Control of souring in an oil field surface facility : biotic and abiotic effects of nitrate
by Darla Marie Goeres

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Environmental Engineering
Montana State University
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Abstract:
A field test was performed in a 5000 barrel produced water tank at Exxon's Hawkins Oil Field in Hawkins, Texas. The test goal was to evaluate the ability of nitrite to inhibit sulfate reducing bacteria (SRB) on a production scale. In the lab, researchers have been able to control souring in porous media columns, batch vials and biological reactors with the addition of 10 ppm-N nitrite. During the test, planktonic SRB concentration in the produced water of the tank decreased 1000-fold five hours after nitrite addition. The basic goal to demonstrate inhibition of SRB was accomplished.

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APPROVAL

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Darla Marie Goeres

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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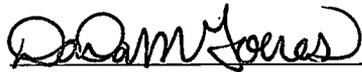
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ACKNOWLEDGMENTS

TABLE OF CONTENTS

TABLE OF CONTENTS - <u>Continued</u>

LIST OF TABLES

LIST OF FIGURES

FIGURES - <u>Continued</u>

ABSTRACT

A field test was performed in a 5000 barrel produced water tank at Exxon's Hawkins Oil Field in Hawkins, Texas. The test goal was to evaluate the ability of nitrite to inhibit sulfate reducing bacteria (SRB) on a production scale. In the lab, researchers have been able to control souring in porous media columns, batch vials and biological reactors with the addition of 10 ppm-N nitrite. During the test, planktonic SRB concentration in the produced water of the tank decreased 1000-fold five hours after nitrite addition. The basic goal to demonstrate inhibition of SRB was accomplished.

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CHAPTER 1: INTRODUCTION

Sulfate Reducing Bacteria (SRB) are a consortium of anaerobic microorganisms that reduce sulfate to hydrogen sulfide while utilizing short chain fatty acids as a carbon source. Oil fields and their production facilities host excellent SRB growth conditions. Especially when recycled water and seawater is injected into oil bearing formations to increase oil recovery from the field, a high production of hydrogen sulfide can be found in many of the formations after some lag time (Burger *et al.*, 1992; Frazer and Bolling, 1991). The presence of hydrogen sulfide defines a field as sour. Hydrogen sulfide can exist both in the aqueous and gas phases.

The problems associated with hydrogen sulfide and SRB growth are numerous and expensive, costing oil companies billions of dollars every year. Hydrogen sulfide is both extremely toxic and corrosive. In addition, the soured crude oil may contain some dissolved H_2S, which would decrease its value and increase refining costs (McInerney *et al.*, 1991). SRB biomass and precipitated iron sulfide are responsible for increased reservoir plugging which retards secondary oil recovery. For these reasons, oil companies are challenged with finding both an effective and economical means of controlling the SRB population and the hydrogen sulfide by-product. This thesis explores nitrite's ability to solve both problems.

Approximately four years ago a consortium of oil companies commissioned The Center for Biofilm Engineering at Montana State University to study and characterize the SRB responsible for souring their oil. Through a series of

experiments performed in batch systems, chemostats and porous media columns, growth kinetics were determined for the consortium of bacteria. In addition, the group at MSU discovered nitrite could be used to inhibit SRB growth. This discovery led to a new set of experiments which determined nitrite's efficiency at acting like a biocide. In conjunction with lab experiments, a computer model was constructed to simulate the results obtained from growing the SRB in a porous media column, including nitrite inhibition. The next logical step was to scale-up the experiments to actual field conditions.

During the scale-up process, the decision was made to use a surface facility to test the new technology. The basis for this decision was the ease of sampling at a surface facility versus the logistic problems of sampling a down-hole application. The surface facility chosen was a produced water tank (PWT) at Exxon's Hawkins Oil Field in Hawkins, Texas. A PWT is the second piece of equipment used in an oil/water separation process. At Hawkins, each PWT is a 5000 barrel tank where the oil is skimmed off the top and the produced water flows out the bottom. The tank influent stream contained both sulfate and short chain fatty acids as well as a significant SRB population. Consequently, hydrogen sulfide and a thick biofilm were present in the tank. Due to EPA regulations and the problems stated earlier that are associated with hydrogen sulfide, Exxon treats their PWTs once a week with a biocide to control the SRB population. The MSU team proposed that Exxon use a nitrite solution in place of their normal biocide application to the PWT to test nitrite's effectiveness in the field.

Before the actual test, a considerable amount of time was spent in the lab testing the PWT's water chemistry as well as studying the SRB consortium

associated with the surface facility. In April of 1995 the actual test was accomplished in a week.

During the test, the concentration of hydrogen sulfide in the PWT's head space changed. Although previous lab experiments had shown nitrite and hydrogen sulfide chemically react (Mueller, 1994; Reinsel, 1995), it was not clear how this reaction may have affected the head space concentration. Consequently, in order to examine the field test results, a new set of experiments was performed which studied the abiotic H_2S/nitrite reaction.

Goals and Objectives

The primary goal of this project was to test nitrite as a SRB inhibitory agent in an oil field surface facility. From this primary goal sprang the possibility of using nitrite to control hydrogen sulfide through an abiotic chemical reaction; the second goal was to examine the potential of nitrite as an abiotic control.

The two above stated project goals were accomplished through the following objectives:

o   A field test protocol was established.

o   The produced water from Hawkins Oil Field was analyzed.

o   The SRB consortium from Hawkins was studied in lab experiments.

o   The inhibitory effect of nitrite on the SRB consortium was studied.

o   The produced water was tested for the presence of denitrifying bacteria.

o   The abiotic H_2S/nitrite reaction was studied by analyzing the effect of pH, nitrite concentration and hydrogen sulfide concentration on the reaction rate.

o   The field test was performed with hydrogen sulfide concentrations, SRB

populations, and nitrite concentrations measured after the nitrite application,

and the results were interpreted.

This thesis presents the results, discussion and conclusions for both goals

and the objectives used to accomplish them.

CHAPTER 2: BACKGROUND INFORMATION

Sulfate Reducing Bacteria-General Review.

A soured oil reservoir is one in which hydrogen sulfide is present. Hydrogen sulfide (H_2S) is produced by a consortia of strict anaerobic microorganisms known collectively as Sulfate Reducing Bacteria (SRB). SRB exist in two forms in an oil reservoir. First, the SRB accumulate as biofilms on the oil reservoir porous media, in the surface facilities and in the piping which connects the latter two together. Secondly, SRB exist as planktonic microorganisms in the oil reservoir's produced water.

In 1988, Widdel defined SRB as a heterogeneous assemblage of microorganisms having in common dissimilatory sulfate metabolism and obligate anaerobiosis (Widdel, 1988; Mueller, 1994). By definition then, the SRB metabolic pathway is the reduction of sulfate to sulfide. Due to injection of sea or brackish water that many oil companies began so as to increase oil recovery, sulfate is present in abundance in almost all secondary oil recovery systems. After a short lag time, previously sweet oil reservoirs begin producing a high concentration of H_2S once water injections are begun (Burger *et al.*, 1992; Frazer and Bolling, 1991).

In the absence of sulfate, SRB are capable of using sulfite or thiosulfate as the terminal electron acceptor (Badziong and Thauer, 1978; Cypionka *et al.*, 1985; Jorgensen and Bak, 1991). Growth of SRB with elemental sulfur as electron acceptor has been observed with some isolates. *Desulfovibrio desulfuricans* and some other *Desulfovibrio* strains, *Desulfobulbus propionicus* and *Desulfobacterium*

catecholicum were all shown to use nitrate as an electron acceptor producing ammonium (Widdel, 1988). Also, sulfate reduction occurs 10 times faster in a monopopulation SRB biofilm than in a mixed SRB sewer system biofilm (Okabe *et al.*, 1992; Nielsen, 1987).

For many years, experts in the field of SRB research, such as Postgate, thought lactate or pyruvate were the sole carbon sources SRB were capable of utilizing. Widdel and Pfennig proved this hypothesis wrong in 1981 when they isolated the species *Desulfobacter postgatei*, which almost exclusively utilizes acetate as its carbon source. The discovery of SRB utilizing short-chain fatty acids changed the way of viewing the microbial ecology of SRB in natural systems (Sorensen *et al.*, 1981).

In an oil reservoir, oxygen is present on an extremely limited basis. Once the make-up water enters the reservoir's closed water system, facultative microorganisms such as fermentative bacteria quickly utilize any dissolved oxygen. In fermentation, the overall oxidation state (e.g. energy state) of the degraded matter remains unchanged (Mueller, 1994). In many natural anaerobic environments, the quantitatively most important fermentation products formed with CO_2 are H_2, acetate, propionate, and butyrate (Sorensen *et al.* 1981; Lundegard and Kharaka, 1990.) If sulfate is present under anaerobic conditions and the redox potential is sufficiently low (Eh < -100 mV), SRB are able to utilize the reduced fermentation products. Sulfate is reduced to hydrogen sulfide, whereas the organic materials are oxidized either completely to CO_2 or incompletely to acetate (Widdel, 1988). The SRB species which completely oxidize the organic substrates to CO_2 are more versatile, but usually have slower growth rates than the species which carry

out an incomplete oxidation (Widdel, 1988). Therefore, SRB in natural environments depend on fermentative bacteria that cleave and ferment the more complex organics to low molecular weight compounds (Jorgensen, 1989).

Although SRB are anaerobic microorganisms, they are capable of growing in marine-sediment micro niches because of the high sulfate concentration of seawater (2800ppm) (Fossing and Jorgensen, 1989). These micro niches are often made visible by blackening of the sediment, which is a result of the formation of ferrous sulfide (FeS) from iron minerals (Cord-Ruwisch et al., 1987). Two factors explain the formation and maintenance of such micro niches. First, the respiration of aerobic bacteria scavenges oxygen and favors growth conditions for SRB below the aerobic bacteria. Secondly, the produced H_2S is a strong reducing agent that reacts with oxygen even at low temperatures. Thus, once established, the SRB can protect themselves against oxygen (Cypionka et al., 1985).

SRB prefer growing in a neutral pH environment. Activity can be observed over a pH range of about pH = 5.5 to pH = 8.5 (Mueller, 1994). Although, SRB have been observed in more acidic waters (Tuttle et al., 1969). The SRB metabolic products establish the following buffer systems: HS^-/H_2S and HCO_3^-/CO_2. These systems help keep the SRB's surrounding environment pH neutral, and so protects the biofilm against extreme pH changes (Crolet et al., 1992; Curd-Ruwisch et al., 1987).

A SRB tolerance to salinity depends upon where the specific consortium originated. A concentration of 20 to 30 g/L NaCl will inhibit the growth of fresh water SRB. Conversely, marine SRB are moderately halophilic by nature, requiring 10 to 30 g/L NaCl for optimum growth (Mueller, 1994). The activity of most SRB

declines drastically if the NaCl concentration exceeds 50 to 100 g/L (Postgate, 1984; Widdel, 1988).

SRB require both phosphate and nitrogen for growth, although about 10 to 20 times less than aerobic bacteria because of their slower growth rate. Okabe reported the stoichiometric ratio of C:N:P for *D. desulfuricans* grown on lactate as the limiting nutrient was in the range of 1000:4:1 with a cell yield of 0.03g cells/g lactate (Okabe *et al.*, 1992).

Sulfate reducing bacteria can be divided into two primary classes based upon optimum growth temperature. Mesophilic SRB (m-SRB) are usually found in environments with temperatures below 45° C (Herbert and Stott, 1983). Most m-SRB have a fairly broad temperature optimum between 30 and 37° C. The second classification is thermophilic SRB (t-SRB), which grow best at temperatures between 55 - 75° C. Many t-SRB can survive at temperatures higher than 75° C by the formation of spores. SRB form spores (Rosnes *et al.*, 1991a) when threatened by environmental factors such as extremely high temperatures, and have been shown to survive a temperature of 131° C for 20 minutes. Some species of t-SRB actively produce H_2S at temperatures up to 80° C (Rosnes *et al.*, 1990) Historically, the majority of SRB research was done using m-SRB, because t-SRB are difficult to isolate and cultivate in the lab (Edwards, 1990).

Recent and Relevant Souring Research

Both m-SRB and t-SRB have been found in injection water for oil reservoirs (Cochran *et al.*, 1988). The current understanding of souring is that it occurs at a mixing zone, where the injection water, formation water, and the oil mix (Ligtheim *et al.*, 1991, Burger *et al.*, 1992, Frazer and Boiling, 1991). In this zone the

essential components supporting SRB growth-- electron donors such as organic compounds or hydrogen, electron acceptor such as sulfate, and other essential nutrients-- are present (Herbert *et al.*, 1985) Mesophilic SRB would generally thrive near the injection well, where cold injection water first enters the formation. The mixing zone moves with the water front and therefore the temperature increases as it moves toward the production well (Mueller, 1994). As the temperature increases, t-SRB growth would be continuously more favored. Two different fields of thought exist on the origin of t-SRB in an oil reservoir. Rosnes *et al.* (1991a) believe t-SRB are indigenous inhabitants of oil field waters, whereas Cochrane *et al.* (1988) think t-SRB are not originally present in the formation, but arrive as spores in the injection water, or mutate from m-SRB.

Fundamental Souring Research Done at CBE

At the Center for Biofilm Engineering (CBE) souring research was done on the characterization of thermophilic SRB consortia from two different soured oil reservoirs. Samples were collected from the Kuparuk (North Slope) and Ninian (North Sea) oil fields. The rationale for choosing these sites was that previous work on characterization had been done by Arco (Burger *et al.*, 1992; Frazer and Bolling 1991) and because there were important differences in operation between the two tested sites such as pressure and temperature differences and length of time each field had been soured. The microbial consortia collected from the two sites were examined and characterized by size and morphology; their activity was then examined on expected preferred substrates at a temperature range from 35 to 75°C. Temperature was found to influence the rate of sulfate reduction, the rate of H_2S production, the rate of substrate oxidation, and the cell morphology (Mueller, 1994).

In addition to the characterization studies, stoichiometry and kinetics were determined for the t-SRB Kuparuk and Ninian consortia. A better understanding of rate and stoichiometry was needed to model a reservoir and to determine the best potential control mechanisms. During these experiments, biofilm formation was examined because Chen *et al.* (1994) had reported that the attached biomass contributed over 99% to the total biomass found in a porous media reactor. The experiments showed stoichiometric ratios were in a similar range for both the Ninian and Kuparuk consortia. Both consortia grew best on butyrate, although the SRB from the Ninian oil field grew at approximately half of the maximum growth rate of the SRB from the Kuparuk field. The biofilm studies showed t-SRB from the Kuparuk field did not exhibit significant differences in cellular growth rate, growth yield, sulfate reduction rate, and stoichiometry between suspended and attached cells (Mueller, 1994). Thus, it is likely that a SRB consortium from other fields would act similarly.

Microbial souring in porous media columns was then studied using the same Ninian and Kuparuk SRB consortia as was used for the characterization, kinetics and stoichiometry experiments. The consortia were grown in anaerobic up-flow fixed bed porous media reactors operated at a thermophilic temperature using the main organic acids found in reservoir waters as carbon and energy sources. The idea was that the laboratory columns be the first step towards a simulation of a reservoir. The results obtained could then be used in reservoir computer model simulations.

The most notable information gleaned from the porous media studies was that similar souring activity (H_2S concentrations, rates of production, and substrate preferences) was seen for both the Kuparuk and Ninian oil field consortia. Activity

in the porous media systems was similar to that seen in batch experiments containing suspended cells. The bulk of souring activity occurred at the beginning of the column, yet when the columns were disassembled an even distribution of biomass was found (Reinsel, 1995). In a porous media experiment done using a Berea sandstone core system, a large permeability decrease resulted from the growth of the Kuparuk SRB consortium. It appeared that plugging due to iron sulfide precipitation was at least as significant as that due to biomass formation (Goeres, 1994).

Concerning the porous media modeling results, the initial model runs using independent estimates of physical and biological parameters from batch culture kinetic studies and literature were not able to adequately describe all features of the experimental results. After additional work, a key parameter identified by the simulations was the "biofilm thickness." The modified model was able to better simulate the experimental system.

Souring Control Mechanisms-General

Essentially, an oil company can choose one of four strategies to control souring in a reservoir and the adjoining surface facilities. One, the injection water can be pretreated. The basis for this strategy is to remove the components necessary for SRB growth to occur, such as sulfate or nutrients, before the injection water ever reaches the reservoir. Two, SRB growth can be inhibited or stopped. This goal can be accomplished through the addition of a biocide either down hole or in a surface facility where souring is a problem, such as a produced water tank. In addition to biocides, treatments such as aeration, radiation, sulfide inhibition, acetate inhibition, control of Eh or pH, or microbial competition can be used to stop

or inhibit SRB growth (see Mueller (1994) and Reinsel (1995) for a detailed discussion). Third, the hydrogen sulfide can be removed from the produced water by chemical or microbial scavenging. Finally, an oil company can choose to ignore the problem, or just modify the steel, etc., in their facilities.

In reality, an oil company most likely uses some combination of all four strategies to control souring in the reservoir and surface facilities. Because of the stiff competition which exists among oil companies, and the vast differences in operation protocol and actual field conditions from site to site, the exact control strategies employed by each oil company at each site are impossible to know. Most importantly, even if the information is available, it is most likely proprietary.

Currently many oil fields add a biocide to the injection water to control souring. In an experiment which compared various biocidal efficacies in controlling SRB, Grab and Theis (1993) reported glutaraldehyde as being the most effective of the biocides tested against both the planktonic and sessile sulfate reducing microorganisms. Reinsel (1995) reported that after glutaraldehyde is removed from a porous media system, H_2S production quickly recovers. This suggests that glutaraldehyde does not have any long-term inhibition effects but instead lowers the SRB population. After glutaraldehyde removal, SRB apparently multiply at their original growth rate and simultaneously produce hydrogen sulfide (Reinsel, 1995).

The use of nitrate (NO_3^-) to control noxious odors caused by SRB in waste water treatment was recognized as early as 1929 (Allen, 1949). Jenneman et al. (1986) reported that nitrate inhibited sulfide accumulation in a variety of anaerobic environments. Nitrate was also found to reduce sulfide concentrations in produced water from a hyper saline oil field (McInerney et al., 1993). In April of 1995,

Hitzman *et al.* received a U.S. patent for the addition of both nitrate and nitrite (NO_2^-) to an aqueous system to control the production of hydrogen sulfide by SRB. Their claim was that denitrifying (DNB) would utilize the nitrate and nitrite first and out-compete the SRB for the available nutrients.

There are several possible mechanisms by which nitrate can prevent the accumulation of sulfide production. First, DNB may out compete SRB for common electron donors such as acetate and longer chained fatty acids. Second, DNB may produce compounds that raise the redox potential of the environment to a level that is inhibitory to the growth of SRB. Third, the SRB preferentially use nitrate instead of sulfate as the electron acceptor. Finally, DNB may use the sulfide produced by SRB as the electron donor for nitrate reduction (Jackson and McInerney, 1994). In the later case the production of sulfide would not be inhibited, but the use of sulfide by DNB would prevent its accumulation (Montgomery *et al.*, 1990). Depending on the microbial populations present any of the above mechanisms or combinations of the mechanisms may be operative in a given ecosystem.

Controlling the SRB population is only part of the solution for controlling souring in aqueous systems. In actuality, the presence of hydrogen sulfide is probably more costly and dangerous than the SRB themselves. Lee and Sublette (1993) demonstrated the use of the sulfide oxidizing bacteria *Thiobacillus denitrificans* to reduce the concentration of sulfide in produced waters. This was in contrast to the historical method wherein sulfide was removed from aqueous systems by reacting the H_2S with sulfur dioxide at high temperatures (120-200° C) to make elemental sulfur.

Nitrite has been used as a corrosion inhibitor in sulfide-polluted waters (Al-Hajji and Reda, 1993), recirculating cooling water systems (Al-Borno et al., 1989) and contaminated and chloride-free carbonated mortars (Alonso and Andrade, 1990).

Souring Control Mechanisms- CBE

Montana State University began their investigation of using nitrate and nitrite to control souring in an oil field reservoir in 1993. Denitrifying bacteria were found present in the produced waters of both the Kuparuk and Ninian oil fields. When nitrate and sulfate were present with a SRB consortia in oil field water, after an initial lag time no measurable sulfate reduction occurred and approximately 80% of the nitrate initially present was converted to nitrite (Mueller, 1994). In the porous media up-flow column experiments, 10 ppm NO_3-N was required to completely inhibit active souring, but once inhibited the concentration could be lowered to 5 ppm NO_3-N and still maintain inhibition. Souring was inhibited for three months with the continuous addition of nitrate to the columns. If the nitrate was removed and the column allowed to sour, a reapplication of nitrate would once again inhibit souring (Reinsel, 1995).

It was discovered by measuring ion concentrations during the column experiments that nitrite was present. From this information came the deduction that DNB were reducing nitrate to nitrite, and the nitrite was the inhibitory agent responsible for the souring suppression. Inhibition of SRB in batch systems was in fact found for nitrite concentrations of 5 ppm NO_2-N or higher. In addition, the produced nitrite chemically reacted with hydrogen sulfide. This reaction was strongly pH dependent (Mueller, 1994).

The researchers at MSU showed the addition of 10-12 ppm NO_2-N completely inhibited souring in a porous media column. Pulses of nitrite were more effective than the same amount of nitrite added continuously. The addition of nitrite raised the Eh in the column, but not to inhibitory levels for SRB (Reinsel, 1995). The addition of nitrite to the Berea sandstone core system caused a significant decrease in pressure drop across the core. This shows nitrite not only addresses the H_2S problem associated with souring, but could also reduce plugging (Goeres, 1994). Nitrite was found to be more effective at inhibiting souring than glutaraldehyde. Glutaraldehyde killed the SRB while nitrite provided a long-term inhibition without killing. Even after the nitrite was removed, the hydrogen sulfide production by SRB was decreased for up to five days (Reinsel, 1995). The inhibition constant for nitrite was proven to be a key parameter in a computer model which simulated inhibition of souring by both DNB and nitrite addition to a porous media column (Reinsel, 1995).

CHAPTER 3: MATERIALS AND METHODS

Introduction

The field test was performed on produced water tanks at Exxon's Hawkins Oil Field in Hawkins, Texas. The primary research work was done at Hawkins Oil Field. In addition to the field work, microbial and kinetic experiments were conducted in the lab at Montana State University. The focus of Chapter 3 is a description of the experimental materials and methods employed for the completion of this project. In addition, this chapter will explain the dynamics of Hawkins Oil Field and the necessary protocol which was developed for a successful field experiment.

Hawkins Oil Field

Background Information

Hawkins Oil Field is located in the north eastern part of Texas, approximately 35 miles due north of Tyler. Exxon Oil Company owns and operates the field. Hawkins was chosen for three reasons: background souring research had previously been done on the surface facilities in 1988 by Sara McMillen (Exxon); the operator was agreeable with performing such a test, and surface facilities are more easy to control and monitor than an entire reservoir. The nitrite addition occurred at a produced water tank battery located at Greer salt water station. Greer has three 5000 barrel produced water tanks arranged in parallel (Figure 3.1). The flow from the field is approximately 20,000 - 25,000 barrels/day, and in theory, is split equally among all three tanks. The tanks serve as a second oil/water separation, so
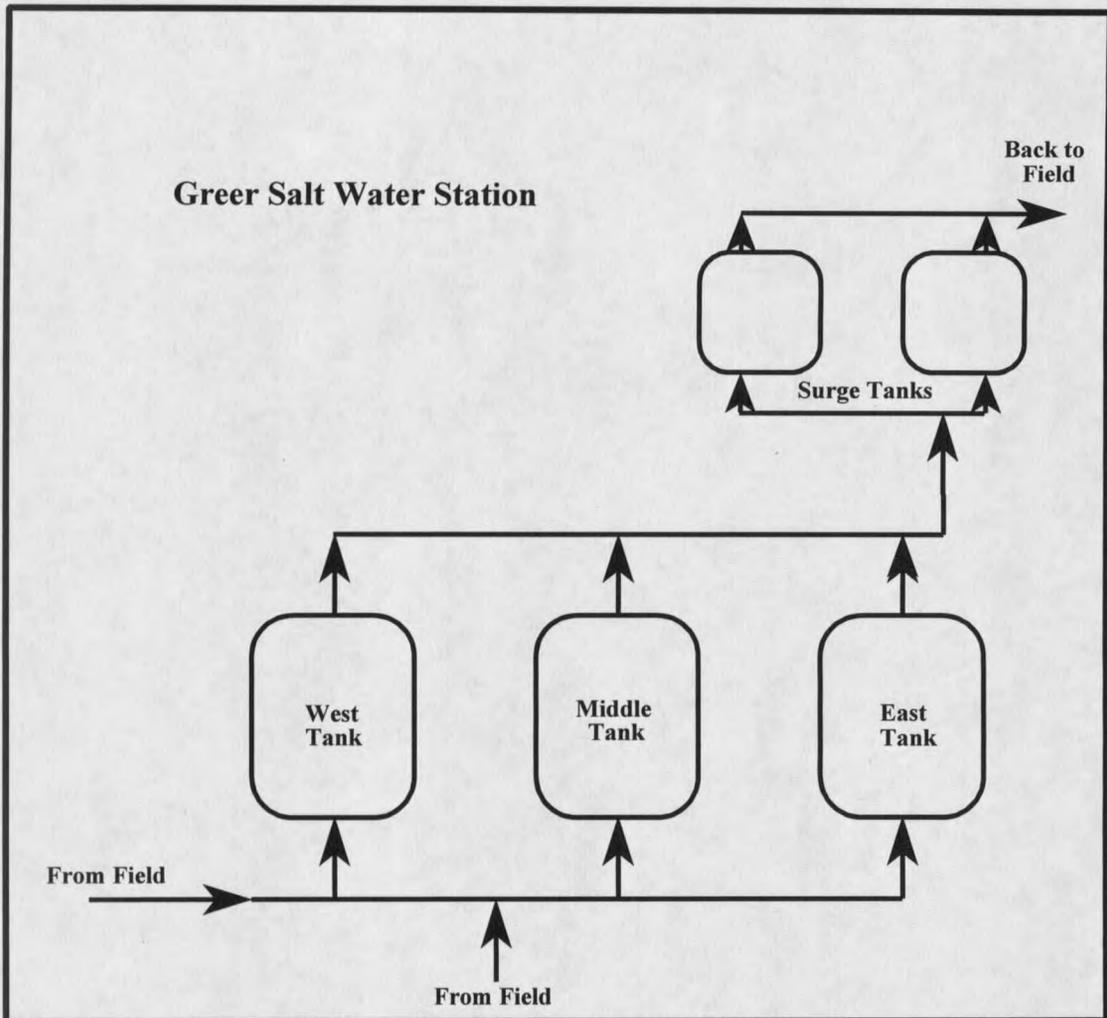
Figure 3.1: A flow diagram for Greer Salt
Water Station, Hawkins, Texas

separated oil flows from the top of the tank and the salt water flows out of the

bottom. The calculated residence time in the tanks is ~12 hours. From the

produced water tanks, the three bottom flows are rejoined and then fed into two

parallel 1500 barrel surge tanks, after which the water is used for re-injection into

the field.

Exxon is currently using the biocide glutaraldehyde to control souring in the

tanks. A dosage of 10 gallons glutaraldehyde/tank has been added once a week.

This calculates to a treatment of 24 ppm glutaraldehyde.

Analytical Methods

o Sulfate was measured using a Dionex ion chromatograph (Model AI-450; Dionex Co., San Francisco) with a pulse electrochemical detector (Model DX 300). Sample preparation included adding 0.1 ml sample to a specified amount of ultra pure water to obtain a desired dilution. After vortexing the diluted sample, it was passed through a Dionex OnGuard-Ag sample pretreatment cartridge to remove any chlorine and cells before being run on the IC. The IC specifications for analysis are presented in Table 3.1. The detection limit for sulfate analysis was less than 0.1 ppm with a standard deviation smaller than 5% for identical samples. All sulfate concentrations are presented in terms of sulfur content.

o Nitrate was tested using the exact same method as was used to measure sulfate concentrations. The IC, when set-up for anion detection, detects nitrite, nitrate, phosphate and sulfate in the stated order. All nitrate concentrations are presented in terms of nitrogen content.

o Nitrite was measured using a Hach chemical test kit (catalog #20596). Although the IC was capable of measuring nitrite, more accurate results were obtained using the Hach test kit because of interference from chlorine, even when the OnGuard-Ag pretreatment cartridges were used. The Hach test kit is a colormetric method. If the sample being tested contained nitrite, a pink color would develop after the reagent was added and the sample vortexed for one minute. For the samples done at MSU, a Milton Roy Spectronic 601 color spectrometer equipped with a tungsten bulb read the absorbance of the

sample. The wavelength was set to 546 nm. The samples taken at Hawkins were read on an older model color spectrometer. Before the field test commenced, a day was spent calibrating the older machine. The calibration curve for nitrite obtained in Hawkins can be found in the Appendix. All nitrite concentrations are presented in terms of nitrogen content.

Table 3.1 Ion Chromatograph Specifications for Analysis		
SPECIFICATION	SULFATE	VFAs
Column type pore size	AS4A-SC 2 mm	AS10 4 mm
Detector	conductivity	conductivity
Eluent concentration	$Na_2CO_3 + NaHCO_3$ 1.8 mM/1.7 mM	$K_2B_4O_7$ 3.5 mM
Regenerant concentration	none	$K_2B_4O_7$ 100 mM
Flow rate	0.5 ml/min	1.0 ml/min

o   Volatile fatty acids (VFAs) were measured using the same Dionex ion chromatograph as the sulfate samples. IC specifications for VFAs are also presented in Table 3.1. Samples diluted with ultra pure water were passed through a Gelman Acrodisc filter with a pore size of 0.2 μm before being run on the IC. Detection limits for acetate, propionate, i-butyrate, and n-butyrate were smaller than 2 ppm. The detection limit for formate was smaller than 4 ppm. The standard deviation among identical samples was less than 5%.

o   Hydrogen sulfide in the aqueous phase was measured using the methylene blue method (Cline, 1969). For a 1:50 dilution, 0.1ml of sample was added to 4.9 ml of 1% zinc acetate. To this solution 0.4 ml of diamine reagent was

added. After 20 minutes, absorbance of any blue color which developed was read on a color spectrometer. The wavelength was set to 670 nm. The color spectrometers used were the exact same as those used for nitrite analysis. The standard curve obtained for H_2S in Hawkins can be found in the Appendix. The detection limit for H_2S was smaller than 50 ppb, with a standard deviation of less than 2% for identical samples. All liquid hydrogen sulfide concentrations are expressed in terms of sulfur. The hydrogen sulfide in the head space of the produced water tanks was measured using a commercial Dragger tube. The H_2S in the head space of the kinetic batch experiments was measured by pulling a syringe full of gas out of the experimental flask through a septum. Then 0.1 ml of gas was slowly bubbled through 4.9 ml of 1% ZnAc. From this point, the method was the same as the liquid samples.

Total cell counts were performed using the acridine orange direct count (AODC) method (Hobbie *et al.*, 1977). Cells were fixed in a 4% buffered formaldehyde solution before the acridine orange (AO) was added to the sample. After the addition of AO, the sample was vortexed for one minute and allowed to stain for 30 minutes before being filtered and placed on a glass slide. A microscope equipped with an ultraviolet light and counting grid was used for cell enumeration. The equation used for calculating the number of cells per ml was:

cell count = average x dilution factor x size factor

where size factor = filter area/grid area

o   <u>Most probable number (MPN)</u> tests were performed at Hawkins using

    commercially produced Postgate Media B with lactate and acetate as the

    carbon sources (Clesceri *et al.*, 1989). The vials were incubated at 35° C.

Laboratory Batch Studies

<u>Growth Vials: Medium Definition</u>

The medium used for the batch growth vials was suggested by Pfennig *et al.*

(1986) for marine type SRBs. This particular recipe was chosen for two reasons.

First, the Pfennig medium is defined. Secondly, Pfennig medium was the medium of

choice for all of MSU's kinetic experiments and was used for this work for

consistency. The medium was prepared with a variety of sterile stock solutions. A

detailed medium composition and preparation procedure can be found in the

Appendix. The sulfate-S concentration in the medium was in excess at 300 ppm.

Sodium chloride was used to adjust the medium's salinity to 2%. After autoclaving,

the medium was saturated with a prepurified gas mixture of 10% CO_2/90% N_2 to

minimize contamination as well as to ensure an oxygen free environment. Dithionite

was also added in small amounts to help maintain anaerobic conditions. The pH of

the medium was 6.5-7.0. The electron donors (carbon source) added to the Pfennig

medium were the following five short chain fatty acids: formic acid, acetic acid,

propionic acid, n-butyric acid and isobutyric acid. Each electron donor was added at

a initial concentration of 200 ppm. The medium could be prepared ahead of time up

to the point just prior to the fatty acid addition and then stored in a refrigerator until

needed.

The Pfennig medium was also simply adapted for support of denitrifying

bacteria (DNB) growth during this thesis project by the addition of nitrate-N in place

of the 300 ppm sulfate-S. All other medium preparation procedures were the exact
same as those described for the SRB medium.

Growth Vials: Experimental Approach

In December of 1994, a field inspection trip was made to Hawkins, Texas for
the following reasons. First, the daily operation of Hawkins field needed to be
understood. Second, water samples from the influent and effluent of the Middle
tank were collected and brought back to Montana State University for evaluation.
To ensure anaerobic water samples, five gallon bottles were filled to overflowing
and immediately capped. Unfortunately, the size of the bottles hindered easy
transportation. Therefore, water was siphoned from the bottom of the bottle into
smaller jugs. The jugs were once again filled to overflowing and immediately
capped.

Immediately upon arrival at MSU, the produced water samples were placed in
an anaerobic tent. Under anaerobic conditions, samples were taken for analysis.
The water was tested for the presence of the chemicals and conditions necessary
for souring to occur. In addition, an AODC was performed to determine the order of
magnitude of cells in a ml of the produced water.

Within twelve hours of arriving at MSU, the produced water was used to
inoculate a series of previously prepared SRB batch vials. The abiotic vials were
removed from the refrigerator and placed in a 40°C incubator for approximately an
hour. Once the vials were heated to the desired temperature, the fatty acids were
added. An anaerobic stock solution had been previously made containing a high
concentration of all five fatty acids for ease in their addition to the vials. Now the
vials were ready to be inoculated with the produced water. In total, eleven SRB

vials were inoculated with five ml of the middle tank's produced water influent and eleven SRB vials were inoculated with five ml of the middle tank's effluent. After inoculation, time zero samples were taken from each vial and analyzed for sulfate, hydrogen sulfide and VFAs. The vials were then incubated at 40° C and allowed to sour. Sulfate, hydrogen sulfide and VFAs concentrations along with AODC were determined for eighteen days.

During the same time as the souring batch vial experiments, an attempt was made to find denitrifying bacteria (DNB) in the produced water samples. The procedure for this task was the same as the one used for the SRB vials, except the previously prepared batch vials contained DNB specific Pfennig medium. In total, six DNB vials were inoculated with five ml of the middle tank's effluent and six DNB vials were inoculated with five ml of the middle tank's influent. Time zero samples were taken from each vial and analyzed for nitrate, nitrite and VFAs. The vials were incubated at 40° C and monitored for eighteen days.

In March 1995, Hawkins sent fresh produced water samples collected from the effluent of the middle tank. These samples were used to inoculate another series of SRB batch vials. The same procedure was used for the second set of experiments as was used for the first.

In addition to the growth experiments, nitrite's effectiveness as an inhibitor was also studied by adding various concentrations of nitrite-N to souring batch vials. For this experiment, an abiotic anaerobic stock solution of nitrite was prepared. Once the SRBs were in logarithmic growth two different concentrations of nitrite were added in duplicate to specified vials. The concentration of nitrite-N added was 20 ppm and 30 ppm. In addition, 30 ppm nitrite-N was added to batch vials prior to

inoculation to test whether the initial presence of nitrite would inhibit souring in a SRB batch vials.

Protocol

General Field Test Protocol

Before any technology is transferred into the field, the following factors are important to know. First, the technology must consistently work under laboratory conditions, where the systems are defined and therefore the results easier to interpret. This includes running experiments using actual field conditions as the defining parameters and water samples from the site where the test will occur. Secondly, the field where the technology will be implemented must be understood and defined as well as possible. The physical conditions such as flow rates and tank placements should be documented. The following chemical conditions should be tested to establish a baseline: pH, temperature, Eh, and water chemistry. An attempt should be made to understand the microbial ecology present- especially the microbes responsible for the problem which is being remediated. The more information gathered before the test, the better the chances of the test being a success.

Once the decision is made to go ahead with the field test, time should be spent deciding the details, which are numerous. These details can include the concentrations of the biocide (or any chemical being tested) to be used and the actual logistics of how the biocide will be added to the surface facility or down hole. Permission must be granted from every person of the company involved with the test. Material Safety Data Sheets, chemicals and equipment all need to be gathered. A testing procedure needs to be decided upon for while the test is in

progress. A field person can be of immeasurable help in gathering the above information and samples.

Once all the details have been decided and everyone concerned gives approval for the go-ahead, the actual test can proceed. While the test is in progress, as much data as possible should be gathered, even though data acquisition may be difficult. The data may indicate that the testing procedure needs to be adapted slightly, while the test is still in progress. The last stage of the field test is analyzing the data and reporting the results to all concerned.

Hawkins Field Test Protocol

After five months of lab work and positive results from the batch tests, the decision was made to proceed to Hawkins, Texas for the actual field test.

As a modification to general protocol, and to help ensure a more conclusive test, during the week prior to the field test the Middle tank was emptied and the sludge on the bottom cleaned out to eliminate the complexity of an existing thick SRB biofilm during the test. In December of 1994, the East tank had also been taken off-line and cleaned. The West tank had not been cleaned for at least two years.

The first day in Texas was spent establishing standardized curves on the oil field's equipment, touring the facility, meeting the workers and learning necessary plant safety regulations. On the second day of the field test, a sodium nitrite-N solution was prepared by adding seventeen 50-pound bags of food grade sodium nitrite into a clean chemical truck containing ten barrels of fresh water. This recipe would produce a nominal 100 ppm N when added to a full produced water tank. The solution was mixed by pumping it through tubing then back into the truck. Prior

to the nitrite addition to the clean Middle tank the H_2S-liquid, H_2S-vapor (in the tank head space) and sulfate concentrations were measured in the influent and effluent streams of the Middle tank. The nitrite solution was then pumped into the tank and the H_2S, sulfate and nitrite concentrations were measured for the rest of the day. The other two tanks were also tested for sulfate and H_2S intermittently during the day. The nitrite addition in the Middle tank was repeated on day four. The same method described previously was employed for the second test. Here an equivalent 100 ppm nitrite-N was added to the East tank by the same procedure described previously. During this entire experiment H_2S-liquid, H_2S-vapor, sulfate and nitrite concentrations were to be continuously measured and recorded as well as MPN tests for SRB using Postgate medium B performed before and after the nitrite addition.

NO$_2$/H$_2$S Abiotic Experiments

Experiments testing the chemical kinetics of the H_2S/nitrite reaction were done using batch reactors. The experiments were done at a pH = 4.3, 7.1 and 8.3. For the three pH concentrations, one liter of Phennig's medium was prepared as stated in the Appendix. Phennig's medium contains approximately 30 ppmS H_2S. The pH of the anaerobic medium was first adjusted up to 9.1 using a concentrated NaOH solution. After adjusting the pH, 30 ml of the medium was injected into six autoclaved, nitrogen purged, sealed vials via a sterile needle and syringe. The vials were then re-nitrogen purged and incubated at 35° C. The pH of the Phennig medium was then lowered using concentrated hydrochloric acid, and the same procedure as was stated above followed for the lower pH vials. For the experiment done at a pH 4.3, the procedure varied slightly. One liter of water was autoclaved

in a flask for 15 minutes. When the water came out of the autoclave, it was cooled with nitrogen to make the solution anaerobic. Enough concentrated H_2S was added to make a 50 ppmS solution. The pH was then adjusted to 4.3 by the addition of concentrated hydrochloric acid. Lastly, 30 ml of the solution added to the anaerobic vials in the same manner as above. At time zero, initial H_2S concentrations were tested. Then nitrite was added, at a noted time, from a concentrated stock solution to obtain 10, 30 and 100 ppm nitrite-N in various test vials. The experiments were timed as H_2S, nitrite and sulfate were monitored over the following days.

CHAPTER 4: RESULTS

Water Chemistry and Microbiology

The produced water samples gathered from the first trip (December 1994) down to Hawkins, Texas were analyzed specifically for the presence of the chemicals and conditions necessary for souring to occur. The information gathered is presented in the table below.

Table 4.1 Results from the Chemical and Microbial Analysis of Greer Salt Water; Hawkins, Texas.	
Tank Temperature	30° - 40° C
Tank pH	5.6
Sulfate concentration	40 ppm-S
Acetate concentration	490 ppm
Propionate concentration	290 ppm
i-Butyrate concentration	290 ppm
n-Butyrate concentration	300 ppm
Formate concentration	270 ppm
Total cell count (AODC)	10^5 cells/ml

Batch Experiments

Microbial experiments were conducted in batch vials using the produced water from Hawkins Oil Field as an inoculum to examine souring potential. Sulfate reducing microorganisms were found to grow in the Pfennig medium anaerobic batch vials. A graph depicting hydrogen sulfide production in four batch vials shows

a typical S-shape from SRB activity (Figure 4.1). Raw data are in the appendix section of the thesis. The Hawkins consortium did not utilize all available sulfate (Figure 4.2). The preferred carbon source was formate (Figure 4.2). In the DNB vials containing 50 ppm nitrate-N, neither nitrate nor fatty acids were utilized (Figure 4.3, Table 4.2). During the second set of batch experiments, in vials which contained both nitrate and sulfate, the nitrate was completely utilized. Nitrite production lasted for only 1-2 days. The nitrite-N concentration reached 1-2 ppm before the vials started to sour.

Table 4.2 Fatty Acid Concentrations in DNB Vial #5					
Day	Acetate (ppm)	Propionate (ppm)	i-Butyrate (ppm)	n-Butyrate (ppm)	Formate (ppm)
0	230	218	217	246	226
2	274	244	259	257	225
6	271	244	259	257	220
8	241	200	215	193	190
11	276	248	232	252	220

Twenty ppm nitrite-N added to souring batch vials inhibited SRB growth (Figure 4.4). All experiments were done in duplicate. In the souring batch vial #9 without any nitrite addition, the cell numbers continued to increase by almost an order of magnitude. In addition, the nitrite addition caused a cessation of H_2S production in the souring batch vial, and over the next 30 days the concentration of H_2S decreased (Figure 4.5). When 30 ppm nitrite-N was added before the vials were inoculated with produced water, no souring or any other microbial growth occurred (Figure 4.6).

Field Test

The addition of nitrite on April 18, 1995 to the Middle PWT inlet at Greer Salt Water Station took about 15 minutes. It resulted in an immediate decrease of hydrogen sulfide in the vapor phase (Figure 4.7). During the nitrite addition, the concentration of sulfate in the tanks varied from 30-100 ppm-S with no apparent trend in the variations. Figure 4.8 illustrates a comparison of the hydrogen sulfide vapor concentration in the three tanks' head space on April 18. These results were reproduced by the second addition of nitrite to the Middle PWT on April 21, 1995 (Figure 4.9). Analysis of Figure 4.7 and Figure 4.9 yields three more results. First, if the following assumptions are incorporated into the analysis: the Middle tank behaved like an ideal CSTR, the input of nitrite was a pulse addition, and negligible chemical disappearance occurred, then a plot of $[NO_2]$ vs time should be an exponential ($e^{-(t/\tau)}$) decrease. The mean residence time can be obtained from this expression. The actual residence time in the tank from this calculation was approximately 2½ - 5 hours, not the assumed twelve (as obtained when the inlet produced water is dispersed equally among all the tanks). Second, the nitrite was completely washed out of the Middle tank in 24 hours. Third, the calculated area under a smoothed nitrite wash-out curve for both Figure 4.7 and Figure 4.9 is equal to approximately 700 ppm-hr. Both the inscribed rectangles and circumscribed rectangles methods were used to calculate the area under the curve. The final number is an average of the results from the two methods mentioned above. If the flow were such that the residence time was 12 hours, this value should be ~2000 ppm-hr. For a residence time near 4 hours, then ~700 ppm-hr is obtained. Thus the material balance and new residence time are consistent.

The concentration of hydrogen sulfide in the liquid phase appears to decrease when there is a presence of nitrite in the effluent at concentrations greater than 25 ppm-N (Figure 4.10). At low hydrogen sulfide concentrations, a high nitrite concentration interferes with the methylene blue test used to determine the concentration of aqueous hydrogen sulfide. At these conditions, the sample was a yellow color instead of the expected shade of blue or being clear. Thus, specific H_2S concentrations cannot be stated. This result is presented further in the NO_2/H_2S Abiotic Experiments section.

Nitrite was also added to the East tank, and H_2S in the vapor decreased as in the Middle tank (Figure 4.11). The measured initial concentration of nitrite-N in the East tank's effluent 20 minutes after the nitrite addition was approximately 24 ppm, about 150 ppm less nitrite-N than what was measured in the Middle tank for essentially the same total nitrite addition. Also, nitrite was measurable for a much greater time after the addition.

The addition of nitrite also produced an orders of magnitude decrease in SRB cell numbers (Figure 4.12). Cell numbers varied from 1E4-1E6 for MPN tests done using effluent water from a tank with no nitrite addition. From a MPN test, the influent cell number was found to be 1E4 in the Middle tank.

The hydrogen sulfide and sulfate concentration in the tanks was not constant and did not significantly change from the influent to the effluent stream (Table 4.3).

Table 4.3 Change in Sulfate and H_2S Concentration in the East Tank			
Date	Time	ΔH_2S (ppm-S)	ΔSO_4^{2-} (ppm-S)
4/18/95	10:45 A.M.	1.01	2
4/18/95	4:00 P.M.	1.09	7
4/19/95	8:30 A.M.	0.26	-5
4/19/95	12:00 P.M.	0.05	-6
4/19/95	3:30 P.M.	-0.16	14
4/20/95	8:00 A.M.	1.76	12
4/20/95	12:00 P.M.	0.1	8
4/20/95	3:30 P.M.	0.13	2

NO_2/H_2S Abiotic Experiments

At pH 4.3, the addition of 100 ppm nitrite-N to an anaerobic batch vial caused the hydrogen sulfide concentration to immediately decrease. After 2.8 days the concentration of H_2S was zero. At the same pH, the addition of 30 ppm nitrite-N to an anaerobic batch vial caused the H_2S concentration to decrease, but not as quickly as the vial containing 100 ppm nitrite-N (Figure 4.13). The H_2S-S concentration was still 5 ppm after five days. In this same experiment at pH 4.3, an addition of 10 ppm nitrite-N resulted in only a 5 ppm decrease in H_2S-S concentration. When no nitrite was added to a control vial, the H_2S concentration did not significantly change (Figure 4.13). On day seven of the experiment, the vials were opened and the pH was read. Figure 4.14 illustrates the pH change which occurred in the vials. The concentration of nitrite added to each vial is shown in Table 4.4. No significant change in nitrite concentration was measured in the vials during the experiment.

In an experiment performed at pH 7.1, the concentration of H_2S-S went from 10 ppm to zero in less than 1.8 days when 100 ppm nitrite-N was added to the vial (Figure 4.15). In this same vial, the nitrite concentration did not significantly change. The pH in this vial increased to a final 8.0. In an experiment done at pH 9.1, neither the concentration of hydrogen sulfide nor the nitrite concentration changed significantly over 10 days when approximately 90 ppm nitrite-N was added to the vial (Figure 4.16). The pH of this vial did not significantly change over the duration of the experiment. Figure 4.17 compares the decrease in H_2S concentration when 100 ppm nitrite-N was added to vials with varying pH concentrations.

A qualitative result observed during this series of experiments is the color change which occurred in the vials where the hydrogen sulfide concentration decreased. As soon as the reaction occurred, the vial would first turn a pale yellow. This yellow color would brighten as the reaction continued. Eventually, in vials that sat for twenty or so days, the shade of yellow would again become pale, but the hydrogen sulfide concentration would remain low. Occasionally, some of the vials also developed a somewhat white cloudy appearance.

An additional qualitative result from this series of experiments is that high concentrations of nitrite interferes with the methylene blue test used to quantitatively measure the aqueous H_2S concentrations. This interference was first noted during the field test. When the slug of nitrite was added to the Middle PWT, the sample testing for H_2S turned yellow when the Diamine-R reagent was added. The initial H_2S-S concentration in the tank was 1.15 ppm. During the nitrite addition to the East tank, though, this interference was not noted. The concentration of

Table 4.4 Concentration of Nitrite Added to Anaerobic Batch Experiments at a pH = 4.3	
Vial number	Nitrite concentration (ppm-N)
1	10
2	10
3	30
4	30
5	100
6	100
CONT	0
CONT	0

nitrite-N was recorded as 25 ppm. During the abiotic H_2S/NO_2 experiments, the H_2S samples turned yellow instead of the expected shade of blue or remaining clear only in vials with a nitrite-N concentration approximately equal to 100 ppm and a hydrogen sulfide-S concentration less than approximately 5 ppm.

Hydrogen Sulfide Production
in control vials
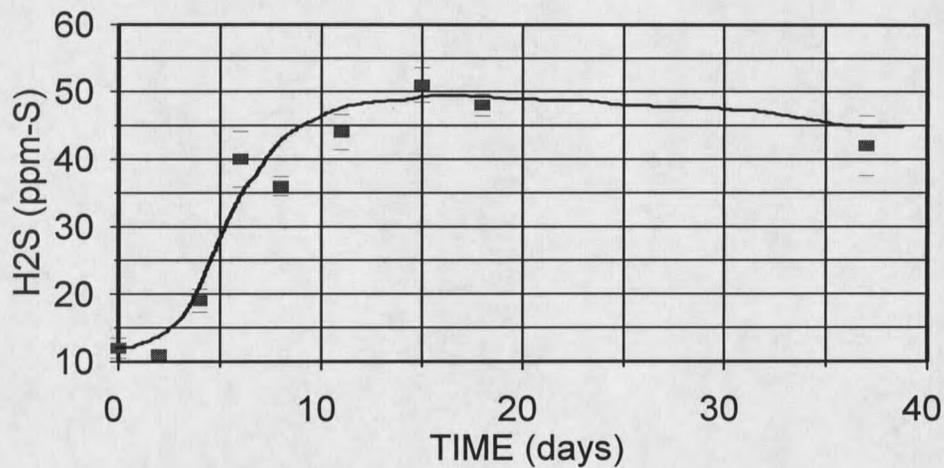
Figure 4.1 Hydrogen sulfide production in control vials. A SRB growth curve based on hydrogen sulfide production in four batch vials incubated at 40° C. Medium contains an initial H_2S-S concentration of approximately 10-14 ppm. Error bars are $\pm$ 3σ.