted to methane. Therefore, methane production was not suppressed due to toxic byproducts from the sulfate reduction process.

4.2.8.2 Competition for Hydrogen

Two syringes were filled with feed for a sulfidogenic reactor and sludge from the sulfidogenic reactor which was not producing methane. One syringe was additionally fed 20 mL molecular hydrogen. Methane production proceeded more quickly and earlier in the syringe fed hydrogen than in the syringe not fed hydrogen (Figure 4.23). Eventually, both syringes produced the same quantity of methane. This showed that although there was competition for hydrogen, the absence of hydrogen did not preclude the production of methane by this biomass with this substrate. This also again verified that there was no inhibition of methanogens due to the production of inhibitory compounds by the SRB.

Sulfate-reducing bacteria have been shown to grow on H_2 plus sulfate as the energy source (Badziong et al. 1982a, b; Pankhania et al. 1986) and even have been shown to out compete methanogens for molecular hydrogen (Lovley et al., 1982; Robinson and Tiedje,

Figure 4.23 Methane production versus time in plastic syringes. Syringes were fed effluent from a stripped sulfidogenic reactor receiving powdered whey and sodium sulfate (R9). One syringe was additionally fed molecular hydrogen (H_2).



1984; Schonheit et al., 1982). Conceivably, then, methanogenesis may have been suppressed due to competition for hydrogen.

Carbon removal in the stripped sulfidogenic reactors R2, R4, and R7 (methane production provided up to 50% of the carbon removal, section 4.1) was similar to purely methanogenic reactors fed comparable quantities of carbon. Since stripped sulfidogenic reactors could be operated consistently with a continuous production of methane (R2, R4), competition for hydrogen was not the reason for the suppression of methane production in R9-R12.

Daniels et al. (1984) showed that methanogenesis can proceed by the decarboxylation of acetate which supports the finding that competition for hydrogen was not the reason for the suppression of methane production in R9-R12.

4.2.8.3 CO_2 Limitation

In the experiments with R1-R8, zinc acetate or ferric chloride were used to absorb H_2S from the gas. At the beginning of the experiment with R9-R12, ferric chloride was used as a stripping solution to absorb H_2S; however, ARI-311C™, a proprietary iron-containing compound which was developed for the efficient, cost-effective removal of H_2S from petroleum refinery and gas well sour gas, was employed during the remainder of the experiment. It can be regenerated by aeration; consequently, it would appear ideal for a continuously operating stripping system, would eliminate the need for changing spent batch solutions of the stripping media, and would maintain a constant level of stripping.

However, it functions most effectively at pH > 7.0 which permits absorption of CO_2.

Gas from the regenerating solution in a continuous regeneration tank was collected and analyzed and was found to be 100 % CO_2. The regenerated solution has a pH>7 which is much more amenable to CO_2 absorption than the fresh solution with a pH<3. Those measurements showed that CO_2 is absorbed by ARI-311C™ when used in the operating system.

For short periods of time during the operation of R1-R12, solutions of NaOH were used to strip the H_2S from the gas. when NaOH was employed as a stripping solution: a) the reactor pH increased up to 1.5 units; b) all methane production ceased; c) the gas pressure in the system became less than the ambient atmospheric pressure resulting in long columns of liquid in the suction line of the gas pump tubing. Frequently, liquid was pulled into the suction side of the gas pumps resulting in diaphragm failure; a result which also frequently occurred when using ARI-311C™.

In order to determine the effects of various stripping solutions upon methane production, an experiment was performed using water, ARI-311C™, and NaOH as the solutions in the stripping tank (see Figure 3.1). The effluent of R9-R12 had high concentrations of acetate in the effluent. Therefore, acetate was chosen as the carbon source for the methanogenic reactor. The reactor was fed acetate as the carbon source and was producing 1.6 L CH_4/d.

When water was used, there was no effect upon the production of methane by the reactor.

When NaOH was used, only traces of CO_2 could be detected in the gas, methane production immediately ceased, the gas pressure in the reactor became negative as evidenced by changes in the liquid level in the effluent split-boxes. When the gas recycle pump was turned off, methane production returned to the pre-stripping level within 36 h.

When ARI-311C™ was used (fresh, pH<3.0), there was no inhibition of methanogenesis by the use of ARI-311C™. Since the pH of the fresh solution was low, it was concluded that carbon dioxide was not immediately absorbed by the solution. However, during the previous operation of stripped sulfidogenic reactors when ARI-311C™ was continually being regenerated, gas bubbles were observed in the regeneration tank. The gas was collected and analyzed and determined to contain 100% CO_2. Therefore, since previous experience had shown that CO_2 was absorbed by the ARI-311C™, under long-term operation, the use of ARI-311C™ most likely resulted in absorption of CO_2 from the reactors.

Methanogens are autotrophic and fix (assimilate) CO_2 during cellular synthesis in the following series of reactions (Daniels et al. 1984, Figure 4.24): CO_2 is fixed to form acetate, acetate combines with CO_2 to form pyruvate; phosphoenolpyruvate (PEP) combines with CO_2 to form oxaloacetate; succinate combines with CO_2 to form α-ketoglutarate.

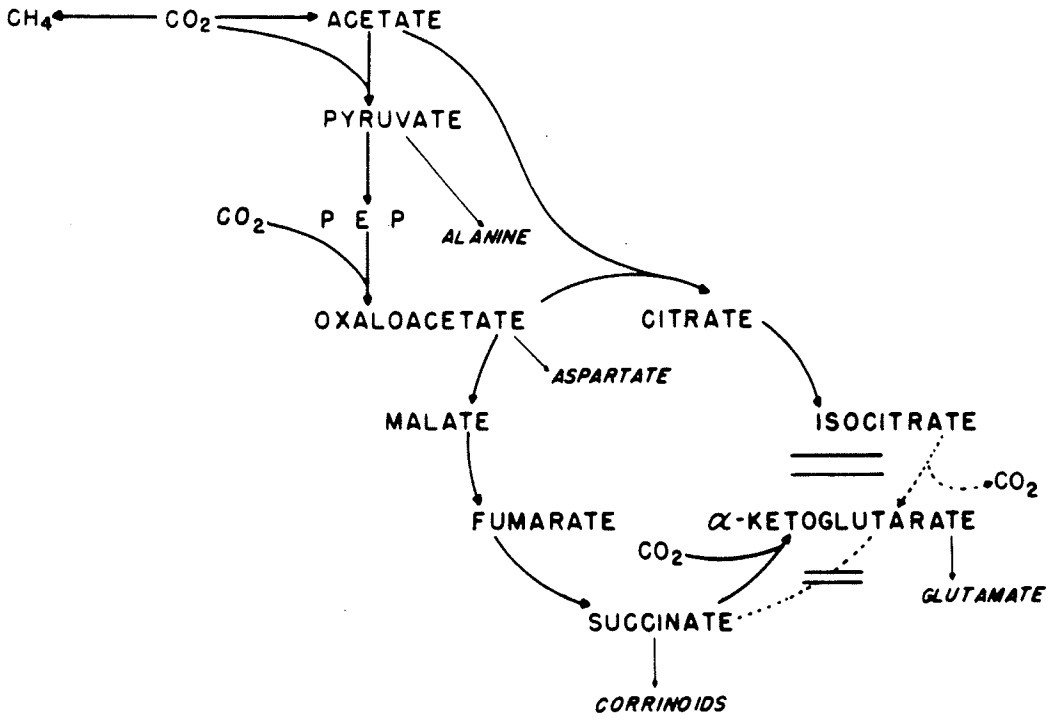


Figure 4.24 Pathways of CO_2 assimilation of methanogenic bacteria. In most genera, α-ketoglutarate is produced via succinyl CoA; in Methanothrix, it is produced via isocitrate, as shown in dashed lines. (After Daniels et al. 1984)

If CO_2 is limited (e.g. nutrient limitation) cell synthesis will cease. If there is no cell synthesis, then there will be no methane production since there is no energy demand by the cell as was shown by the removal of all residual CO_2 from a methanogenic reactor. Since it has been shown that methanogenesis can be suppressed by stripping of CO_2, this verifies that CO_2 is necessary for the metabolism of methanogens and was a limiting factor. The threshold requirement for CO_2 for methane production was not established but could be a basis for future research. Desulfovibrio baarsii has been shown to grow on CO_2 and CO as sole carbon sources (Jansen et al. 1985). Desulfovibrio vulgaris has been shown to use CO as the sole electron donor for growth and sulfate reduction (Lupton et al. 1984). According to Postgate (1984), however, sulfate reducing bacteria are mixotrophic rather than autotrophic. This means that CO or CO_2 are assimilated in the presence of other compounds such as acetate or formate.

There was no difference in sulfate reduction when NaOH was employed as a stripping solution than when $FeCl_3$ or ARI-311C™ were used. Therefore, it was concluded that sulfate reduction was not limited by the availability of CO_2 in this experiment.

Whatever stripping solution is employed, absorption of CO_2 must be avoided in order to prevent suppression of methanogenesis. Due to absorption of CO_2, ARI-311C™ is not acceptable as a stripping solution, at least when it is used at an alkaline or neutral pH. If NaOH is employed as the absorbent, it is imperative that a means be devised which precludes significant absorption of CO_2.

4.2.9 Population Distribution in R9 - R11

Taylor and Parkes (1983) showed that Desulfobacter, Desulfovibrio, and Desulfobulbus can be identified by particular phospholipid markers (C12-C18 phosphated fatty acids). For example, 10-methylhexadecanoic acid is specific to Desulfobacter. Ratios of the markers to the C16 (16:0) acid which is common to all bacteria, give the relative proportion of the particular microorganism in the sample.

Samples of sludge were collected from R9-R11 at day 225. lyophilized, and set to Dr. David C. White at the University of Tennessee, Knoxville, TN for quantification of the phospholipids.

The ratios of markers to the 16:0 acid for sulfate reducing bacteria showed large proportions of Desulfovibrio and Desulfobulbus. The laboratory report said that there were some Desulfobacter but the report sheet did not show the specific markers for Desulfobacter.

Desulfovibrio and Desulfobulbus both are incomplete oxidizing SRB. This means that the operating conditions in R9-R11 promoted the growth of incomplete oxidizing SRB and not the growth of complete oxidizing SRB such as Desulfobacter which was not unexpected according to other laboratory workers (Nanninga 1985). This was in spite of reactor acetate concentrations as high as 3000 mg/L (acetate) and reactor sulfate concentrations in excess of 500 mg/L (as SO_4). The lack of acetoclastic SRB and the abundance of incomplete oxidizing SRB suggests that the incomplete oxidizing SRB may have outcompeted the acetoclastic

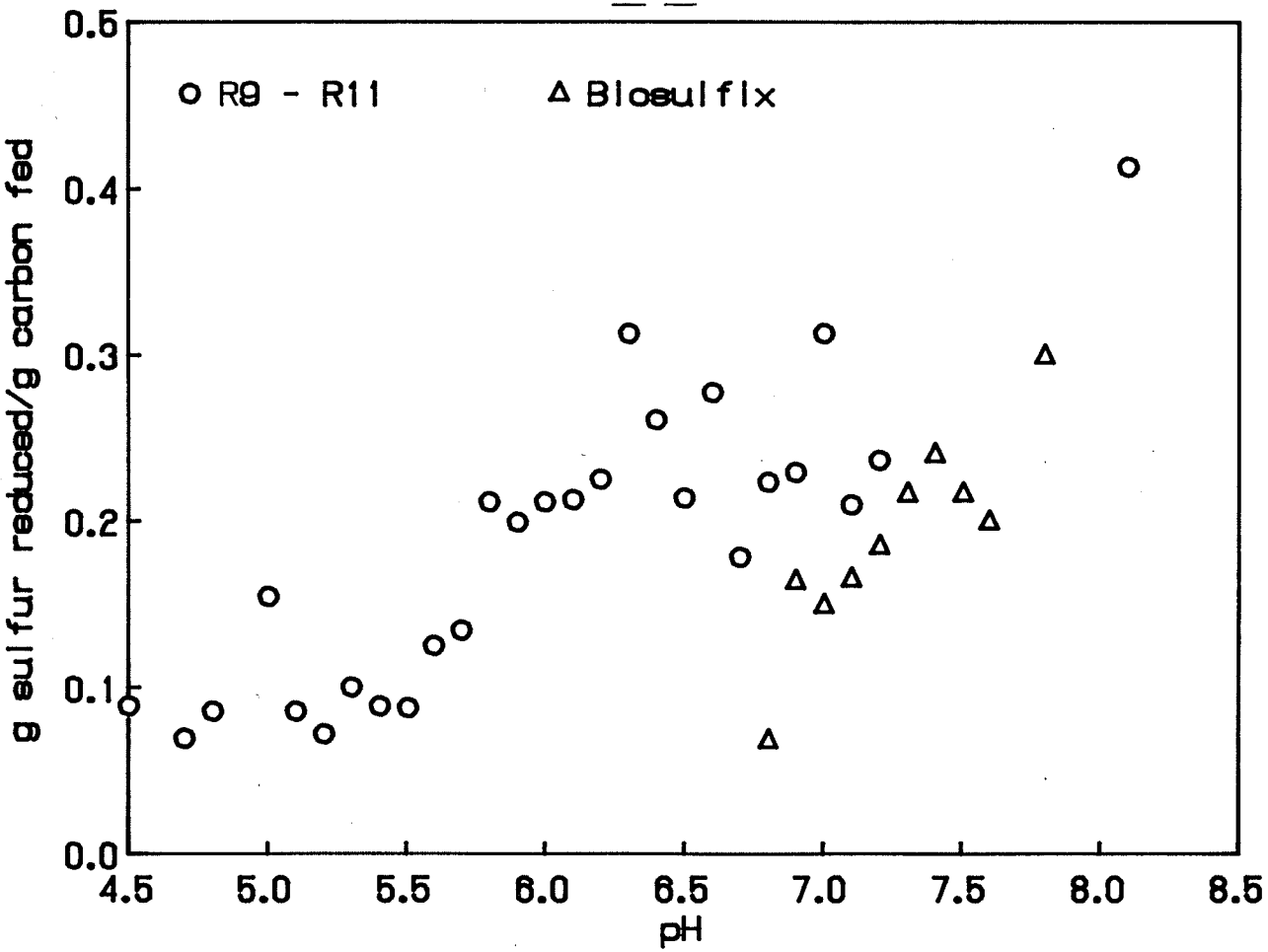
SRB for a common substrate. Since acetate was abundant and the addition of nutrients such as biotin made no difference, the common substrate may have been hydrogen. These results do strongly suggest that incomplete oxidizing SRB and acetoclastic SRB will not grow syntrophically, especially in a flow-through reactor.

4.2.10 The role of pH in the operation of stripped sulfidogenic reactors

The results of operation of the four reactors, R9 - R12, showed that sulfate reduction increased with increases in pH. In Figure 4.25, data from the Biosulfix® pilot plant (Olthof et al. 1986) has been added to the data from Figure 4.19. The data showed that the S_r/C_o increased with increases in the reactor pH, regardless of whether the feed was powdered whey and sodium sulfate in laboratory scale reactors or industrial sludge and gypsum as in the pilot plant. A regression line could be drawn through the data points which would show a linear increase in S_r/C_o with an increase in the pH.

There are two basic sets of data points: below pH 5.7 and above pH 5.8. From pH 4.5 - 5.7, S_r/C_o = 1; from pH 5.8 - 7.8, S_r/C_o = 0.2 - 0.3. The values in the figure are average values of S_r/C_o for each pH value. The raw data showed a wider variation than represented in the figure. The general trend of the data follows the same trend shown for lactic acid production as presented by Zoetemeyer et al. (1984) (Figure 1.2).

Figure 4.25 S_r/C_o versus pH for stripped sulfidogenic reactors. R9-R12 were fed reconstituted powdered whey and sodium sulfate. The Biosulfix® reactor was fed industrial sludge and calcium sulfate (gypsum) from a phosphate base fertilizer industry (Biosulfix® data from Olthof et al. 1986).



This is further evidence that, in sludge bed reactors fed whey (or other lactate precursors), the incomplete oxidizing SRB predominate in this type of system.

4.2.11 Optimal sulfur reduction in relationship to influent carbon (S_r/C_o)

The highest S_r/C_o for R9-R12 was 0.3 although the average was 0.22. The DLA Biosulfix® pilot plant (Olthof et al. 1986) operated at S_r/C_o = 0.15 - 0.25. Neither the research presented here nor the work of Olthof et al. (1986) suggest that all the available carbon in the feed could be oxidized or assimilated in support of sulfate reduction.

In the mixed culture and complex feed experiments with R1-R8, in the work of DLA (1982), and the work of Isa et al. (1986a, b), complete utilization of carbon was only attainable when both sulfate reduction and methane production occurred. These findings are in contrast with the research of Middleton and Lawrence (1977) where acetate was the sole carbon source and Saw et al. (1987) where edible oil wastes were the feed. Isa et al. (1986a, b) found that methanogens easily outcompeted sulfate reducing bacteria for acetate in the absence of a hydrogen donor such as ethanol.

Middleton and Lawrence (1977) demonstrated that acetate could be the sole carbon source for sulfate reducing bacteria. Schonheit et al. (1982) and Kristjansson et al. (1982) showed that in pure culture, below 180 mg/L acetate, SRB could outcompete MPB for acetate and H_2. Above 180 mg/L acetate, the rate of

methane production was equal to the rate of sulfur reduction which coincides with the findings with R1-R8 and the published findings of Isa et al. (1986a, b).

The studies of Middleton and Lawrence (1977), Schonheit et al. (1982) and Kristjansson et al. were conducted under conditions of low concentrations of S^{2-} in the reactors. The precise S^{2-} concentrations were not stated. However, Middleton and Lawrence (1977) added sufficient $FeCl_3$ to precipitate all the S^{2-}. Schonheit et al (1982) and Kristjansson et al. (1982) purged with 80% N_2/20% CO_2 at 50 mL/min to purge H_2S. Saw et al. (1987), on the other hand, demonstrated that better than 90% of the available carbon could be used in the support of sulfate reduction in an unstripped mixed culture reactor receiving edible oil plant wastewater.

The research presented here for R1-R12, the work of Olthof et al. (1986), DLA (1982), Maree and Strydom (1987), and Isa et al. (1986a, b) indicate that in mixed culture reactors, especially under conditions of complex feed, sulfate reducers do not outcompete methanogens for acetate. With few exceptions (R1, day 184), in the absence of significant methane production, carbon removal is limited to 40-45% efficiency.

In other work yet unpublished, McKinney (1986) has shown that in the presence of 1000 mg/L sulfides and at a reactor pH 8.4, methanogens outcompete sulfate reducers for acetate and provide over of 90% removal efficiency with little carbon flow having been diverted towards sulfate reduction. Kroiss and Wabnegg (1983) and Kroiss (1987) found that sulfate reduction and methane production

occur concurrently in full scale reactors treating citric acid plant wastes (COD = 17 g/L, SO_4-S = 0.95 g/L, CH_4 = 0.324 L/g COD removed, COD removal = 90%, S_r/C_o = 0.03). The calculated reactor sulfide concentration in Kroiss' (1987) reactor was 450 mg/L. His specific methane yield was 7.4% less than the expected 0.35 L/g which means that 7.4% of the carbon flow (0.42 g TOC/L) was diverted towards sulfate reduction which was the same as reported by Isa et al. on acetate.

In all the above noted research, in the presence of complex substrates which can be fermented to lactate, incomplete oxidizing SRB perform sulfate reduction; the remaining carbon is removed by methanogens. In only one case (Saw et al. 1987) has a complex substrate reportedly been completely utilized in support of sulfate reduction.

The sulfur reduction for several different substrates and reactors is shown in Table 4.6. Sulfur reduction for sulfidogenic reactors ranged from 0.08-1.43 S_r/C_o.

In the research presented here, consistent steady-state sulfur reduction yielded a S_r/C_o = 0.32-0.42 which was the same as found by Olthof et al. (1986). The stripped sulfidogenic reactor R2 exhibited the best S_r/C_o = 0.55 but still only attained 73% removal of organic carbon, although this evaluation was based upon a small sample size.

The low S_r/C_o noted at day 180 for R2 indicates that long acclimation times were required in order to achieve the optimal S_r/C_o which reflected the low growth rates of the acetoclastic

Table 4.6. Sulfide yields from various substrates during high-sulfate anaerobic digestion in mixed cultures.

Carbon	Sulfide yield S_L g/L·d	S_r/C_o (g/g)	S_r/C_r (g/g)	U_S	% Rem	System	mL CH_4 / g COD rem	S^{6+} to S^{2-} (%)	Reference
Pet Food	0.45	.16	.23		75	A.G.	*	75-85	Celanese (1984)
Primary sludge	.75			0.04	50	S.G	0		Smith & Middleton (1980)
Acetate	1.1	1.4				A.G.	0	92	Cork (1982)
Municipal Sludge	0.5			0.01	44	S.G.	0		Olthof et al. (1986)
Industrial Sludge	0.7	.25	.59	0.07	37	B.	0.07		Olthof et al. (1986)
Acetate	0.16	0.08			97	A	0.24	31	Isa et al. (1986)
Ethanol	.5	.167			91	A.G.	0.34	99	Isa et al. (1986)
Molasses	0.29	.53	1.2		50	A.G.		75-95	Maree et al. (1986)
Whey Unstripped	0.8	.32			75	S.B.	0	63-93	this study
Whey Stripped	0.48	.56			57	S.B.	0.33	40	this study
Whey Stripped	0.72	.34			92	S.B.	0.17	95	this study
Whey Stripped	1.5	.34			73	S.B.	0.17	67	this study
Whey Stripped	1.3	.42	.67	0.08	40	S.B.			this study
Edible Oil		.56	.63			S.G.			Saw et al. (1987)
Edible Oil		.48	.59			A.G.	0		Saw et al. (1987)

S.G.: Suspended growth; A.G.: Attached growth; B: Biosulfix; S.B.: Sludge Bed;
* Not measured but 70% CH_4 in gas

SRB. This long period could explain the observation that methane production in stripped sulfidogenic reactors gradually decreased. This optimal utilization occurred at a B_V = 2.0 g/L·d (TOC) which was well below the 3-6.5 g/L·d TOC loading used for R9 - R12. If R9-R12 had been operated at this lower rate, greater stability might have been achieved. The work of Olthof et al. (1986) corresponded with the experience with R9-R12 where sulfur reduction was limited by the production of lactate.

The work of Middleton and Lawrence (1977) implied that in the presence of acetate, sulfate reduction should proceed when more than 30 mg/L sulfate is present. Schonheit et al. (1982) and Kristjansson et al. (1982) showed that sulfate reducers (K_S = 0.2mM acetate) could outcompete methanogens (K_S = 3 mM acetate) for acetate when the acetate concentration was less than 180 mg/L. However, above 300 mg/L acetate, the carbon flow was equally distributed between sulfur reduction and methane production.

Based upon the above mentioned work, it was expected that all the carbon fed to the stripped reactors R9-R12 would be diverted towards sulfate reduction. This did not occur; less than 40% of the carbon was utilized and more than 3000 mg/L acetate (1200 mg/L TOC) and more than 500 mg/L sulfates (167 mg/L S^{6+}) remained in the effluent which would have been expected if incomplete oxidizing SRB rather than completely oxidizing SRB were active. An analysis of the microbial population verified that the sulfate reducing bacterial population was primarily composed of

incomplete oxidizing sulfate reducing bacteria, Desulfovibrio spp. and Desulfobulbus spp.

Hydrogen has been shown to be necessary for sulfate reduction by Desulfovibrio (Odum and Peck, 1981). Isa et al. (1986a, b) showed that the addition of hydrogen or an hydrogen donor (ethanol) enhanced sulfate reduction in reactors fed acetate or formate in the presence of high concentrations of sulfate. Saw et al. (1987) showed that the entire carbon flow could be diverted to sulfate reduction with a carbon removal of 90% when using edible oils as the carbon source. Under aerobic conditions, fatty acids are dissimilated by β-oxidation (Zubay 1984). If an analogous process occurs under anaerobic conditions, then it would be expected that a large quantity of hydrogen would be available to sulfate reducers. This availability of hydrogen could explain the success of Saw et al. (1987) when using edible oils.

It has been shown that incomplete oxidizing SRB predominated when reconstituted powdered whey was supplied as the carbon source. It has also been shown that both incomplete and complete oxidizing SRB require hydrogen for sulfate reduction.

Incompletely oxidizing SRB have a high growth rate with hydrogen ($\mu = 0.21\ h^{-1}$, Badziong and Thauer 1978). Therefore, because of the requirement for hydrogen, there should only be a very limited success in maintaining the incomplete and complete oxidizers in the same reactor.

In conclusion, when lactate or lactate precursors are present in the feed of an anaerobic reactor receiving high sulfate wastes,

sulfate reduction will proceed via the incomplete oxidation of lactate to acetate and carbon dioxide. This explains the results of Olthof et al. (1986), DLA (1982), Maree and Strydom (1987) and the results for R1 - R14 as described in this research.

4.2.12 Maximum Sulfur Yield

The maximum rate of sulfur reduction, S_r was 1.3 g/L·d (Figure 4.16) at B_V = 2.2 g TOC/L·d. This is nearly as high as has been reported for sulfidogenic reactors (Table 4.6). In reactor R9 - R12, the carbon loading was 3-6.5 g/L·d. At this loading rate, pH control was difficult and frequently dropped below 6.0.

4.2.13 Kinetics

Many attempts were made to calculate the k_d, μ_m, V_{max}, K_m, K_S, and K using Monod, Michaelis-Menton, and Grau equations (Benefield and Randall, 1980; Gaudy and Gaudy, 1980; Grau et al., 1975). The data are not reported here because there was a large scatter in the data which resulted in a very large uncertainty in the results. The first conclusion following such failure would be that there were gross errors in the experimental technique and that steady-state conditions were not achieved. However, examination of Figures 4.11-4.12 shows that steady-state conditions were achieved with respect to sulfur reduction.

Data from Olthof et al. (1986) (SOC removed, C_r, vs SOC load, B_V, Figure 4.19) showed a similar wide variation in carbon removal. Examination of that data for sulfur reduction showed a similar non-steady-state sulfur reduction. Based upon the comparison of data from R9-R11 and the Biosulfix® data, the day to

day variation in sulfur removal and TOC removal from this process is due to factors other than those associated with the experimental procedure.

4.2.14 Alkalinity Generation

The products of the mixed acid anaerobic fermentation of glucose (acetate, butyrate, carbon dioxide, ethanol, formate, hydrogen, lactate and propionate) are dependent upon the particular microorganism and reactor pH (Gaudy and Gaudy 1980; Zoetemeyer et al. 1982). The fermentation of whey powder has given similar results (Chartrain and Zeikus 1986). Zoetemeyer et al. (1982) showed that the proportion of each fermentation product is dependent upon the pH and dilution rate.

The ratio of volatile acids/alkalinity must be less than 0.5 g/g (1 M/M) in order to maintain stability in an anaerobic reactor. When this ratio is exceeded, the reactor becomes unstable and becomes acidogenic, maintaining a low pH with the accompanying loss of methane production and, in this study, loss of sulfate reduction.

If one assumes that 82% of lactose is fermented to lactic acid and 18% to other VFA (Chartrain and Zeikus 1986), then for each gram of lactose (0.4 g TOC), 10.25 mM (820 mg/L) lactate will be generated and 2.5 mM (150 mg/L) acetate will be generated. If all the lactate is incompletely oxidized to acetate and CO_2 from the reduction of Na_2SO_4, then the result is 10.25 mM $NaHCO_3$ (861 mg/L), and a total of 13.25 mM (795 mg/L) acetate. This would result in an equilibrium pH of approximately 5.6. In order to

maintain an equilibrium pH of 7.0, 13.25 mM (663 mg/L) alkalinity (as $CaCO_3$) addition would be required.

If all the acetate is converted to methane, then there would be 10.25 mM $NaHCO_3$ (861 mg/L) and 13.25 mM CO_2 (583 mg/L) generated. Depending upon the residual CO_2 in solution, the pH could be as high as 8.4.

4.2.15 Summary

Sulfur reduction was a direct function of the available carbon, both in quantity (B_V) and in form, primarily lactate. This was evidenced by a direct relationship to pH and enhanced sulfur reduction in direct proportion to the addition of lactate to the feed. It was hypothesized that the availability of carbon was due to pH dependent changes in the products of fermentation as shown by Zoetemeyer et al. (1982).

The generation of excess alkalinity in a sulfidogenic reactor was more than offset by the generation of organic acids and CO_2. It was shown that the organic acids, primarily acetate, must be removed in order to take advantage of the additional alkalinity. In the absence of acetoclastic SRB or MPB, pH control became difficult and resulted in unstable reactor operation.

The generation of methane requires the availability of free CO_2. It was shown that removal of CO_2 from a methanogenic reactor (and by extrapolation from a stripped methanogenic reactor) suppressed methane production.

4.2.16 Conclusions

1. The production of methane is required if maximum carbon removal is to be achieved.

2. The absorption of CO_2 from a bioreactor will suppress methanogenesis by limiting available CO_2 for cell synthesis. Consequently, the use of NaOH or any other solution such as ARI-311C™ which absorbs CO_2 is inappropriate due to the absorption of CO_2.

3. In the absence of methane production, additional alkalinity must be added to the feed or reactor when using a low alkalinity complex feed such as reconstituted powdered whey. Sulfate reduction alone did not generate sufficient alkalinity to offset the acid generation phase.

4. Excess alkalinity was generated in sulfidogenic-methanogenic reactors due to the conversion of organic acids to methane and CO_2 thereby minimizing any need for the addition of alkalinity. Therefore, the requirement for the addition of alkalinity is a function of the removal of acidity from the reactor.

5. The maximum rate of sulfur reduction was less than 1.5 g S_r/L·d. It is hypothesized that this was due to reactor limitations upon the generation of lactate.

6. Sulfate reduction in stripped sulfidogenic reactors requires maintenance of an optimal pH between 6.0 and 7.5. This is due to the relationship between pH and the production of acids and not activity of the SRB.

7. Analyses of biomass from R9, R11, and R14 showed that incomplete oxidizing SRB (Desulfovibrio and Desulfobulbus) were the predominant species of sulfate reducing bacteria.

8. It is hypothesized that incompletely oxidizing SRB were predominant due to their ability to out-compete acetoclastic SRB for hydrogen.

4.3 BATCH STUDY RESULTS

The preceding continuous-culture studies left unanswered questions concerning the effects of total sulfides and un-ionized hydrogen sulfide upon lactose utilization, methanogenesis, and sulfate reduction. The following batch culture studies were conducted in an attempt to answer those question.

4.3.1 Effects of sulfides upon lactose utilization

Reconstituted powdered whey, 68% lactose, was the carbon and nutrient source in R9 - R11; liquid whey was used in R1 - R6. Lactose, (a major component of milk solids and whey), is a disaccharide, galactose-β-1,4-glucose (Zubay, 1984). One mole of lactose is dissimilated to two moles of glucose-6-phosphate during glycolysis (Zubay 1984). In this study, the rate of utilization or uptake of lactose is used as a measure of glycolysis.

The effects of differing sulfide concentrations upon lactose utilization at different pH values is shown in Figure 4.26. The most rapid uptake of lactose was observed at pH 6.0 in the absence of sulfides. At 100 mg S^{2-}/L, no difference in the uptake rate could be detected, regardless of the pH. Above 100 mg S^{2-}/L, the optimum uptake occurred at pH 8.0; minimum uptake occurred at pH 6.0.

The induction period (period before uptake commenced) decreased with increases in the pH. At 1000 mg S^{2-}/L, lactose uptake was completed after 10 h at pH 8.0, and 24 h at pH 7.0; uptake remained incomplete after 200 h at pH 6.2.

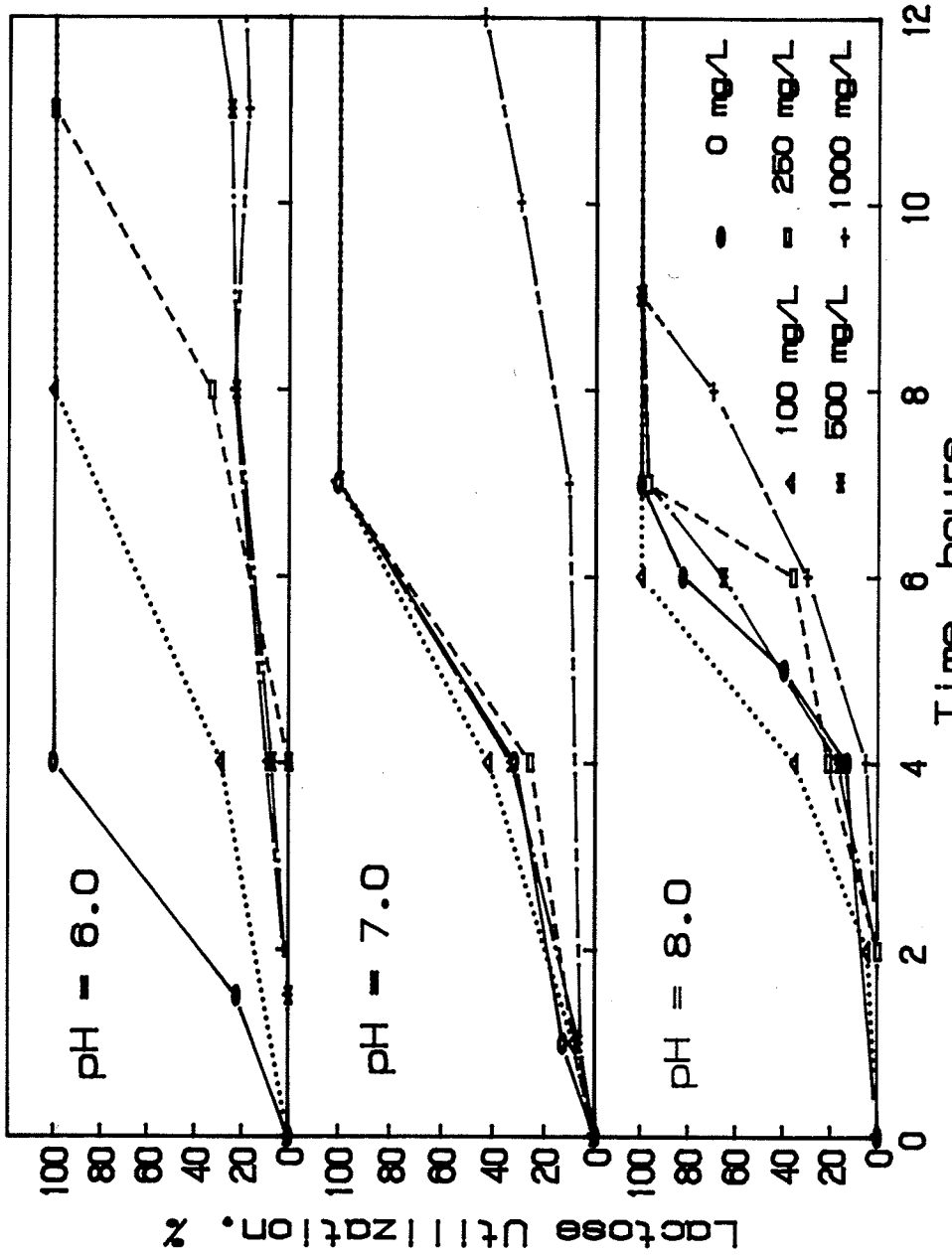


Figure 4.26 Lactose utilization or uptake versus time for syringes fed reconstituted whey powder and varying concentrations of sodium sulfide. The syringes were run in three series at initial pH values of 6.0, 7.0, and 8.0

The lactose uptake gradually increased with increasing concentrations of sulfides. It must be concluded that sulfides inhibit and retard the metabolism of the microbes which utilize lactose, presumably the fermentative or glycolytic microorganisms.

Since lactose uptake decreased with a decreased pH at a given concentration of sulfides, and since un-ionized H_2S decreases with increasing pH (Equation 1.7, Figure 1.2), these results show that sulfide toxicity to the rate of lactose uptake can be minimized simply by increasing reactor pH. As an example, in Figure 4.26, at pH 8.0, 1000 mg S^{2-}/L corresponds to 89.3 mg H_2S/L (Figure 1.2, equation 1.7). Comparable concentrations of un-ionized H_2S were 180 mg S^{2-}/L at pH 7.0 and 104 mg S^{2-}/L at pH 6.2.

Inhibition of lactose uptake due to un-ionized H_2S is shown in Figure 4.27. The data base is the same as in Figure 4.26. A lowered lactose uptake implies a lowered rate of fatty acid formation. After 4 hours, 20-30% uptake of the lactose was observed up to 250 mg/L H_2S. After 10 hours, 100% lactose uptake was observed up to 250 mg/L H_2S. There was little difference in lactose uptake between 10 hours and 24 hours when the un-ionized H_2S was above 250 mg/L. However, after 228 hours (9 days), 100% uptake was achieved at 500 mg/L H_2S and 80% at 950 mg/L H_2S.

The latter finding demonstrates that the effects of sulfide toxicity can be ameliorated by increasing the retention time. This finding implies that the metabolic rate of the microorganisms was lowered by the presence of high concentrations of un-ionized H_2S.

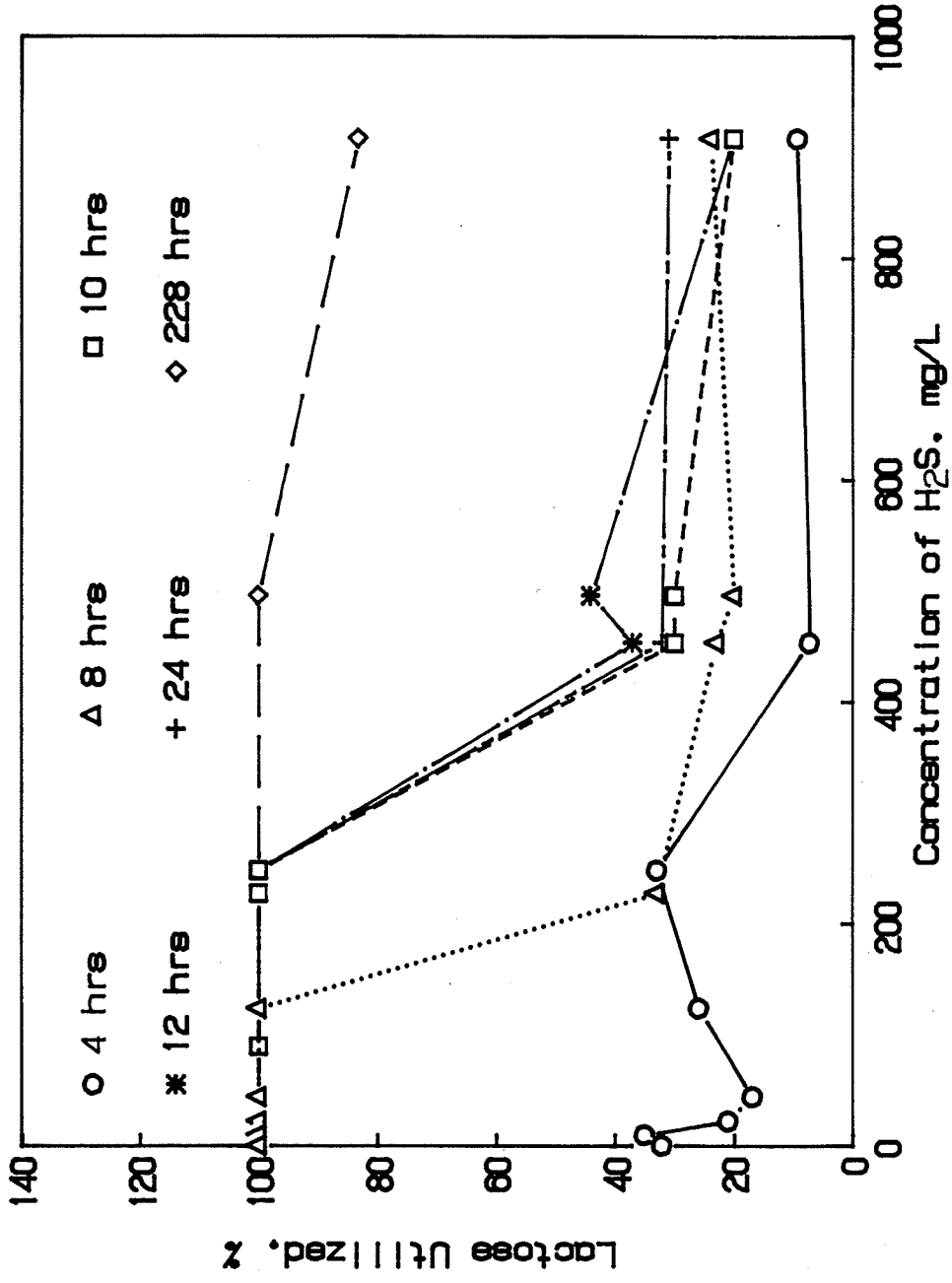


Figure 4.27 Lactose utilization versus concentration of calculated unionized H_2S. The syringes were fed reconstituted whey powder and varying concentrations of sodium sulfide. The syringes were run in three series at initial pH values of 6.0, 7.0, and 8.0.

In terms of reactor engineering, this would mean increasing the retention time in the reactor. This increased retention time could be in the form of an increase in either the hydraulic (HRT) or solids residence times. The nature of the batch test precluded differentiating between SRT and HRT.

Kroiss and Plahl-Wabnegg (1983) noted a decrease in the volatile fatty acid concentration and a decrease in the COD removal in digesters in which un-ionized H_2S was greater than 200 mg/L. This means that in the presence of high concentrations of H_2S, the complex carbohydrates fed to an anaerobic reactor will not be broken into the simpler fatty acids which are the source of energy and carbon for either sulfate reducing bacteria and methanogens.

If lactose utilization is inhibited, then process failure in an anaerobic reactor will occur due to substrate limitation to the succeeding bacterial groups (SRB, MPB). Since lactose utilization was inhibited by H_2S and not total sulfides, sulfide toxicity can best be controlled either by maintaining a pH > 8.0 or by increasing the retention time in the reactor.

4.3.2 Effects of Sulfides upon Methanogenesis

The activity of methanogenic bacteria was measured in terms of acetate utilization. Figure 4.28 is a plot of percent acetate utilization versus time. The initial pH values were 6.0, 7.0, and 8.0 with final pH values of 6.5, 7.4, and 8.0. At pH 6.5, acetate utilization was completely inhibited at 500 mg/L S^{2-} which corresponds to 450 mg H_2S/L.

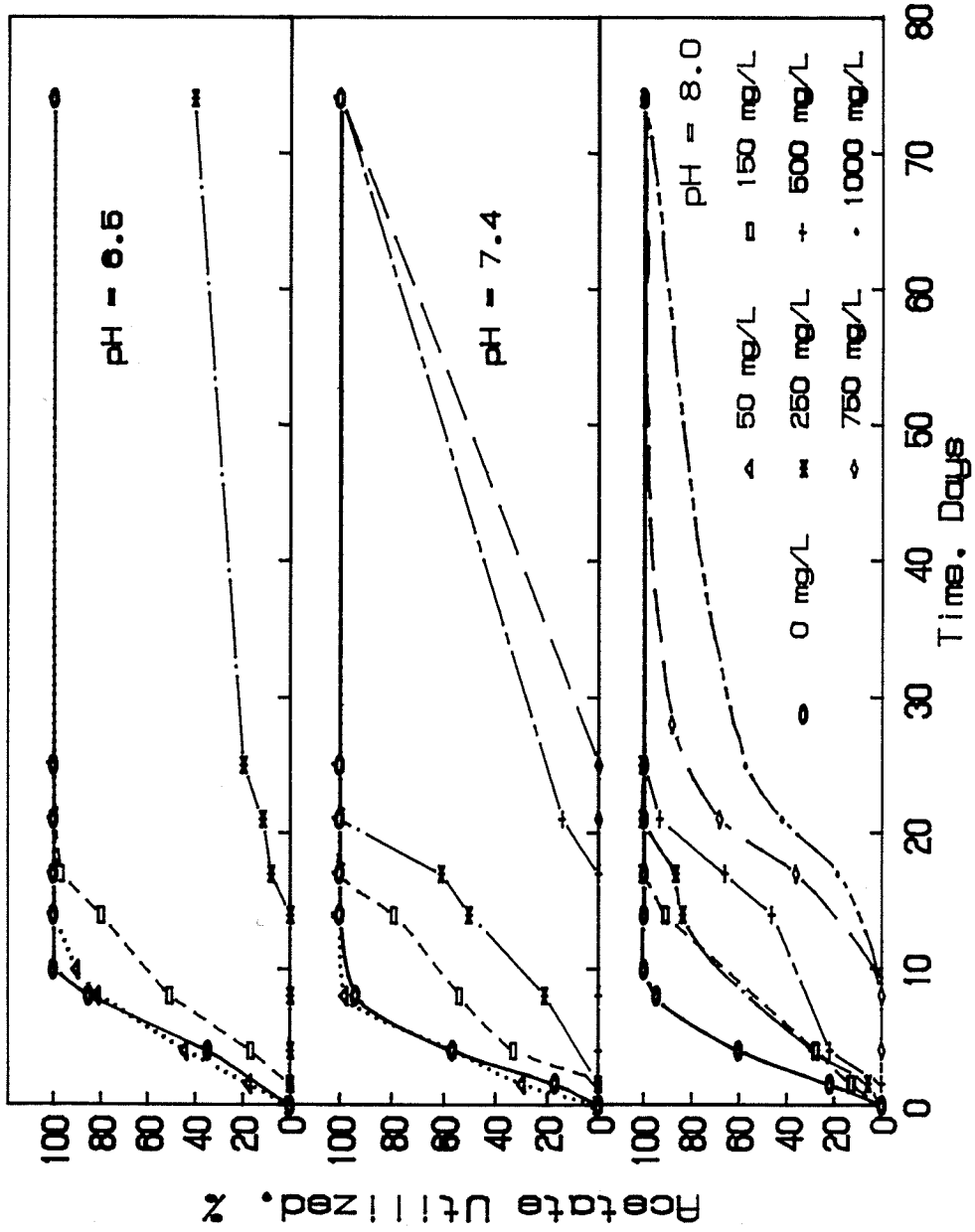


Figure 4.28 Acetate utilization versus time for syringes fed acetate and varying concentrations of sodium sulfide at three final pH values, 6.5, 7.4, and 8.0.

Figure 4.29, using the same data base as Figure 4.28, presents acetate removal versus the calculated un-ionized H_2S. This figure shows that with increased time, acetate was completely removed at greater and greater concentrations of H_2S. After 8 days, 100% of the acetate was removed at 20 mg/L H_2S but only 50% of the acetate was removed at 45 mg/L H_2S. At day 17, 100% was removed at 60 mg/L H_2S and 60% at 90 mg/L H_2S. After 25 days, 100% was removed at 90 mg/L H_2S. After 75 days, 100% was removed at 175 mg/L H_2S.

The lowered rates of acetate removal at increasing concentrations of H_2S show that the metabolism of the methanogens was decreased by increased concentrations of un-ionized H_2S. This decrease in the rate of utilization suggests that longer retention times will be required to maintain maximum carbon removal in reactors with elevated concentrations of sulfides. This corresponds with the finding with lactose uptake where the effects of sulfide toxicity were ameliorated by increasing the retention time.

Kroiss and Plahl-Wabnegg (1983) concluded that in the presence of inhibition due to H_2S toxicity, an increased sludge age was necessary for optimal process efficiency. This corresponds with the findings of this study which have demonstrated that the effects of sulfide toxicity can be minimized by increasing the retention time. Kroiss (1987) has reported success in the full-scale upflow sludge bed reactor treatment of citric acid plant waste in the presence of more than 400 mg/L S^{2-} (117 mg/L H_2S).

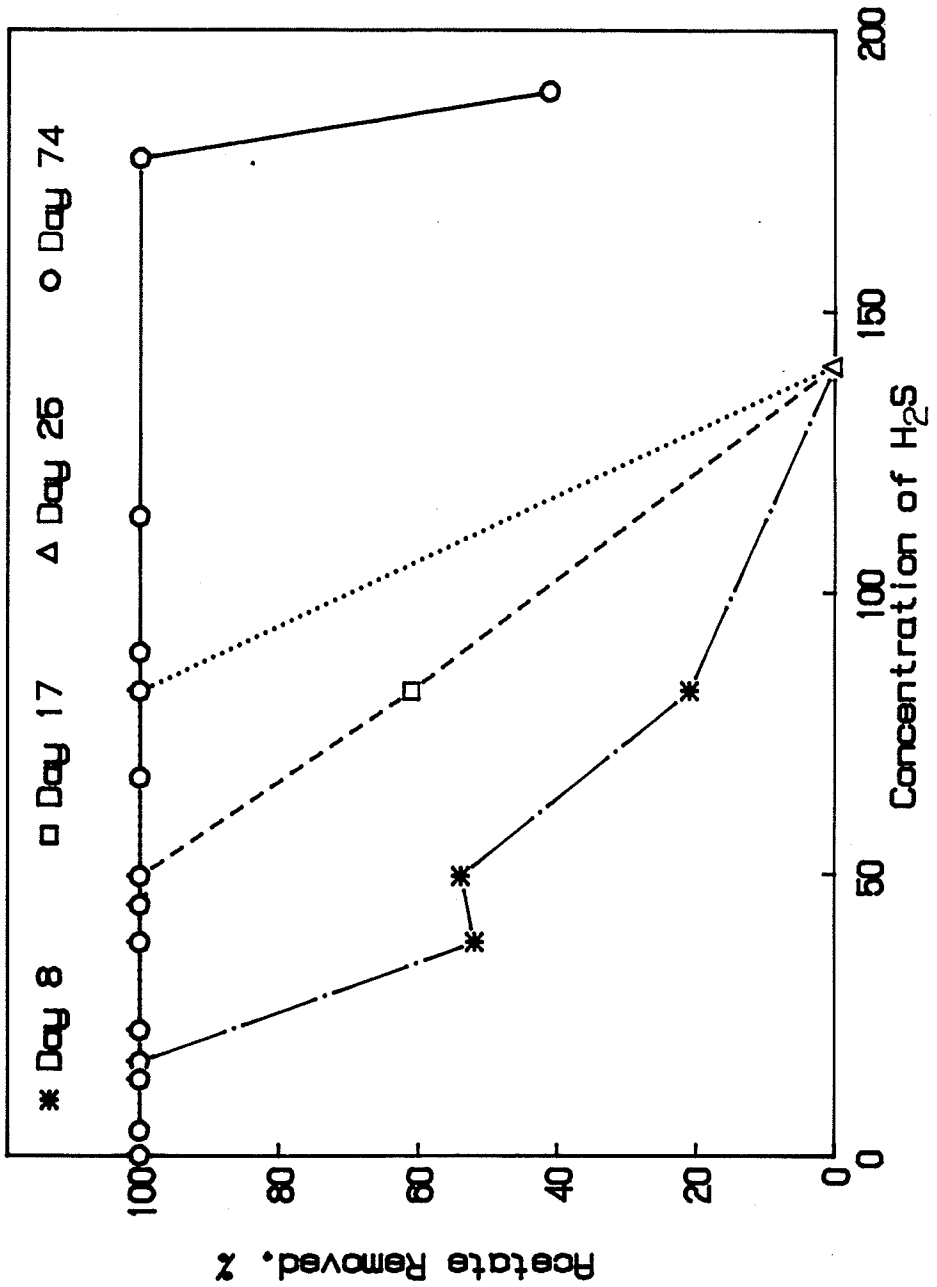


Figure 4.29 Acetate removal versus concentration of calculated unionized H_2S.

Koster et al. (1986), using granular sludge, found that acetate uptake was lower at pH 6.0 than at pH 8.0. This contrasts with the study presented here where acetate uptake was independent of pH in the absence of sulfides (Figure 4.28). However, both studies have shown inhibition at un-ionized H_2S concentrations as low as 50 mg/L (Figure 4.24). Koster et al. (1986) found that inhibition of methanogenesis due to total sulfide concentration was similar at pH 7.0-7.2 and at pH 7.8-8.0 which contrasts with the findings of the research reported here where inhibition of methanogenesis was directly correlated to the concentration of un-ionized H_2S.

Extrapolation of the data of Koster et al. (1986) suggests that 100% inhibition of methanogenesis would occur at 700 mg/L total sulfides at pH 6.0 (630 mg/L H_2S) and 2000 mg/L sulfides at pH 7.0-8.0 (1000 mg/L H_2S at pH 7.0, 180 mg/L H_2S at pH 8.0). Extrapolation of that data verifies that increased retention times can be used to compensate for the lowered acetate utilization (and by implication, methane production) due to metabolic inhibition by un-ionized H_2S.

4.3.3 Effects of Sulfides upon Sulfate Reduction

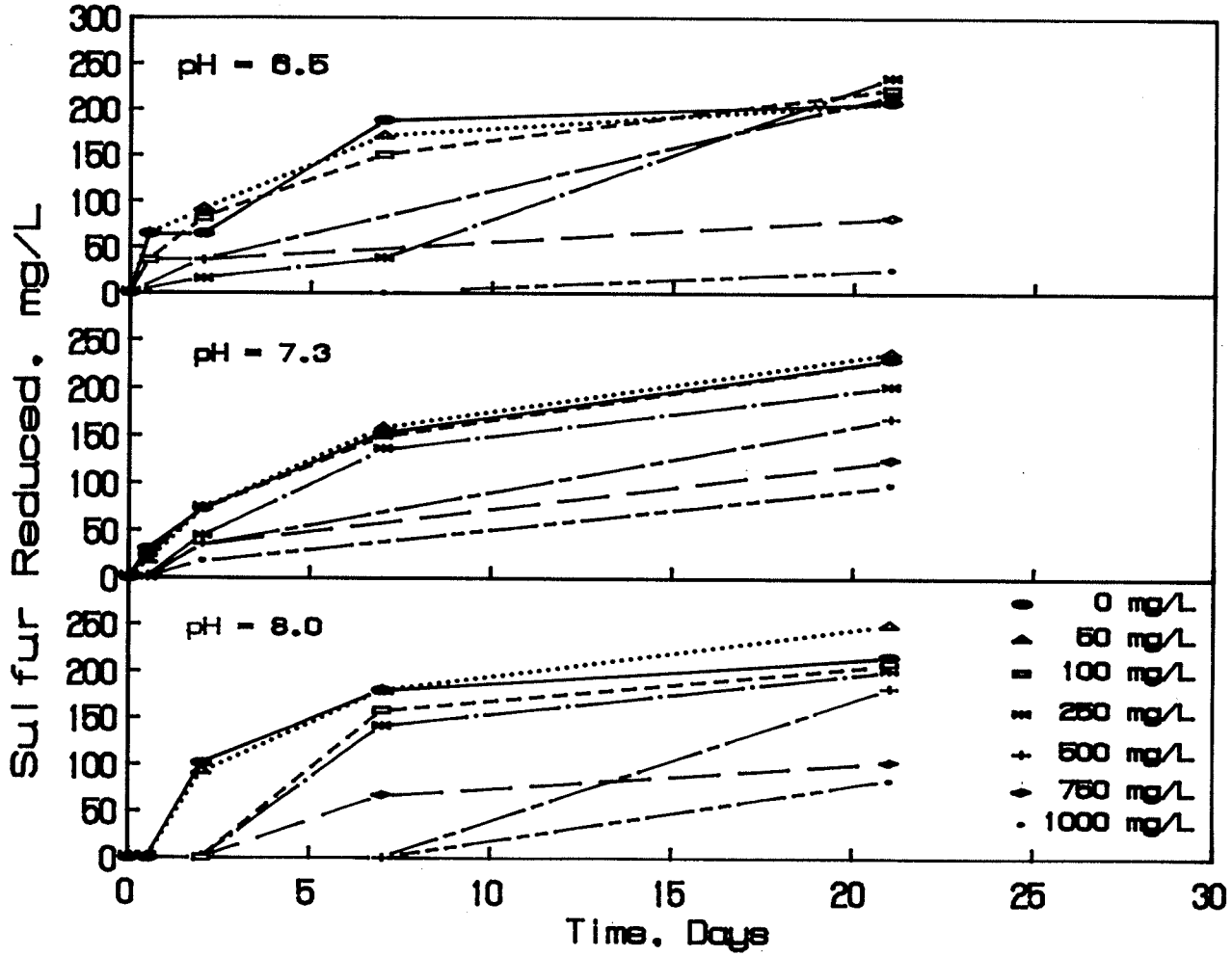
The operation of the continuous flow sulfidogenic reactors raised questions regarding the effects of increased concentrations of sulfides upon the sulfate reducing process. It was shown that the incompletely oxidizing sulfate reducing bacteria were predominant in reactors R9 and R11. The lactate ion, the preferred carbon source for incomplete oxidizers, was the carbon source for

this batch culture experiment which was conducted in glass syringes in a 35°C water bath.

The sulfate reduction rate decreased with increases in sulfide concentration (Figure 4.30) which suggests that increased concentrations of sulfides affect sulfate reduction by decreasing the metabolic rate of the SRB. This is similar to the conditions observed for lactose uptake and methanogenesis in which increased concentrations of sulfides directly retarded the rate of metabolism. However, in the cases of lactose uptake and acetate uptake, inhibition due to S^{2-} was alleviated with increased pH values. In the case of sulfate reduction, inhibition was not alleviated by increasing the pH. This is illustrated in Figure 4.31 where sulfate reduced was dependent upon the initial sulfide concentration, regardless of the pH.

The relationship between sulfur reduced, initial sulfide concentration and pH in syringes fed lactate and sodium sulfate is shown in Figure 4.31. Sufficient lactate (C_o = 440 mg/L TOC) was fed for 222 mg/L sulfur to be reduced by incomplete oxidizing SRB. The data from the 21 day point was used to plot sulfate reduction versus the initial sulfide concentration. The data values for all three pH values are similar. If inhibition were due to un-ionized H_2S rather than total S^{2-}, then more sulfur would have been reduced at pH 8.0 than at pH 6.4 for any given initial sulfide concentration.

Figure 4.30 Sulfur reduction versus time for syringes fed lactate, sodium sulfate, and varying concentrations of sodium sulfide. The results are for three series of syringes with the final pH values, 6.5, 7.3, and 8.0.



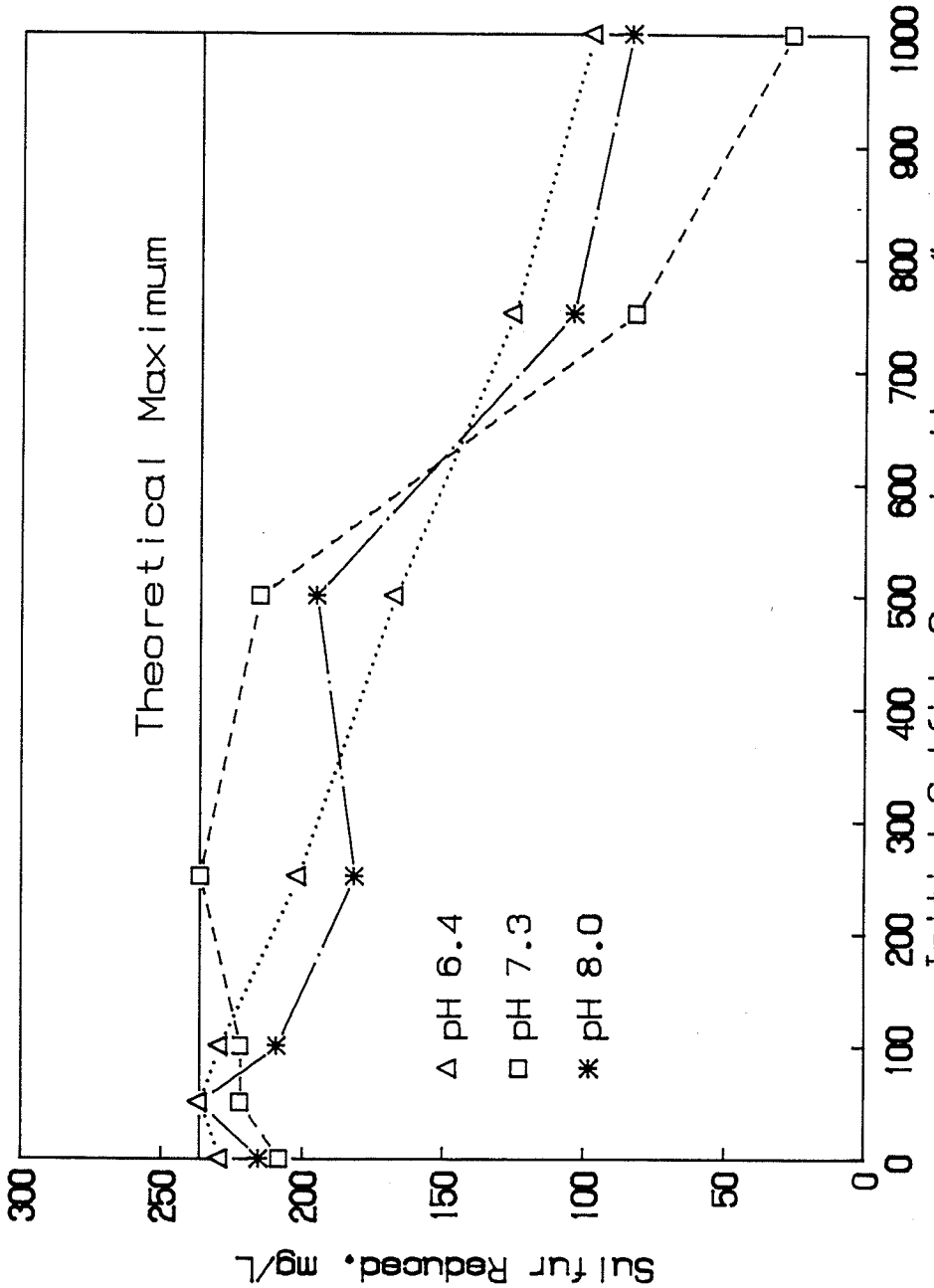


Figure 4.31 Sulfur reduced versus initial sulfide concentration for syringes fed lactate (C_o = 440 mg/L), sodium sulfate (S_o = 533 mg/L), and varying concentrations of sodium sulfide.

A different set of syringes was maintained at pH 7.0, with sufficient lactate (C_o = 6000 mg/L TOC) to permit the reduction of 2000 mg S^{6+}/L (Figure 4.32). No sulfur reduction and no carbon removal were measured in the syringe containing 1600 mg S^{2-}/L. The maximum sulfur reduction was 1095 mg S^{6+}/L when the initial sulfide concentration was less than 100 mg S^{2-}/L. There was little difference in sulfur reduced when the initial sulfides were less than 150 mg/L. However, above 150 mg/L S^{2-}, there was a significant inhibition of sulfur reduction as related to the maximum value.

Figure 4.32 shows that sulfate reduction would not proceed when the total sulfide concentration was above 1100 mg/L S^{2-}, regardless of the availability of carbon or sulfate.

The data from Figures 4.31 and 4.32 are combined in Figure 4.33, a plot of final total sulfides versus initial sulfides in syringes fed lactate and sulfate. The solid line through the origin represents the concentration of sulfides added to the syringes in the form of Na_2S which is the lower limit of the final concentration of S^{2-} in the syringes. The upper dashed line is the final total sulfide concentration (initial plus reduced) for the syringes fed 6000 mg/L TOC and 2000 mg/L sulfate-sulfur. The total sulfide concentration was less than 1200 mg/L S^{2-} and plotted as a horizontal line. This is the maximum concentration attainable.

The middle set of lines represent the total reduced sulfur for syringes at the final pH values of 6.4-8.0 in which sulfate reduction was intentionally carbon limited. The three lines are nearly congruent which again demonstrates that variations in pH did not

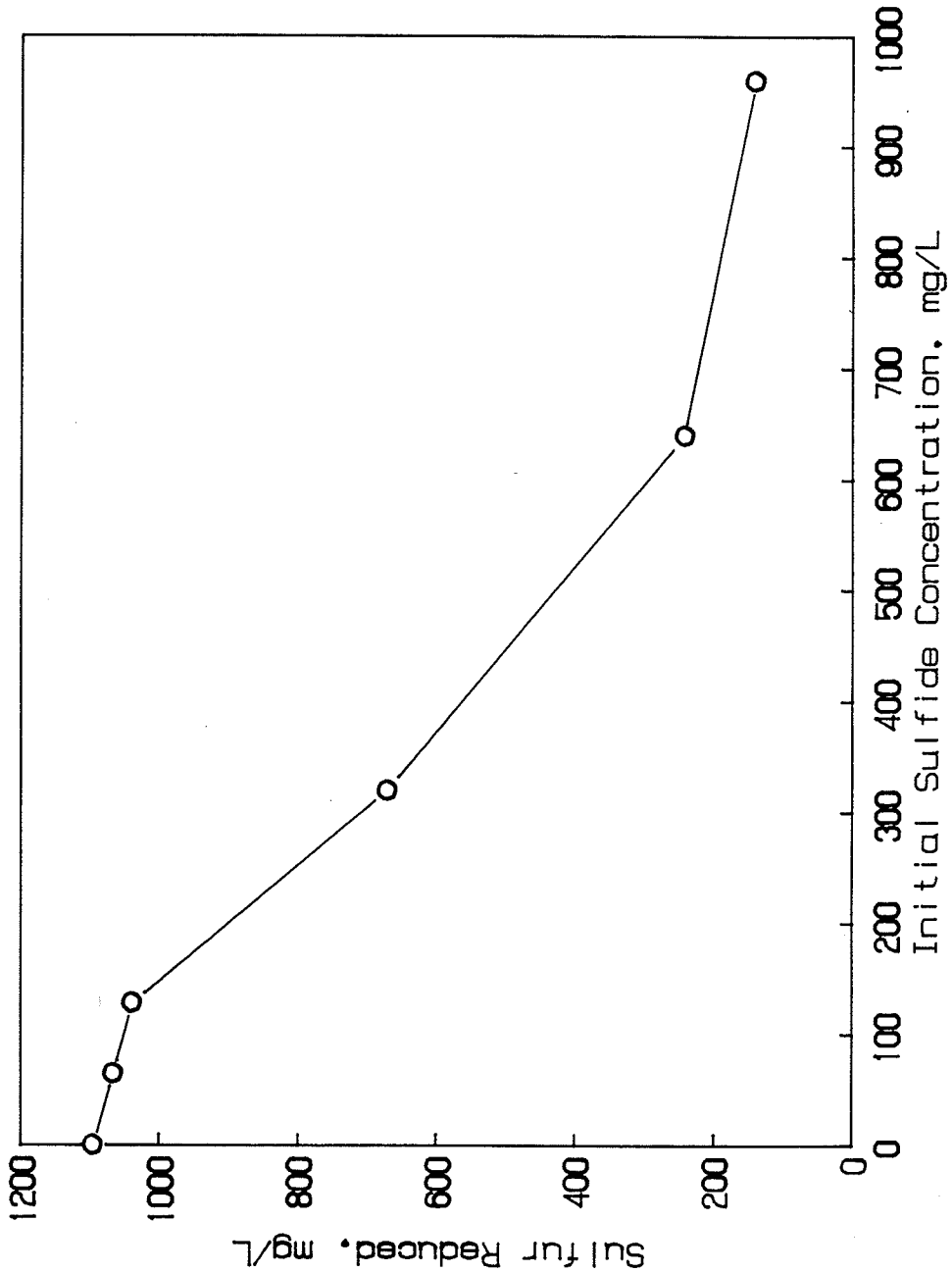
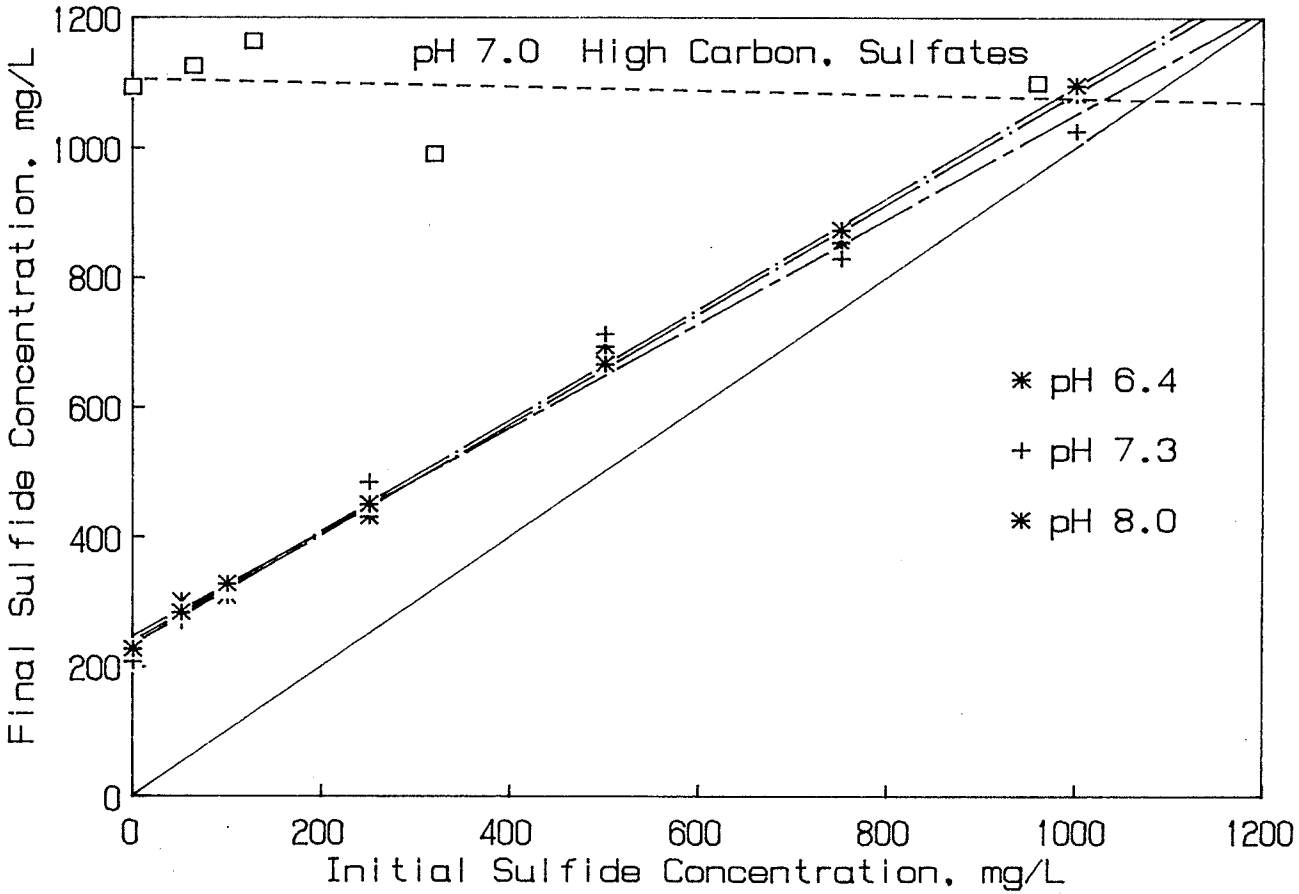


Figure 4.32 Sulfur reduced versus initial sulfide concentration for syringes fed lactate (C_o = 6000 mg/L), sulfate (S^{6+} = 2000 mg/L), and varying concentrations of sodium sulfide.

Figure 4.33 Final syringe sulfide concentration versus initial syringe sulfide concentration. Syringes were fed lactate, sulfate, and varying concentrations of sodium sulfide. The initial pH values were 6.0, 7.0, and 8.0. The final sulfide concentration is the summation of the initial sulfide concentration and sulfur reduced.



affect sulfate reduction. Therefore, sulfate reduction was inhibited by the concentration of total S^{2-}, not the concentration of un-ionized H_2S.

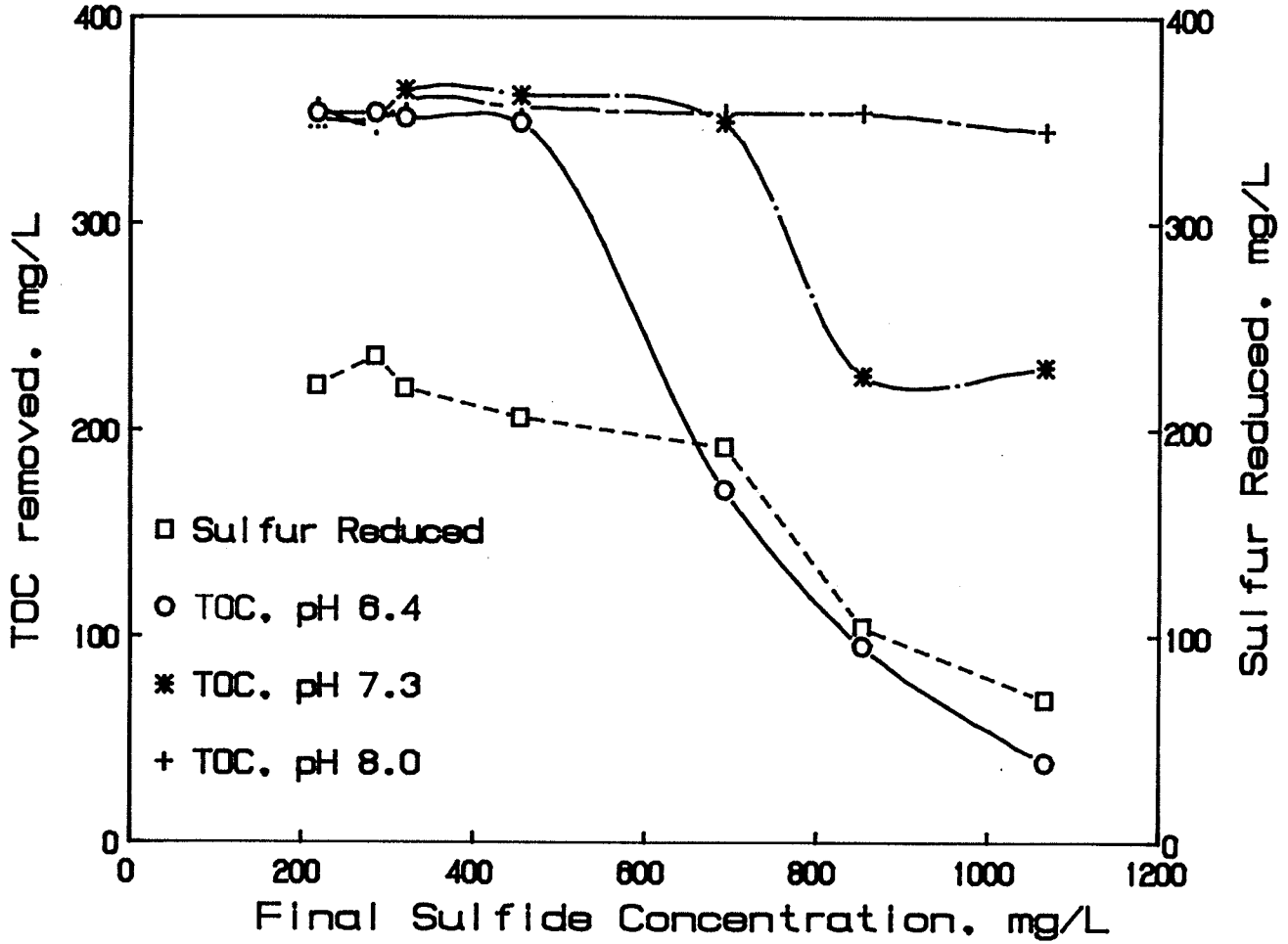
4.3.4 Carbon utilization in the presence of high concentrations of sulfides

TOC removal and sulfur reduction were plotted against the final sulfide concentration in the syringes fed lactate and sulfate (Figure 4.34). The data points for sulfur reduction represent the average values for all three pH values. The data are from the syringes in which sulfate reduction was studied (Section 4.3.3).

Sulfur reduction was inhibited with increased concentrations of total sulfides as was also shown in Figure 4.24, regardless of pH. TOC removal was directly related to pH and final sulfide concentration. At a final pH = 6.4 and 1100 mg/L total sulfides, only 45 mg/L TOC was removed whereas 350 mg/L TOC was removed at 220 mg/L total sulfides. At pH 8.0 (initial and final pH) and 1100 mg/L total sulfides, 350 mg/L TOC, was removed.

The findings of the research in Section 4.3.2 showed that methanogenesis was inhibited above 200 mg/L un-ionized hydrogen sulfide. At 1100 mg/L total sulfides and 450 mg/L un-ionized H_2S, methane production was 50% of the methane production observed in the syringes operated at pH 8.0 and the syringes with less than 400 mg/L total sulfides.

Figure 4.34 Sulfur reduced and TOC removed versus final sulfide concentration in syringes fed lactate, sodium sulfate and varying concentrations of sodium sulfate. Three series of syringes were maintained at a final pH of 6.4, 7.3, and 8.0.



It has been shown in Section 4.3.2 that acetate utilization was inhibited by un-ionized H_2S, not total S^{2-}. In Section 4.3.3, it was shown that sulfate reduction was inhibited by the concentration of total S^{2-}, not un-ionized H_2S. These mechanisms were shown to occur in the same syringes. The data in Figure 4.34 were taken from the same syringes used in Figure 4.31 with the addition of the data for total carbon utilization. These data were collected 21 d after the beginning of the experiment.

These results indicate that at high sulfide concentrations, if a high pH is maintained, sulfate reduction can be inhibited whereas methane production can proceed uninhibited. This product inhibition of sulfate reduction provides conditions whereby the carbon flow in an anaerobic reactor can be diverted from sulfur reduction to the production of methane, even in the presence of the preferred carbon source for the incomplete oxidizing sulfate reducing bacteria.

Schonheit et al. (1982), Kristjansson et al. (1982), and Lovley et al. (1980) showed that the acetoclastic sulfate reducing bacteria had a lower half-saturation constant, K_S, than acetoclastic methane producing bacteria which provided a mechanism whereby sulfate reducers could out-compete methanogens at low substrate concentrations. Middleton and Lawrence (1977) implied that the entire carbon flow in a reactor receiving acetate as the carbon source could be diverted to sulfur reduction. On the other hand, Isa et al. (1986a, b), Kroiss (1987), Szendry (1984) and McKinney (1986) have shown that methane production can proceed nearly

uninhibited in the presence of sulfates in concentrations of 1000 mg/L. The experience of Szendry (1984) was with distillery wastes; that of Kroiss (1987) was with citric acid plant effluent; that of Isa et al. with acetate and ethanol; that of McKinney with acetate and sodium sulfate in the laboratory.

The reactors of Kroiss and Plahl-Wabnegg (1983) and Kroiss (1987) were operated with total dissolved sulfides as high as 450 mg/L at pH 7.3. McKinney (1986) operated his reactors at 1000 mg/L sulfides at pH 8.4. He noted that sulfate reduction was minimal at 1000 mg/L sulfides and that the carbon flow was diverted towards methanogenesis. In all three cases, the un-ionized H_2S was maintained less than 200 mg/L.

Schonheit et al. (1982) and Kristjansson et al. (1982) used nitrogen and CO_2 to strip sulfides. Middleton and Lawrence (1977) used $FeCl_3$ to precipitate sulfides. Isa et al. (1986a, b), Kroiss (1987), Szendry (1984) and McKinney (1986) employed no mechanical stripping or other control of dissolved sulfides other than natural stripping via the generation of methane. Kroiss and Plahl-Wabnegg (1983) developed equations using the concentration of H_2S in the gas to show the concentration of sulfides in the reactor liquid. Those equations showed that Kroiss (1987) had over 400 mg/L dissolved S^{2-} in the reactor.

The self-inhibition of sulfate reducers by the buildup of product is a mechanism whereby methanogens are able to out-compete sulfate reducing bacteria for carbon. Under conditions of high sulfide concentrations and an elevated pH, the carbon flow

can be diverted from sulfate reduction to methane production rather than from methane production to sulfate reduction.

This finding explains the apparent contradiction between the findings of Schonheit et al. (1982), Kristjansson et al. (1982) and Middleton and Lawrence (1977) as opposed to the findings of Isa et al. (1986a, b), Kroiss (1987), Cappenberg (1975), and McKinney (1986). In the former case, sulfate reduction accounted for the entire carbon flow. In the latter case, the carbon flow was primarily diverted towards methanogenesis.

The engineer can choose the path of carbon utilization. On the one hand, sulfate reduction can be optimized at the expense of methane production by minimization of dissolved sulfides. On the other hand, methane production can be optimized by maintenance of pH control and by not controlling dissolved sulfides.

4.3.5 Conclusions

1. Un-ionized H_2S, not total S^{2-} inhibited lactose utilization.
2. Un-ionized H_2S, not total S^{2-} inhibited acetate utilization.
3. Sulfate reduction was completely inhibited at 1100 mg S^{2-}/L.
4. Sulfate reduction was inhibited in proportion to the total sulfide concentration, not the concentration of un-ionized H_2S.
5. The methanogenic bacteria were found to be more sensitive to un-ionized H_2S than the lactose utilizing bacteria.
6. Methanogens outcompeted sulfate reducing bacteria for carbon in the presence of >600 mg/L total S^{2-} at pH 8.0.
7. Toxicity due to H_2S was ameliorated by increasing the retention

time. It was postulated that this was due to a decreased metabolic rate in the microorganisms resulting from sulfide toxicity.

5. ENGINEERING SIGNIFICANCE AND APPLICATION

The fundamental significance of this research lies in the descriptions of the interrelationships between the various groups of microorganisms responsible for anaerobic treatment of high sulfate wastes. The basic process of anaerobic treatment of high sulfate wastes involves a series of metabolic and biochemical processes. These processes form the foundation of two basic needs of living organisms: assimilation of compounds for growth, dissimilation of compounds for the extraction of energy.

The primary interest of the environmental engineer in the design of a waste treatment system is the removal of dissolved organic carbon from a waste stream. One of the advantages of the anaerobic treatment process is the low growth rate of the biomass in relationship to the dissimilation of complex carbon compounds to CO_2 and CH_4. CO_2 normally is sufficiently abundant that it could be expected that it would not be a limiting nutrient. However, when CO_2 was stripped from the reactor, it was shown to be a limiting factor for the growth of methanogens.

The importance of assimilative needs was underscored by the demonstrated assimilative requirement for CO_2 by methanogens. This was shown by the total inhibition of methanogenesis by the removal of CO_2 from a stripped sulfidogenic reactor. The finding that methanogenesis can be suppressed by the exclusion of CO_2 provides the means for intentional suppression of methanogenesis. Conversely, it demonstrated the need for care in the selection of a

stripping system if maximum carbon removal is desired in the design and operation of a sulfidogenic-methanogenic reactor.

Sulfate reduction can be maximized or minimized by the selection of operating parameters for the reactors. High rate sulfate reduction requires the presence of lactate although sulfate reduction does proceed in the presence of H_2, acetate, and ethanol. The research presented here has shown that optimal sulfate reduction can be achieved in reactors fed lactate or lactate precursors.

Optimal sulfate reduction occurs under the following conditions: pH 6.2 - 7.6, lactate precursor in the feed, minimal sulfide concentration, preferably less than 100 mg/L, $S_o/C_o < 0.4$.

Alkalinity generation in sulfidogenic reactors contributes to reactor stability only in the presence of methanogenesis through the removal of excess acidity contributed by the acetate ion. In the case of suppression of methanogenesis, the reactors will stabilize in the acidogenic mode unless alkalinity is added to the waste stream or the reactors.

The growth rate of the incompletely oxidizing SRB is so great that it is not possible to maintain a culture of both incomplete and completely oxidizing SRB. This means that if the substrate will support growth of the incomplete oxidizers, then there will be few complete oxidizers (SRB) present. If stripping is practiced, the maximum $S_r/C_o = 0.4$ if lactate precursors are present. Further work with a two-stage system to separate the incomplete and complete oxidizers might succeed in utilizing all the

available carbon for sulfate reduction. Consideration should also be given to experimentation with retained growth reactors such as a packed bed reactor, anhybrid reactor, or fluidized bed reactor.

The sulfate reducing bacteria are inhibited by the concentration of product, total sulfides, whereas methanogens are primarily inhibited by un-ionized H_2S. This product inhibition most likely was due to the chemical reactions in the process of sulfate reduction rather than specific metabolic inhibition due to sulfide toxicity. Therefore, the carbon flow in an anaerobic reactor treating a high sulfate waste can be diverted from sulfate reduction to methanogenesis by the maintenance of a pH >8.0 and by not stripping sulfides from the reactor.

Toxicity of sulfides to methanogens can be minimized by elevation of the reactor pH. However, the addition of chemicals such as $NaCO_3$ most likely would be prohibitively expensive in full-scale operation. Batch studies showed that the rate of methanogenesis is lowered in the presence of sulfides but is not completely inhibited. Therefore, increases in the retention time (SRT or HRT) will overcome the handicap of a lowered metabolic rate. This means that the design engineer must employ a system which will promote maximum retention of the biomass.

6. SUMMARY AND CONCLUSIONS

6.1 Summary

6.1.1 Carbon/Sulfur Ratio.

In anaerobic treatment, previous thinking has held that sulfide toxicity is a function of the C_o/S_o. This ratio is an artifact of statistical testing or mathematical relationships. The conclusion that C_o/S_o is significant is based upon a sufficiently high C_o that sulfate reduction was not carbon limited. When there is sufficient carbon present and in a form utilizable by SRB, sulfate will be reduced (assuming SRB are present).

Oxidized sulfur compounds can be likened to oxygen; a terminal electron acceptor. Sulfur will only be reduced so long as there is an electron donor. Sulfate reduction may occur under a low E_h, reducing conditions. However sulfate reduction involves oxidation of the electron donor, whether it is carbon or hydrogen. The presence of concentrations of oxidized sulfur in excess of that required for oxidation of the electron donor will have no effect upon the reaction.

The primary concern with the presence of oxidized sulfur compounds is the generation of the byproduct of the reaction, H_2S. This research, as well as all other published research, has shown that sulfide toxicity is strictly a function of the concentration of sulfides in the reactor. The primary reason the C_o/S_o ratio has been shown to be significant is because sufficient carbon (C_o) was present for the generation of toxic concentrations of H_2S. Secondarily, a portion of the carbon flow was diverted from

methanogenesis to sulfidogenesis. This was shown in Figure 4.3 where the specific production of methane decreased in a stripped, sulfidogenic reactor, with an increase in the ratio of S_o/COD_o until the $S_o/COD_o = 0.1$. Above a $S_o/COD_o = 0.1$, increased concentrations of oxidized sulfur had no effect upon the production of methane in a stripped reactor.

The presentation of data and figures in this thesis has primarily been based upon the premise that sulfate reduction is strictly dependent upon an available electron donor. The experiments with R9-R14 and the batch study experiments were conducted under conditions where oxidized sulfur was well in excess of the requirement for sulfur as the terminal electron acceptor. All the experiments were designed to be carbon limiting with respect to sulfur reduction.

The research project presented herein primarily dealt with lactate or lactate precursors as the carbon source for sulfate reduction. However, during the early phases of the research (R1-R8), beef broth, methanol, acetate, and glucose were also used as the sources of carbon. During those periods of time, sulfate reduction was at a far lower rate than after whey was introduced as the carbon source.

The research presented in this thesis as well as published research has shown that S_r/C_o is dependent upon the carbon source. If acetate or methanol are the predominant source of carbon, methanogenesis will predominate and the S_r/C_o will be as low as 0.08 (Table 4.6). On the other hand, if lactate or lactate

precursors are present and total sulfides are limited, maximum sulfur reduction will occur at S_r/C_o = 0.4 (Table 4.6) which is the maximum which can be expected. As an example, if S_r/C_o = 0.4 and C_o = 100 mg/L, then S_r = 40 mg/L S^{2-} which is not a toxic or inhibitory concentration of S^{2-}, regardless of the form. On the other hand, if S_r/C_o = 0.4 and C_o = 1000 mg/L, then (provided that there are enough sulfates available) S_r = 400 mg/L S^{2-}, which may or may not be toxic to acidogens or methanogens, depending upon the fraction of unionized H_2S. In the latter case, if S_o/C_o = 0.4, then little residual oxidized sulfur would be present; however, if S_o/C_o = 1.0, then approximately 600 mg/L residual oxidized sulfur would be present.

In the former case, when C_o = 100 mg/L, if S_o/C_o = 10.0, there still would not be sulfide toxicity *per se* because only 40 mg/L S^{2-} would be generated.

6.1.2 Toxicity of Sulfates to anaerobic treatment.

The engineering community as a whole accepts that the presence of sulfates adversely affects anaerobic treatment since methane production normally diminishes inversely with increased concentrations of sulfates. The production of methane is diminished as a function of the diversion of carbon flow as well as toxicity of sulfides to methanogens and acidogens. However, a diminished production of methane *per se* cannot be the sole criterion for establishing toxicity, be it sulfides or anything else.

The research presented in this thesis and previously published as well as work by DLA (DLA, Inc. 1982; Olthof *et al.* 1986)

unequivocally demonstrated that complete carbon utilization can occur in the presence of high concentrations of OSC yet methane production can be significantly diminished due to the diversion of carbon flow from production of methane to the reduction of sulfur.

6.1.3 Sulfide inhibition to sulfate reduction.

Sulfate reduction was found to be a function of product inhibition, not the form of the product (H_2S, HS^-). If sulfate reduction were inhibited solely as a function of unionized H_2S, then, given an initial sulfide concentration, the quantity of sulfate reduced would be different at pH 8.0 than at pH 7.0 or ph 6.0. Experiments demonstrated that there was no difference in the quantity of sulfates reduced due to pH but only as a function of the initial sulfide concentration. Minimal sulfur reduction occurred when the total reactor sulfide concentration was 1000 mg/L. No sulfur reduction occurred when the total reactor sulfide concentration was 1600 mg/L S^{2-}.

If inhibition to sulfate reduction were due to unionized H_2S, then it would be expected that the sulfur reduced after 70 d retention time would significantly greater than that observed at 21 d. In fact, the sulfur reduced after 70 d was unchanged from the values observed after 21 d.

Finally, if sulfate reduction were a function of unionized H_2S and not S^{2-} *per se*, the carbon flow should have been towards sulfate reduction at 1000 mg/L S^{2-} and pH 8.0, not methane generation as shown in Figure 4.34. The carbon flow was diverted from sulfate reduction to methane production, as long as un-ionized

H_2S was minimized. Sulfate reduction did not show a similar variation, depending upon pH. Therefore, this is more evidence that sulfate reduction is a product limited process.

6.1.4 Sulfide toxicity to acidogenic, methanogenic processes.

Toxicity by un-ionized H_2S was manifested as a decreased rate of substrate utilization. Complete substrate utilization for lactose and acetate was achieved by increasing the retention in batch cultures. It is hypothesized that an increase in the SRT, HRT or F/M of continuous-flow reactors would alleviate the effects of sulfide toxicity. The batch studies presented here did not distinguish between SRT and HRT, however most kinetic models presume growth rates and solids retention times.

6.1.5 Carbon utilization.

The literature has suggested that sulfate reducers would always outcompete methanogens for substrate. Consequently, it was expected that a syntrophic culture of both incompletely oxidizing sulfate reducing bacteria and completely oxidizing sulfate reducing bacteria could be established with 80-90% carbon removal in the absence of methane production.

Maximum carbon removal in reactors treating high sulfate wastes only occurred in stripped sulfidogenic reactors with syntrophic generation of methane. Steady-state production of methane was maintained as long as CO_2 was not stripped from the reactors. There was no significant difference in total carbon removal between stripped sulfidogenic reactors or methanogenic reactors fed the same carbon source. The only difference in

performance of the two types of reactor was in the production of methane. In the sulfidogenic reactor, part of the carbon flow was diverted towards sulfate reduction whereas the entire carbon flow was diverted towards methanogenesis in the methanogenic reactor.

Two genera of incompletely oxidizing sulfate reducing bacteria were predominant, Desulfovibrio and Desulfobulbus. There were only traces of one genus of completely oxidizing sulfate reducing bacterium, Desulfobacter. Therefore, under the conditions of this research, only incompletely oxidizing sulfate reducing bacteria predominated. It is hypothesized that it is not possible to maintain a syntrophic culture of incompletely and completely oxidizing sulfate reducing bacteria due to interspecific competition for H_2. It is hypothesized that the maximum $S_r/C_o = 0.4$ for anaerobic sludge bed reactors.

6.1.6 Competition for substrate.

Sulfate reducing bacteria only outcompeted methanogens for carbon when sulfide concentrations were less than 100 mg/L S^{2-} and when lactate or lactate precursors were the carbon source. In batch culture studies, when un-ionized H_2S was maintained below 200 mg/L but total S^{2-} were high, (>200 mg/L), methanogens outcompeted the rapidly growing incompletely oxidizing sulfate reducing bacteria for carbon even when the preferred carbon source, lactate was provided.

6.1.7 Generation of alkalinity.

Alkalinity generation in sulfidogenic reactors was more than offset by the generation of acids, both organic acids and carbonic

acid. The benefits of increased generation of alkalinity in sulfidogenic reactors could only be realized when organic acids were removed by methanogenesis and when carbonic acid was removed by artificial stripping of CO_2.

6.2 Conclusions

A detailed list of conclusions for each phase of the research has been included at the end of each subchapter in sections 4.1.6, 4.2.15, and 4.3.6. Therefore, only the most significant conclusions are listed.

6.2.1 Continuous flow studies

1. Incompletely oxidizing SRB (Desulfovibrio and Desulfobulbus) were the predominant SRB. It is hypothesized that incompletely oxidizing SRB were predominant due to their ability to out-compete acetoclastic SRB for hydrogen.

2. Sulfate reduction in stripped sulfidogenic reactors required maintenance of an optimal pH between 6.0 and 7.5. This was due to the effects of pH upon the type of acids generated during glycolysis and not the activity of the SRB.

3. The removal of CO_2 from bioreactors suppressed methanogenesis by limiting available CO_2 for cell synthesis. Consequently, the use of NaOH or any other solution such as ARI-311C™ which absorbs CO_2 as a stripping solution requires care in design and operation in order to minimize the absorption of CO_2.

4. Carbon removal and methanogenesis were unaffected by the addition of high concentrations of sulfate to the waste stream for a waste stream which normally is amenable to methanogenesis

whenever the carbon source (or precursors) preferred by incomplete oxidizing SRB was absent.

5. The alkalinity generated by sulfate reduction in the sulfidogenic-methanogenic reactors provided more pH stability than that in purely methanogenic reactors, thereby reducing the requirement for the addition of buffers to the system. However, in the absence of methanogenesis in sulfidogenic reactors, the addition of buffering was required to offset generated acidity not used in the process of sulfate reduction.

6. The reduction of sulfates proceeded best in the gas-stripped reactors.

7. At organic loads above 1.1 g/L·d TOC and sulfate loads above 0.5 g/L·d S^{6+}, stripped reactors outperformed unstripped reactors. This difference was most likely due to sulfide toxicity in the unstripped reactors.

8. Inhibition due to sulfide accumulation affected the VFA removal phase rather than the acid-generating or acidogenic phase of the anaerobic biodegradation pathway.

9. The methanogenic and stripped sulfidogenic reactors receiving SSL provided comparable COD removals. However, the production of methane was significantly greater in the purely methanogenic reactor. This difference clearly demonstrated a diversion of organic carbon utilization from methane production to sulfate reduction.

10. The stripped, sulfate loaded reactors worked in a syntrophic manner, generating both methane and sulfides. The generation of

methane was accountable for up to 65% of the removed COD (assuming 0.35 L CH_4/g COD at STP = 100%).

11. The maximum S_r/C_o = 0.40 is the highest value to be expected from a complex waste such as whey which will ferment to generate lactate. Other wastes such as distillery wastes which will not generate lactate will yield a lower S_r/C_o.

12. In a stripped sulfidogenic reactor, methane production decreased to 0.15 L CH_4/g COD removed up to an S_o/COD_o = 0.11. Methane production remained constant above an S_o/COD_o = 0.11.

6.2.2 Batch studies

1. Methanogens outcompeted sulfate reducing bacteria for carbon in the presence of high concentrations of sulfides (>600 mg/L S^{2-}) and at increased pH values (8.0).

2. The effects of sulfide toxicity were ameliorated by increasing the retention time.

3. Sulfate reduction was inhibited in proportion to the total sulfide concentration not due to un-ionized H_2S. Sulfate reduction was completely inhibited at 1100 mg S^{2-}/L.

4. Methanogenesis and acetate uptake were inhibited by increased concentrations of un-ionized H_2S, not total S^{2-}.

5. The methanogenic bacteria were found to be more sensitive to un-ionized H_2S than the lactose utilizing bacteria.

7. FUTURE RESEARCH

7.1 Competition for hydrogen

This research has shown that in the presence of lactate or lactate precursors in the presence of high concentrations of sulfates, incomplete oxidizing SRB will predominate in the culture. It was hypothesized that the incomplete oxidizing SRB outcompeted the acetoclastic SRB for hydrogen.

This is significant because the exclusion of the acetoclastic SRB means that far more carbon is required for sulfate reduction than would be required if both groups of SRB were able to reduce sulfates.

This hypothesis can be tested as follows: Operate two parallel, stripped sulfidogenic reactors fed with reconstituted whey powder and sodium sulfate. Add measured quantities of molecular hydrogen to one reactor. Compare TOC, SO_4, VFA, lactose from each reactor. Test each reactor for membrane bound phospholipids and determine the population composition of each reactor.

7.2 Sulfide inhibition of sulfate reduction

Sulfate reduction in batch syringes was inhibited by high concentrations of total sulfides which permitted diversion of the carbon flow towards methanogenesis. This can be verified in operating reactors by the operation of parallel reactors. Operate one reactor at a high pH with no sulfide stripping and a parallel reactor at the same pH with sulfide stripping.

The stripped reactor should divert all the carbon flow towards sulfate reduction. The unstripped reactor should primarily divert the carbon flow towards methanogenesis. Sulfate reduction in the unstripped reactor should be limited to sufficient sulfate reduction to maintain a steady-state concentration of total sulfides.

7.3 Effects of sulfides upon distribution of products of glycolysis

The research presented here demonstrated that sulfides affected the rate of lactose uptake in batch culture. Further research could define the change in the products of glycolysis resulting from varying concentrations of sulfides and at varying pH values. It is hypothesized that changes in the products of glycolysis under conditions of high sulfides is due to a shift in the population composition. It is also hypothesized that the formation of compounds such as acetate and lactate is minimized in the presence of high sulfide concentrations. If this is true, then methanogenesis and sulfidogenesis would be inhibited due to a lack of a suitable carbon source. The population composition could be evaluated to prove or disprove this hypothesis.

7.4 Engineered control of toxicity

Sulfide toxicity was manifested by a decreased microbial metabolism. Complete conversion of TOC to methane can be achieved given sufficient time. This concept can be applied to the study of many toxicants by the design of systems with high SRT's and/or high HRT's.

Based upon this concept, the practical upper limit of sulfide toxicity (as un-ionized H_2S) could be tested by using reactors with

HRT's/SRT's far in excess of current practice for anaerobic reactors. This concept could well be applied to most other toxic compounds.

8. REFERENCES

A.P.H.A. 1985. Standard Methods for the Examination of Water and Wastes, 16th Ed., American Public Health Association, New York, 1265 p.

Aulenbach, D. B. and Heukelekian, H. 1955. Transformation and effects of reduced sulfur compounds in sludge digestion. **Sewage and Industrial wastes. 27,** pp. 1147-1159.

Badziong, W., R. K. Thauer, and J. G. Zeikus 1978a. Isolation and characterization of Desulfovibrio growing on hydrogen plus sulfate as the sole energy source. **Archives of Microbiology, 116,** pp. 41-49.

Badziong, W. and R. K. Thauer 1978b. Growth yields and growth rates of Desulfovibrio vulgaris (Marburg) growing on energy sources. **Archives of Microbiology, 117,** pp. 209-214.

Benefield, L. D., and C. W. Randall 1980. Biological process design for wastewater treatment. Prentice-Hall, Inc., Englewood Cliffs, NJ 526p.

Burgess, S. G., and L. B. Wood 1961. Pilot plant studies in production of sulfur from sulfate-enriched sewage sludge. **Journal of the Science of Food and Agriculture, 12,** pp. 326-335.

Cappenberg, Th.E. 1975. A study of mixed continuous cultures of sulfate-reducing and methane-producing bacteria. in **Microbial Ecology, 2,** pp. 60-72.

Chartrain, M. and J. G. Zeikus 1986. Microbial ecophysiology of whey biomethanation: characterization of bacterial trophic populations and prevalent species in continuous culture. **Applied and Environmental Microbiology, 51,** pp. 188-196.

Daniels, L., R. Sparling, and G. D. Sprott 1984. The bioenergetics of methanogenesis. **Biochimica et Biophysica Acta, 768,** pp. 113-163.

DLA, Inc. 1982. Unpublished data on anaerobic sequencing batch reactors. Pittsburgh, PA.

DLA, Inc. 1982. Hydrogen sulfide recovery from calcium sulfate--Phase I. A quantitative feasibility study. Final Report to W. R. Grace Co.

Domka, F., and M. Szulczynski 1979. The effect of organic substrate concentration on activity for microbiological reduction of sulfates. **Acta Microbiologica Polonica, 28,** pp. 237-244.

Domka, F., M. Szulczynski 1981. Studies on sulfite reduction by Desulfovibrio vulgaris. **Acta Microbiologica Polonica, 30,** 3, pp. 247-253.

Eis, B. J., J. F. Ferguson, M. M. Benjamin. 1983. The fate and effect of bisulfate in anaerobic treatment. **Journal Water Pollution Control Federation, 55,** pp. 1355-1365.

Gaudy, A. F., Jr., and E. T. Gaudy 1980. Microbiology for environmental scientists and engineers. McGraw-Hill, New York 736p.

Grau, P., M. Dohanyos, and J. Chudoba 1975. Kinetics of multicomponent substrate removal by activated sludge. **Water Research, 9,** pp. 637-642.

Imhoff-Stuckle, D., N. Pfening 1983. Isolation and characterization of a nicotinic acid-degrading sulfate-reducing bacterium, Desulfococcus niacini. **Archives of Microbiology, 136,** pp. 194-198.

Isa, Z., S. Grusenmeyer, W. Verstraete 1986. Sulfate reduction relative to methane production in high-rate anaerobic digestion: technical aspects. **Applied and Environmental Microbiology, 51,** 3, pp. 572-579.

Isa, Z., S. Grusenmeyer, W. Verstraete 1986. Sulfate reduction relative to methane production in high-rate anaerobic digestion: microbiological aspects. **Applied and Environmental Microbiology, 51,** 3, pp. 580-587.

Jansen, K., G. Fuchs and R. K. Thauer 1985. Autotrophic CO_2 fixation by Desulfovibrio baarsii: demonstration of enzyme activities characteristic for the acetyl-CoA pathway. **FEMS Microbiology Letters, 28,** pp. 311-315.

King, G. M. 1984. Metabolism of trimethylamine, choline, and glucine betaine by sulfate reducing and methanogenic bacteria in marine sediments. **Applied and Environmental Microbiology, 48,** pp. 719-725.

Koster, I. W., A. Rinzema, A. L. de Vegt, and G. Lettinga, 1986. Sulfide inhibition of the methanogenic activity of granular sludge at various pH levels. **Water Research, 20,** pp. 1561-1567.

Kristjansson, J. K., P. Schonheit, and R. K. Thauer. 1982. Different K_S values for hydrogen of methanogenic bacteria and sulfate reducing bacteria: an explanation for the apparent inhibition of methanogenesis by sulfate. **Archives of Microbiology, 131,** pp. 278-282.

Kroiss, H., F. Plahl-Wabnegg 1983. Sulfide toxicity with anaerobic waste treatment. in Advanced Anaerobic Waste Treat. Conf., Amsterdam, AWWT Symposium Secretariat, The Hague pp. 72-85.

Kroiss, H. 1987. Personal communication regarding the operation of full-scale anaerobic reactors treating high sulfate citric acid plant wastes.

Laanbroek, H. J., and N. Pfening 1981. Oxidation of short-chain fatty acids by sulfate reducing bacteria in freshwater and in marine sediments. **Archives of Microbiology, 128,** pp. 330-335.

Lawrence, A. W., P. L. McCarty, and F. Guerin 1964. The effects of sulfides on anaerobic treatment. In Proceedings of the 19th Industrial Waste Conference. Engineering Extension Series No. 117, Purdue University, Lafayette, Ind. pp. 343-357.

Lovley, D. R., D. F. Dwyer, and M. J. Klug 1982. Kinetic analysis of competition between sulfate reducers and methanogens for hydrogen in sediments. **Applied and Environmental Microbiology, 43,** pp. 1373-1379.

McKinney, R. 1986. Personal Communication. Department of Civil Engineering, University of Kansas, Lawrence, KS.

Maree, J. P., and W. F. Strydom 1987. Biological sulphate removal from industrial effluent in an upflow packed bed reactor. **Water Research** (G.B.), **21,** pp. 141-146.

Middleton, A. C., and A. W. Lawrence 1977. Kinetics of microbial sulfate reduction. **Journal Water Pollution Control Federation, 49,** pp. 1659-1669.

Mountfort, D. O. and R. A. Asher 1979. Effect of inorganic sulfide on the growth and metabolism of Methanosarcina barkeri. **Applied and Environmental Microbiology, 37,** pp. 670-675.

Nanninga, H.J. 1985. Personal communication. Laboratorium voor Microbiologie, Holland.

Nanninga, H.J., J.C. Gottschal 1985. Amino acid fermentation and hydrogen transfer in mixed cultures. **FEMS Microbiological Ecology, 31,** pp. 261-269.

Odum, J.M. and H. D. Peck Jr. 1981. Hydrogen cycling as a general mechanism for energy coupling in the sulfate-reducing bacteria, Desulfovibrio sp. **FEMS Microbiology Letters, 12,** pp. 47-50.

Olthof, M., W.R. Kelly, J. Oleszkiewicz, H. Weinreb 1986. Development of anaerobic treatment process for wastewaters containing high sulfates. in Proceed. 40th Annual Industr. Waste Confer., Purdue University, May, 1985. pp. 871-877.

Oremland, R. S., and Polcin, S. 1982. Methanogenesis and sulfate reduction: competitive and non-competitive substrates in estuarine sediments. **Applied and Environmental Microbiology, 44,** pp. 1270-1276.

Pankhania, I. P., L. A. Gow, and W. A. Hamilton 1986. The effect of hydrogen on the growth of Desulfovibrio vulgaris (Hildenborough) on lactate. **Journal of General Microbiology, 132,** pp. 3349-3356.

Pipes, W. O., Jr. 1960. Sludge Digestion by Sulfate Reducing Bacteria. Proc. 15th Ind. Waste Conf., Purdue Univ., pp. 308-319.

Postgate, J.R. 1984. The sulphate-reducing bacteria, Second Edition, Cambridge University Press, New York, 208 p.

Puhahka, J.A., J.A. Rintala, P. Vuoriranta 1985. Influence of sulfur compounds on biogas production from forest industry wastewater. in Anaerobic treatment of forest industry wastewaters., **Water Science and Technology, 17,** 1, pp. 281-288.

Robinson, J. A., and J. M. Tiedje 1984. Competition between sulfate-reducing and methanogenic bacteria for H_2 under resting and growing conditions. **Archives of Microbiology, 137,** pp. 26-32.

Ronnow, P. H. and Gunnarson, L. A. H. 1982. Response of growth and methane production to limiting amounts of sulfide and ammonia in two thermophilic methanogenic bacteria. **FEMS Microbiology Letters, 14,** pp. 311-315.

Rudolfs, W., and H. R. Amberg. 1952. White water treatment II. Effect of sulfides on digestion. **Sewage and Industrial Wastes, 24,** 1278.

Sadana, J. C., and A. V. Morey 1962. Microbial production of sulfide from gypsum. **Journal of Science and Industrial Research, 21C,** pp. 124-127.

Saw, C. B., G. K. Anderson, J. A. Sanderson 1987. Comparison of the anaerobic contact and packed bed processes for the treatment of edible oil wastewaters. Proceed. 41st Industrial Waste Conf., Purdue Univ. pp. 178-187.

Schonheit, P., J.K. Kristjansson, R.K. Thauer 1982. Kinetic mechanism for the ability of sulfate reducers to out-compete methanogens for acetate. **Archives of Microbiology, 132,** pp. 285-288.

Sobkowicz, A. M. and H. A. Klemm 1986. The syringe biomethane potential test. Unpublished paper. Badger Engineers, Inc, Cambridge, MA.

Szendry, L.M. 1983. Startup and operation of the Bacardi Corporation anaerobic biofilter. in Proceed. of 3rd International Symposium on Anaerobic Digestion, Evans and Falkner, Inc., Watertown, MA, pp. 365-377.

Taylor, J., and R. J. Parkes. 1983. The cellular fatty acids of the sulphate-reducing bacteria, Desulfobacter sp., Desulfobulbus sp., and Desulfovibrio Desulfuricans. **Journal of General Microbiology, 129,** pp. 3303-3309.

Wellinger, A. and L. Wuhrmann 1977. Influence of sulfide compounds on the metabolism of Methanobacterium strain AZ. **Archives of Microbiology, 115,** pp. 13-17.

Winfrey, M. R., and Zeikus, J. G. 1977. Effects of sulfate on carbon and electron flow during microbial methanogenesis in freshwater sediments. **Applied and Environmental Microbiology, 33,** pp. 275-281.

Zoetemeyer, R. J., J. C. van den Heuvel, and A. Cohen 1982. pH influence on acidogenic dissimilation of glucose in an anaerobic digestor. **Water Research, 16,** pp. 303-311.

Zubay, G. 1983. Biochemistry. Addison-Wesley, Reading, MA 1268p.

APPENDIX 1. NOTATION

B_v	Organic carbon load, g/L·d, kg/m^3·d
C_e	Effluent soluble organic carbon concentration, mg/L
C_o	Influent organic carbon concentration, mg/L
C_r	Soluble Organic carbon removed or oxidized, C_o-C_e, mg/L
COD_e	Effluent COD, mg/L
COD_o	Influent COD, mg/L
COD_r	COD removed, COD_o-COD_e, mg/L
k_d	Decay coefficient, d^{-1}
K_m	Half-velocity saturation constant (Michaelis-Menten)
K_s	Half-velocity saturation constant (Monod)
S_e	Effluent oxidized sulfur concentration, mg/L
S_o	Influent oxidized sulfur concentration, mg/L
S_r	Sulfur reduced, S_o-S_e, mg/L
S_L	Sulfur load, QS_o/V·d, g/L·d
μ	Observed cellular growth rate, g/L·d
μ_m	Maximum cellular growth rate, g/L·d
Θ	Hydraulic retention time, d
Θ_c	Solids retention time, d
V_{max}	The maximum velocity or rate of removal of substrate from a medium (Michaelis-Menten)

APPENDIX 2. NOMENCLATURE

assimilation. Biological incorporation of compounds into the cellular molecular structure.

COD. Chemical Oxygen Demand. The quantitiy of oxygen required to oxidize a compound to CO_2 and H_2O.

TOC. Total Organic Carbon, non-filtered

SOC. Soluble organic carbon; filtered sample

acetoclastic. Biological decomposition of acetate for the purpose of obtaining energy. Ex: acetate to methane and CO_2

acidogenesis. Biological decomposition of compounds to form organic acids during fermentation.

dissimilate. Biological decomposition or breakdown of compounds to simpler components for the purpose of obtaining energy.

glycolysis. Biological degradation of glucose. Results in the uptake of glucose from the medium and the formation of simpler compounds such as acetate, lactate, and ethanol.

sulfidogenic reactor. A biological reactor in which an active biomass of sulfate reducing bacteria has been established and large quantities of sulfides are being generated.

stripped sulfidogenic reactor. A sulfidogenic reactor in which mechanical stripping of sulfides is employed in order to minimize the concentration of sulfides in the reactor.

methanogenic reactor. An anaerobic bioreactor in which carbon removal is performed by the generation of methane.

incompletely oxidizing sulfate reducing bacteria. Sulfate reducing bacteria which incompletely oxidize a carbon compound such as lactate or pyruvate to a less complex carbon compound such as acetate plus CO_2 in the process of reducing an oxidized species of sulfur to sulfide.

completely oxidizing sulfate reducing bacteria. Sulfate reducing bacteria which completely oxidize a carbon compound such as acetate to CO_2 in the process of reducing an oxidized species of sulfur to sulfide.

sulfidogenesis. Dissimilatory reduction of oxidized sulfur to sulfides for the generation of energy to the sulfate reducing bacteria.

APPENDIX 3. Data Sets

Figure 4.1

Day	Unst % rem	Str % rem	S load	COD load
37	68	76	0.54	1.90
39			0.52	1.85
51	62	84	0.60	2.77
79	93		0.46	
80	88			
92	63	64		2.27
99	64	67	2.52	
101	79		0.47	2.59
106			0.40	2.80
115	82		0.44	2.40
122	82	80	0.47	2.00
127		84	0.37	2.00
129		82	0.47	3.95
134			0.43	3.95
136		85	0.43	5.46
141		90	0.43	5.72
143	77	90	0.47	5.80
150	33	92	1.04	5.60
155		94		5.92
164	22	81	1.77	8.84
169	18	84	2.97	6.36
171		89	2.47	4.68
176	15	84	2.34	6.36
184	19	78	2.49	6.76
190	22	76	2.46	9.32

Figure 4.2

Influent COD	COD_e
Stripped, R2	
4625	2900
4625	2475
3000	1800
2500	1700
2500	1750
2750	1700
2050	1800
1500	1240
2700	1700
2650	1750
3650	2900
3500	2000
2450	2000
2500	2000
5100	3550
5100	3350
6400	4500
6800	3850
6100	4500
6100	4700
Unstripped, R1, low sulfur load	
3600	3350
5000	4600
5200	4825
6500	5850
6300	5700
6100	5600
5600	5300
5800	4850
4500	4000
5800	4850
Unstripped, R1, high sulfur load	
6000	2000
6700	2900
5800	1300
4500	800
6200	1500
6200	950
6100	1150
5500	1200

Figure 4.3

S_r/C_o	L CH_4/g COD removed
0.124	0.154
0.187	0.155
0.101	0.164
0.083	0.230
0.295	0.145
0.338	0.149

Figure 4.4

pH	% removal
7.1	55.5
7.5	67.0
7.7	68.0
8.2	78.0
8.0	75.0
8.7	79.0
7.8	72.5
7.4	70.0
7.6	64.0
7.3	70.0
7.0	56.0
5.9	33.0
5.7	22.0
6.3	43.0

Figure 4.5

COD load mg/L.d	pH
945	8.20
4995	7.80
3940	7.10
2556	7.50
1920	7.70
1830	7.50
2618	7.60
1910	8.20
2050	8.00
2257	8.20
2268	7.50
263	8.70
1176	8.00
1960	7.80
2100	8.00
4080	7.40
4080	7.60
5734	7.30
6310	7.10
6100	7.00
5953	6.40
6168	5.90
6566	6.40
6472	6.70
7068	6.60

Figure 4.6

pH	gas, mL
6.5	0.
6.6	0.
6.7	0.
6.8	0.
8.3	2300.
7.9	1200.
8.2	100.
8.0	1500.
7.9	1600.
7.9	1000.
8.1	1200.
8.2	1200.
7.4	500.
7.5	300.
7.5	300.
8.3	1600.
8.1	1200.
8.0	1000.
7.6	850.
7.8	550.
7.8	800.
7.8	600.
7.4	500.
7.2	400.
7.4	400.
7.4	400.
7.6	400.
7.4	300.
7.2	100.
7.3	200.
7.2	250.
6.4	0.
6.0	0.

Figure 4.7

Day	R3 %R	R4 %R	Sulfur g/L·d	COD load g/L·d	SSL load g/L·d
51	63	73	0.7	2.5	
79	58	68	1.4	4.3	0.72
80	60	83	1.5	3.2	0.72
81	75	76	1.4		0.72
92	37	49	0.3	2.2	0.57
99	49	75	0.4	3.1	1.0
101	52	83	0.4	2.9	0.8
106	67	76	0.4	3.3	1.0
115	48	74	0.6	3.1	0.8
120	52	79	0.5	4.2	0.8
122	46	66	0.5	4.0	
127	46	62	0.5		0.8
129	42	66	0.5	4.8	0.8
134	21	14	0.62	6.8	4.3
141	5	7	0.48	4.0	3.4
143	28	37	1.1	3.8	3.4
150	37	62	0.32	3.2	1.2
155	12	30	0.6	5.3	2.6
164	25	57	0.6	2.5	3.0
169	45	64	2.1	1.1	3.0
171	25	57	2.1	2.5	4.0
176		83	2.6		4.0
184	48	48	1.2	9.4	4.0
186		21	1.2	8.6	4.0
190	9	76	1.2		

Figure 4.8

Day	R4 %R	R6 %R	COD load	Day	SSL load
51	84	73	2.45	50	0.80
79	68	68	3.60	79	
80	75	83		80	
81	81	76		81	
92	71	49	1.93	92	
99	78	75	3.38	95	0.80
101	73	83	1.84	95	1.20
106	58	76	3.18	122	1.20
115	77	74	3.50	122	2.00
120	67	79	3.61	130	2.00
122	65	66	3.50	130	2.30
127	58	62	4.44	135	2.30
129	58	66	4.56	135	4.00
134	45	14	7.45	144	4.00
136	42		2.60	144	2.80
141	31	7	3.87	162	2.80
143	23	37	3.40	162	2.00
150	67	62	3.03	178	2.00
155	54	30	5.76	178	5.00
164	50	57	2.29	190	5.00
169	67	64	1.60		
171	50	57	2.29		
176	87	83	4.16		
184	43	48	5.96		
186	5	21	8.99		
190	21	76	8.99		

Figure 4.9

COD in	R4 %R
2450	73
2500	68
2900	76
3800	83
4000	76
4100	74
4750	79
4500	67
5800	66

COD in	R6 %R
2750	73
3975	79
4000	83
4100	87
4400	81
4600	77
5700	67
5900	54

Figure 4.10

Day load	R7 %R	R8 %R	Day	COD conc	Day	C O D
100	39	90	100	2100	177	3300
136	51	90	136	1150	179	2500
138	49	97	138	1200	187	3100
143	67	88	143	1380	192	3500
156	77	91	156	2200	194	2600
165	51	94	165	2200	212	1800
177	75	83	177	2850	216	2500
179	70	92	179	2530		
182	64	73	187	2700		
184	75	83	192	2500		
202	65	76	194	2000		
206	66	70	212	1650		

Figure 4.11

Day	S_r	S_o	C_r	C_o
30	1196	1622	807	3546
31	1233	1667	1175	3560
32	1222	1622	1275	3370
33	1289	1622	800	3000
34	1022	1622	1171	3546
35	1134	1590	2477	4602
36	1343	1590	1902	4602
37	1094	1517	1430	4595
38	1415	1590	1876	4602
39	1454	1590	2210	4610
40	1234			
41	1065			
48	1403	1940	4660	
52	1289	1700	1639	4380
53	1337	1746	1821	4306
54	1263	1751	1542	4454
55	1433	1741	1521	4454
59	1257	1741	1458	4270
60	1332	1631	1732	4383
61	1142	1710	1618	4496
62	1408	1610	1742	4383
63	1238	1671	1699	4300
64	1286	1466	1806	4300
66	1322	1466	1545	4300
67	1282	1461	1828	4300
68	1371	1466	1592	4184
69	1291	1527	1750	4300

Figure 4.12

Day	S_r	S_o	C_r	C_o
130	710	1466	1204	4562
131	762	1521	1471	4496
132	626	1941	576	3830
133	641	1898	1528	4496
134	873	1941	2230	5236
135	722	1941	1785	4932
136	738	1962	994	4424
137	674	1674	898	4379
138	689	1729	1150	4424
139	689	1674	974	4469
140	702	1620	984	4661
141	632	1596	1169	4738
142	692	1515	877	4661
143	753	1596	1463	4661
144	756	1596	2071	4866
145	823	1695	2079	4820
146	913	1668	1995	4866
148	124	1695	2039	4866
149	130	1860	2404	5484
150	113	1691	1888	4891
151	1101	1691	1133	4891
152	1053	1715	1601	4891
153	1021	1691	1898	4910
154	1084	1667	2235	4930
155	1207	1691	1737	4910
156	1055	1439	1854	5616
158	1050	1439	2319	5213
159	1093	1496	2378	4994
160	1110	1495	2456	4994

Figure 4.13

F/M	TOC removal %
0.28	43
0.31	21
0.29	23
0.30	22
0.30	41
0.28	45
0.27	40
0.42	32
0.36	18
0.30	32
0.70	37
0.46	35
0.61	41

Figure 4.14

pH	S_r g/L·d	C_r g/L·d
4.4		0.527
4.5		0.460
4.7		0.518
4.8	0.144	0.490
4.9	0.147	0.474
5.0	0.261	0.907
5.1	0.324	0.604
5.2	0.381	0.965
5.3	0.269	0.921
5.4	0.409	1.188
5.5	0.464	0.843
5.6	0.506	0.905
5.7	0.590	1.225
5.9	0.742	1.164
6.0	0.779	1.119
6.2	0.718	1.359
6.3	1.058	1.286
6.4	1.070	1.325
6.6	1.103	2.064
6.8	1.007	2.051
6.9	1.274	1.589
7.0	1.248	1.835
7.1	1.312	
7.2	1.339	2.178
7.3		1.781
8.1	1.905	

Figure 4.15

B_V	pH
3.63	4.4
3.32	4.5
3.92	4.7
3.23	4.8
3.46	5.0
3.22	5.1
3.78	5.2
3.57	5.3
3.50	5.4
3.24	5.5
3.51	5.6
3.59	5.7
3.66	5.8
3.85	5.9
4.19	6.0
3.50	5.6
3.59	5.7
3.66	5.8
3.85	5.9
4.19	6.0
3.63	6.1
3.67	6.2
3.43	6.3
3.76	6.4
3.60	6.5
4.09	6.7
4.18	6.8
3.83	7.0

Figure 4.16

pH	S_r/C_o
4.5	0.0890
4.7	0.0690
4.8	0.0853
5.0	0.1540
5.1	0.0854
5.2	0.0720
5.3	0.0998
5.4	0.0885
5.5	0.0870
5.6	0.1250
5.7	0.1340
5.8	0.2110
5.9	0.1990
6.0	0.2110
6.1	0.2130
6.2	0.2250
6.3	0.3130
6.4	0.2600
6.5	0.2140
6.6	0.2770
6.7	0.1780
6.8	0.2230
6.9	0.2290
7.0	0.3120
7.1	0.2090
7.2	0.2360
7.3	0.5680
8.1	0.4130

Figure 4.17

C_o	S_r
3.463	0.261
3.219	0.324
3.755	0.381
3.568	0.269
3.500	0.409
3.240	0.464
3.510	0.506
3.592	0.590
3.848	0.742
4.185	0.779
3.674	0.718
3.432	1.058
3.756	1.070
4.179	1.007
4.668	1.248
4.960	1.312
4.780	1.339

Figure 4.18

B_v g/L·d	C_r g/L·d
2.704	1.007
2.638	0.864
2.638	1.001
2.638	0.871
3.424	1.415
3.424	1.069
3.424	1.396
3.424	1.638
4.647	1.526
4.647	1.944
4.647	1.670
4.647	1.564
3.991	1.416
3.229	1.117
3.229	1.357
3.3	.997

Figure 4.19

Influent SOC, g/L·d	SOC removed, %
1.18	21.0
1.17	19.0
1.46	33.0
1.24	31.0
1.23	41.0
1.23	45.0
1.23	33.0
1.20	42.0
1.18	38.0
1.03	50.0
1.10	44.0
1.23	36.0
1.14	30.0
1.02	30.0
1.24	46.0
1.14	38.0
1.21	36.0
1.20	29.0
1.21	28.0
1.45	30.0
1.38	30.0
1.35	35.0
1.93	40.0
1.92	20.0
1.71	12.0
1.88	18.0
1.39	22.0

Figure 4.20

C_r	S_r
0.490	0.144
0.474	0.147
0.907	0.260
0.604	0.324
0.965	0.381
0.921	0.269
1.188	0.409
0.843	0.464
0.905	0.506
1.225	0.590
1.164	0.742
1.119	0.779
1.359	0.718
1.286	1.058
1.325	1.070
2.064	1.103
2.051	1.007
1.589	1.274
1.835	1.248
2.178	1.339

Figure 4.21

Day	Primary %	Secondary %
113	29	43
114	28	
115	27	40
116	46	59
117	39	56
118	24	42
119	24	42
120	12	34
122	14	25
123	20	24
124	18	25
125	20	24
126	21	25
127	20	24
128	23	26
129	25	29
130	33	35
131	28	33
132	34	
133	33	36
134	36	39
135	22	32
136	21	32
137	26	30
138	21	27
139	21	
140	23	24
141	19	27

Figure 4.22

Day	Primary %	Secondary %
113	22	23
114	32	
115	39	
116	41	
117	43	57
118	17	55
119	19	52
120	20	41
122	24	32
123	21	32
124	35	48
125	35	44
126	35	46
127	34	35
128	40	41
129	39	40
130	37	40
131	42	44
132	32	33
133	33	36
134	45	
135	43	
136	41	
137	40	41
138	47	49
139	43	48
140	49	53
141	41	51

Figure 4.25

pH	S_r/C_o R9-R11	S_r/C_o Biosulfix
4.5	0.089	
4.7	0.069	
4.8	0.085	
5.0	0.154	
5.1	0.085	
5.2	0.072	
5.3	0.100	
5.4	0.089	
5.5	0.087	
5.6	0.125	
5.7	0.134	
5.8	0.211	
5.9	0.199	
6.0	0.211	
6.1	0.213	
6.2	0.225	
6.3	0.313	
6.4	0.260	
6.5	0.214	
6.6	0.277	
6.7	0.178	
6.8	0.223	0.068
6.9	0.229	0.164
7.0	0.312	0.149
7.1	0.209	0.165
7.2	0.236	0.184
7.3		0.216
7.4		0.240
7.5		0.216
7.6		0.199
7.8		0.299
8.1	0.413	

Figure 4.26

pH 6.0 Sulfide Concentration, mg/L

Time	0	100	250	500	1000
1.5	81				
4	363	103			
8		360	120	84	86
11			359	91	65
13				134	
23				117	113
27				144	120
36				365	
180					238
228					303

pH 7.0 Sulfide Concentration, mg/L

Time	0	100	250	500	1000
1	41	29	29	21	19
4	102	139	86	106	
7	322	329	333	319	36
10					97
12					140

pH 8.0 Sulfide Concentration, mg/L

Time	0	100	250	500	1000
2		14			
4	42	109	66	54	15
5	122				
6	254	311	113	203	93
7			301	301	
8					218

Figure 4.27

H_2S	% Uptake					
	4 hours	8	10	12	24	228
0	32	100	100	100	100	100
9	35	100	100	100		
23	21	100	100	100		
45	17	100	100	100		
90			100	100		
124	26	100	100	100		100
227		33	100	100		
247	33		100	100	100	
453	7.4	23	30	37	32	
495	20	30	44			
907	9.3	24	20	20	31	83

Figure 4.28

Time (d)	pH 6.4	pH 7.3	pH 8.0
0 mg/L S^{2-}			
0	0	0	0
1.5		17	22
4	35	57	60
8	85	94	95
10	100		100
14	100	100	
17	100		
21	100		
25	100		
74	100		
50 mg/L S^{2-}			
0	0	0	
1.5	18	30	
4	44	56	
8	81	98	
10	90		
14	100	100	
17	100	100	
21	100	100	
25	100	100	
74	100	100	
150 mg/L S^{2-}			
0	0	0	0
1.5	0	0	13
4	17	33	28
8	51	54	
14	80	79	91
17	97	100	100
21	100	100	100
25	100	100	100
74	100	100	100

250 mg/L S^{2-}

0	0	0	0
1.5	0	0	6
4	0		29
8	0	21	
14	0	50	84
17	8	61	87
21	12	100	100
25	20	100	100
74	41	100	100

500 mg/L S^{2-}

0	0	0	0
1.5	0		0
4	0		22
8	0		
14			46
17	0		66
21	14		93
25	100		100

750 mg/L S^{2-}

0	0	0	0
1.5			0
4			0
8			0
14			0
17			36
21			68
25			88
74	100		100

1000 mg/L S^{2-}

0	0
8	0
17	18
21	42
25	57
74	100

Figure 4.29

Acetate Utilized, %

H_2S	8 d	17 d	25 d	74 d
0	100	100	100	100
4				100
13		100	100	100
16	100	100	100	100
22			100	100
37	52	100	100	100
44			100	100
49	54	100	100	100
67				100
82	21	61	100	100
113				100
140	0	0	0	
177				100
189				41

Figure 4.30

Initial Sulfide Concentration, mg/L

Days	0	50	100	250	500	750	1000
	Sulfur Reduced, mg/L Initial pH 6.0						
0.0	0	0	0	0	0	0	0
0.5	65	65	37	37	37	27	0
2.0	64	92	83	17	36	36	73
7.0	188	171	150	38	15	15	0
21.0	208	208	222	236	215	82	26
	Sulfur Reduced, mg/L Initial pH 7.0						
0.0	0	0	0	0	0	0	0
0.5	30	17	24	0	0	0	0
2.0	73	73	73	45	36	36	18
7.0	153	158	150	135			
21.0	229	236	229	201	167	125	97
	Sulfur Reduced, mg/L Initial pH 8.0						
0.0	0	0	0	0	0	0	0
0.5	0	0	0	0	0	0	0
2.0	101	92	0	0	0	0	0
7.0	180	180	157	142	0	67	0
21.0	215	250	209	201	181	104	83

Figure 4.31

Initial S^{2-}	Sulfur Reduced, mg/L
pH 7.0	High Carbon, High Sulfur
0	1094
64	1065
128	1037
320	671
640	242
960	140

Figure 4.32

Initial S^{2-}	Sulfur Reduced, mg/L
pH 6.4, Low Carbon	
0	229
50	236
100	229
250	201
500	167
750	125
1000	97
pH 7.3, Low Carbon	
0	208
50	222
100	222
250	236
500	215
750	82
1000	26
pH 8.0, Low Carbon	
0	215
50	250
100	209
250	181
500	195
750	104
1000	83

Figure 4.33

S^{2-} initial	Sulfur Reduced
pH 7.0, High Carbon	
0	1094
64	1128
128	1166
320	991
960	1100
pH 6.4, Low Carbon	
0	229
50	286
100	329
250	451
500	667
750	875
1000	1097
pH 7.3, Low Carbon	
0	208
50	272
100	322
250	486
500	715
750	832
1000	1026
pH 8.0, Low Carbon	
0	215
50	300
100	309
250	431
500	695
750	854
1000	1083

Figure 4.34

Final S^{2-} mg/L	Sulfur Reduced mg/L	TOC removed pH 6.4	pH 7.3	pH 8.0
217	222	353	350	356
286	236	353	351	347
320	220	351	364	359
456	206	348	362	356
692	192	171	348	353
854	104	95	226	353
1068	69	38	230	344