



Control of microbial souring of oil in a porous media column
by Mark Andrew Reinsel

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in
Chemical Engineering
Montana State University
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Abstract:

Oil reservoir souring is a major problem in industry due to H₂S toxicity, corrosion, plugging and sulfide concentrations in natural gas. Reservoir souring was simulated in Berea sandstone columns and cores using native mixed microbial consortia from two oil fields. Similar souring activity (substrate preference, and rates of substrate consumption and H₂S production) were seen with both consortia growing as a biofilm in columns and cores at 60°C. Most of the souring activity occurred at the beginning of the columns. This simulated injection well souring, in which sulfate-reducing bacteria (SRB), sulfate and carbon sources are all available at the wellbore. Biomass and precipitated iron sulfide accumulated uniformly along the column length and caused plugging. More plugging was found in systems with lower initial permeabilities, and most of this plugging was due to iron sulfide precipitation.

Several novel methods were discovered to inhibit souring in the columns and cores. Addition of ten ppm nitrate stimulated both native consortia to quickly and completely inhibit active souring, and five ppm maintained the inhibition. Nitrite was produced at 80-100% of the nitrate level added through incomplete denitrification by nitrate-reducing bacteria (NRB), and nitrite was determined to be the inhibitory agent. Direct continuous addition of 10-12 ppm nitrite also inhibited souring and pulse addition was found to be more effective than continuous addition at the same total addition rate. In the 20-hour residence time of the reactor, 10-20% of the nitrite was consumed. Nitrite addition appeared to decrease plugging in the core system. Removal of nitrate or nitrite allowed souring to resume after it had been inhibited for as long as three months. Nitrite was a more effective and longer-lasting inhibitor than was glutaraldehyde, a biocide routinely used in oil fields and other industrial applications.

Initial attempts to model souring and nitrate reduction in a column using kinetics from batch studies yielded predictions that differed significantly from the experimental data. Biofilm thickness and K_{η} the Monod inhibition constant for nitrite acting against SRB, were found to be key parameters in the model and were thus adjusted. A final model captured the qualitative behavior of the system.

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ABSTRACT

Oil reservoir souring is a major problem in industry due to H_2S toxicity, corrosion, plugging and sulfide concentrations in natural gas. Reservoir souring was simulated in Berea sandstone columns and cores using native mixed microbial consortia from two oil fields. Similar souring activity (substrate preference, and rates of substrate consumption and H_2S production) were seen with both consortia growing as a biofilm in columns and cores at $60^\circ C$. Most of the souring activity occurred at the beginning of the columns. This simulated injection well souring, in which sulfate-reducing bacteria (SRB), sulfate and carbon sources are all available at the wellbore. Biomass and precipitated iron sulfide accumulated uniformly along the column length and caused plugging. More plugging was found in systems with lower initial permeabilities, and most of this plugging was due to iron sulfide precipitation.

Several novel methods were discovered to inhibit souring in the columns and cores. Addition of ten ppm nitrate stimulated both native consortia to quickly and completely inhibit active souring, and five ppm maintained the inhibition. Nitrite was produced at 80-100% of the nitrate level added through incomplete denitrification by nitrate-reducing bacteria (NRB), and nitrite was determined to be the inhibitory agent. Direct continuous addition of 10-12 ppm nitrite also inhibited souring and pulse addition was found to be more effective than continuous addition at the same total addition rate. In the 20-hour residence time of the reactor, 10-20% of the nitrite was consumed. Nitrite addition appeared to decrease plugging in the core system. Removal of nitrate or nitrite allowed souring to resume after it had been inhibited for as long as three months. Nitrite was a more effective and longer-lasting inhibitor than was glutaraldehyde, a biocide routinely used in oil fields and other industrial applications.

Initial attempts to model souring and nitrate reduction in a column using kinetics from batch studies yielded predictions that differed significantly from the experimental data. Biofilm thickness and K_i , the Monod inhibition constant for nitrite acting against SRB, were found to be key parameters in the model and were thus adjusted. A final model captured the qualitative behavior of the system.

CHAPTER 1

GENERAL INTRODUCTION

Problem Definition

Souring, the production of hydrogen sulfide (H_2S) in oil reservoirs which have been subjected to water flooding (injection), costs oil companies billions of dollars every year (Lee, 1990). Problems associated with H_2S production in oil reservoirs include H_2S toxicity, corrosion, and reservoir plugging by SRB biomass and precipitated iron sulfide, both of which retard secondary oil recovery. In addition, H_2S production significantly raises the sulfur content of the produced natural gas, where pipeline standards limit the H_2S concentration to several ppm. The problem becomes more serious when sea water, which contains a high concentration of sulfate, is injected into oil reservoirs (Rosnes et al., 1991a). Unfortunately, sea water is used in many areas because of its proximity and availability.

The souring process is believed to be mediated by sulfate-reducing bacteria (SRB) present in the reservoirs (Cochrane et al., 1988; Cord-Ruwisch et al., 1987). These bacteria are strict anaerobes that may accumulate in biofilms on reservoir rock (porous media) and reduce sulfate (SO_4^{2-}) in injection water to hydrogen sulfide (H_2S). Major carbon and energy sources used by SRB in reservoirs are believed to be short-chain organic acids, which are probably the products of the incomplete bacterial oxidation of hydrocarbons under aerobic conditions (Barth, 1991), or may be formed by thermal degradation of oxygen-containing compounds in the kerogen

that is the precursor for crude oil (Subcasky, 1995). However, recent studies have found that petroleum hydrocarbons may also serve as electron donors for sulfate reduction (Jackson and McInerney, 1994; Reuter et al., 1994).

The reservoir souring process depicted in Figure 1.1 includes water injection, the oil-bearing formation, biotransformation (souring), and separation of the formation mixture (oil + water + gas). Produced water separated from the formation mixture is recycled in some applications for water injection of the oil reservoirs.

Research Goals

The overriding goal of this research was to characterize microbial souring in porous media and to examine control of souring in a laboratory setting. "Control," in this case, meant to partially or completely limit the concentration of H_2S seen in the laboratory system. This could be achieved by either limiting sulfate reduction (so that H_2S was not produced), by consuming produced H_2S (chemically or biologically), or by a combination of the two. Another goal was to understand the mechanism for any control strategy, and to then use this knowledge to explore methods which may be more appropriate for application in the field. This research was very industry-driven: we could not afford to pursue a control mechanism which would work only on a small scale or in a controlled laboratory environment. Another part of the control strategy, for both industrial and academic reasons, was to explore a biofilm model which could simulate and explain different phenomena experienced in laboratory experiments. With these goals in mind, as the research

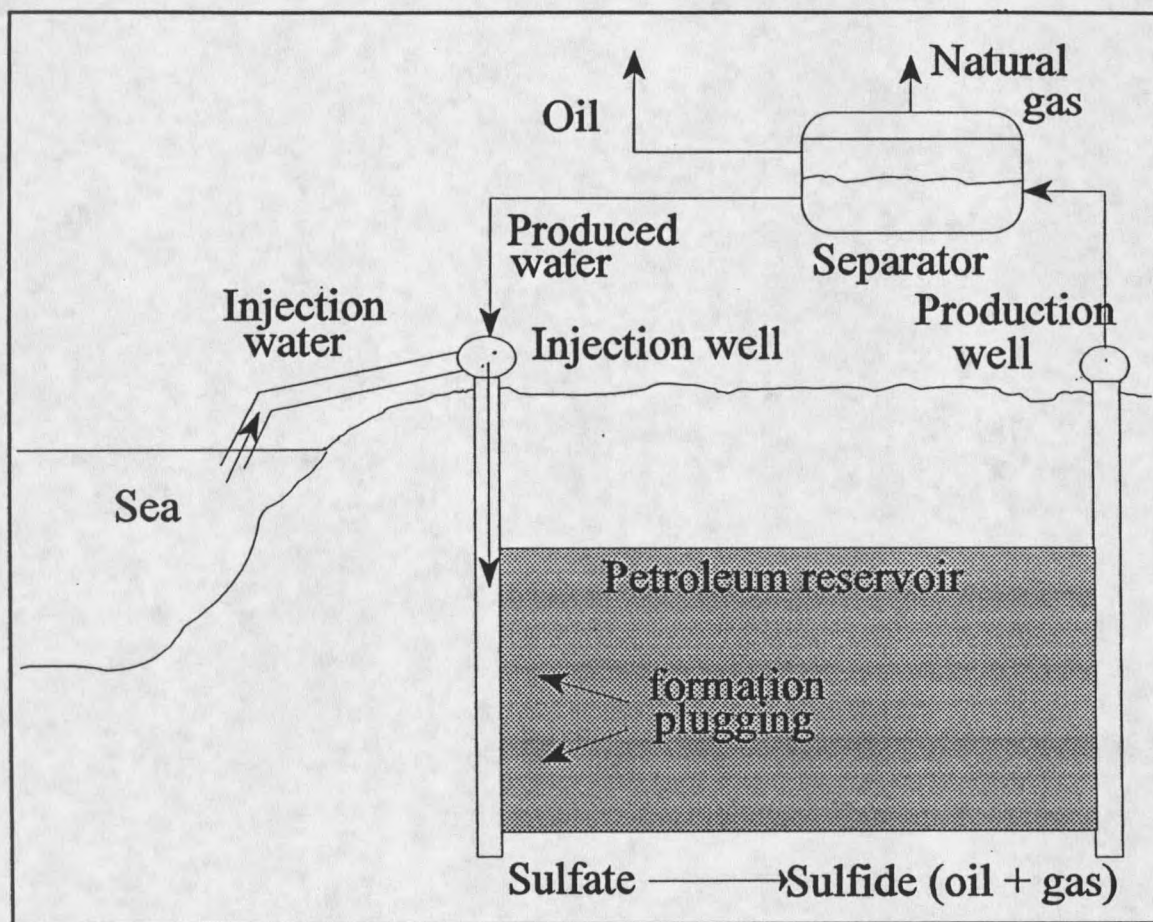


Figure 1.1. Schematic of a water-injected oil reservoir.

progressed, five main objectives for this work on the souring project became clear:

- 1) **Characterize microbial souring in the lab.** Rates of souring (as seen by consumption of electron acceptor and electron donors, production and accumulation of products), stoichiometry (the relationships between substrates consumed and biomass produced), and changes in environmental conditions caused by souring needed to be identified. This information was vital to provide a firm baseline for souring activity before any control measures were attempted.
- 2) **Determine the effects of nitrate addition on a souring system.** Nitrate was identified as a possible control agent and proved to be very effective in limiting H_2S production. Data similar to that gathered in the first objective were used both to determine the effectiveness of nitrate, and to gain information on determining the inhibition mechanism.
- 3) **Determine the effects of nitrite addition to a souring system.** Research suggested that nitrite, the product of incomplete microbial denitrification, was the inhibitory agent for H_2S production. Experiments were run with direct addition of nitrite, and chemical, physical and biological data were again taken from the column to determine the effectiveness of this treatment.
- 4) **Create a biofilm model to explain the inhibitory effects seen.** Using kinetic parameters from previous research, it was desired to create a mathematical model to simulate the microbial behavior seen experimentally. An

appropriate model could be used to explore different phenomena far more easily than by running additional experiments.

5) Determine the implications for a sour or potentially sour oil reservoir.

What do the previous results mean in terms of achieving control of microbial souring in the field? Which technology has the highest probable chance for success in various souring scenarios and what are the costs compared to current control methods?

Background

Sulfate-reducing bacteria are most notable for their end product, hydrogen sulfide (which will be referred to as H_2S or "sulfide"), which by its chemical properties and physiological effects is a far more conspicuous substance than the substrate sulfate (Widdel, 1988). Sulfate is fairly chemically inert, non-volatile and non-toxic. It is commonly found in rocks, soils and waters (water is the concern here, as sulfate is present in water injected into oil reservoirs during secondary recovery). Hydrogen sulfide, in contrast, is chemically reactive. In aqueous habitats where it is formed, sulfide often blackens the sediment due to formation of ferrous sulfide from iron-containing materials. As a reducing agent, hydrogen sulfide reacts with oxygen and may be converted to sulfur. Hydrogen sulfide, which even at low concentration (≥ 0.2 ppm) is recognized by its distinctive smell, is toxic to plants, animals and humans (Widdel, 1988). At high concentrations in the gas phase, sulfide may not be detected because it anaesthetizes the olfactory nerves.

Physiology of SRB

SRB are a metabolically diverse group of anaerobic organisms using sulfate as the terminal electron acceptor and reducing it to sulfide. Most SRB can grow with sulfite or thiosulfate as electron acceptors instead of sulfate (Badziong and Thauer, 1978; Cypionka et al., 1985; Jorgensen and Bak, 1991). In the absence of sulfate or other inorganic electron acceptors, several types of SRB can grow by fermentation of certain organic substrates (Widdel and Pfennig, 1981; Postgate, 1984). The range of carbon sources used for cell growth is very wide. Growth is possible on CO₂ (Brysch et al., 1987), on a range of organic compounds including benzoate but excluding sugars and hydrocarbons, and on organic acids.

SRB can be classified into two broad nutritional groups. Species of the first group carry out an incomplete oxidation of organic substrates with acetate as an end product. Species of the second group oxidize organic substrates, including acetate, completely to CO₂. These complete oxidizers are more versatile but usually have slower growth rates (Widdel, 1988). Recently, Aeckersberg et al. (1991) isolated from an oil production plant SRB which oxidize a saturated hydrocarbon (*n*-hexadecane); the authors theorized that some oil hydrocarbons may be directly utilized by SRB. These SRB came from a mesophilic environment (28-30°C).

The optimum temperature for most SRB is 20-40°C (Widdel, 1988). Relatively few types of SRB are known to grow at temperatures of 55-80°C (Postgate, 1984; Cochrane et al., 1988; Rosnes et al., 1991a). The physiology of

SRB and their ecological impacts have been described by Postgate (1984). Nine genera of SRB have now been recognized, and representative species are described in the literature (Postgate, 1984; Widdel, 1988).

Ecology

Significant activities of SRB are measured in salt-marsh or marine sediments because of the high sulfate concentration ($28 \text{ mM} = 2.7 \text{ g SO}_4^{2-}/\text{L}$) of seawater (Jorgensen, 1983). Despite the inhibitory effect of oxygen on SRB, they are sometimes active in aerobic aquatic environments, where SRB thrive in anaerobic microniches (Canfield and Des Marais, 1991; Lee, 1992). Formation and maintenance of such microniches is explained by two factors. First, the respiration of aerobic bacteria scavenges oxygen and favors growth conditions for SRB. Second, H_2S produced by SRB is a reductant that reacts with oxygen; once established, colonies of SRB can protect themselves against oxygen (Cypionka et al., 1985). SRB have been observed in unfavorable pH environments because the metabolic products of SRB are buffers (the $\text{H}_2\text{S}/\text{HS}^-$ and the $\text{CO}_2/\text{HCO}_3^-$ systems) which protect them against extreme pH values.

SRB and methanogenic bacteria both operate in anaerobic environments. In sulfate-rich habitats, SRB growth is favored while methanogens dominate in freshwater (sulfate-poor) environments (Widdel, 1988). In thick biofilms, even in a saline environment, methanogens may be favored because of slow sulfate

diffusion (Nielsen, 1987). At higher temperatures ($>55^{\circ}\text{C}$), SRB may be limited and methanogens more active (Widdel, 1988). No SRB are reported to utilize methane.

SRB often depend on fermentative bacteria to degrade polymers into lower molecular weight substrates such as butyrate (Widdel, 1988). Fermenters can, however, become competitive organisms as well, since lactate (Widdel, 1988), and propionate and butyrate (Brock and Madigan, 1991) can be fermented further into acetate, CO_2 or H_2 . SRB may aid these potential competitors by consuming H_2 , a fermentation product (Rosnes et al., 1991b).

Denitrifying bacteria (DNB) are facultative anaerobes which reduce nitrate (usually to N_2) in the absence of oxygen. DNB growth is favored over that of SRB and methanogens because of the greater energy obtained (McCarty, 1972). Some species of SRB are also known to reduce nitrate (Widdel, 1988), usually to NH_3 .

Temperature Effects

Both mesophilic ($20\text{--}40^{\circ}\text{C}$) and thermophilic ($>40^{\circ}\text{C}$) SRB have been found in injection water for oil reservoirs (Cochrane et al., 1988). Mesophiles would generally thrive near the injection well, where cold injection water first enters the formation, while thermophiles are favored deeper in the formation. The origin of thermophilic SRB (which have not been studied extensively) in an oil reservoir is a point of debate. Some researchers (Rosnes et al., 1991a) believe they are indigenous inhabitants of oilfield waters. Others (Cochrane et al., 1988) believe thermophilic SRB are not originally present in the formation; they arrive as spores

in the injection water, or mutate from mesophilic SRB.

Thermophilic SRB are an important part of the mixed-population consortia in the reservoir. Thermophiles, defined by Edwards (1990) as those organisms having optimum growth at temperatures greater than 55°C, are difficult to isolate and cultivate in the lab. Advantages possessed by thermophilic systems include lower liquid viscosity (better mixing) and more efficient killing of pathogens (Edwards, 1990). Finally, many thermophiles can survive at higher temperatures than they can actually grow. SRB can form spores (Rosnes et al., 1991a) when threatened by environmental factors such as extremely high temperature and can survive a temperature of 131°C for 20 minutes. Some species of SRB actively produce H₂S at temperatures up to 80°C (Rosnes et al., 1990).

Control Strategies

Biocides

Extensive research has been conducted to develop effective biocides with the goal of inhibiting SRB growth and hence sulfide production. For example, in secondary oil recovery, injection water used in flooding operations is treated routinely with a biocide (typically glutaraldehyde) to control SRB growth in the injection well, reservoir and piping (Brunt, 1986). Eagar et al. (1988) reported that glutaraldehyde was an effective agent for controlling *Pseudomonas fluorescens* biofilm growth and activity. Also, field experiments indicated that glutaraldehyde

was sufficiently persistent in the distribution system to remain at a high level of efficacy, and reduced the corrosion rate to an acceptable rate.

Biocide addition is often of limited effectiveness because SRB and other anaerobic bacteria form biofilms, which coat the surfaces of pipes and other materials. Within these biofilms, SRB are somewhat protected because biocides do not effectively penetrate through the biofilm. Reports have shown that bacteria within biofilms are much more difficult to control with biocides than their planktonic counterparts (Galyarde and Johnston, 1983; Ege et al., 1985; Dewar, 1986). In an oil reservoir, SRB have been found to be effectively killed by biocide only near the injection well (Cochrane et al., 1988). Consequently, biocide treatment may not be an ultimate means to control SRB activity because of rapid microbial regrowth, cost effectiveness and environmental concerns.

Nutrient Limitation

Reducing the concentration of an essential nutrient (e.g., phosphorus, nitrogen and/or sulfate) to below the limiting concentration is a possible means of controlling SRB activity. Maree and Strydom (1987) examined the feasibility of microbial sulfate removal from industrial effluent using an upflow packed bed reactor with photosynthetic sulfur-oxidizing bacteria to prevent the release of sulfide, and confirmed the successful performance of the reactor. Bakke et al. (1992) reported the removal of sulfate from injection water by membrane filtration but this process is very expensive. There is no information in the literature that

addresses control of SRB activity by removing phosphorus or nitrogen. This appears to be infeasible in an underground oil reservoir because of the low levels of phosphorus and nitrogen necessary to limit cell growth (Okabe, 1992), coupled with the nearly infinite supply of these nutrients available from rock in a reservoir.

Aeration

Oxygen is the cheapest and most effective inhibitor of SRB activity. If a system is maintained in an aerated condition (even though the dissolved oxygen concentration may be very low), SRB remain dormant but are not killed (Hardy and Hamilton, 1981; Cypionka et al., 1985). However, this measure is of limited effectiveness because SRB are generally found in mixed-culture biofilms. Oxygen does not effectively penetrate to SRB in these biofilms due to consumption of oxygen by surrounding aerobes (Jorgensen, 1977). As demonstrated by Lee et al. (1992), SRB activity at the substratum beneath an aerobic biofilm can be extensive, even at high bulk water dissolved oxygen concentrations. Furthermore, introducing oxygen into some industrial water systems increases the oxidative corrosion of facilities (Lee et al., 1992). Even though anaerobic corrosion is a major problem associated with H_2S production, oil companies are very hesitant to introduce oxygen into the system because of the likelihood of increased corrosion.

Radiation

Use of ionizing radiation to control SRB activity and growth has also attracted attention, as ultraviolet radiation was used to kill SRB in injection waters

by Ege et al. (1985). Gamma radiation was also applied as water entered the oil reservoir to control SRB at the bottom of well bores (Agaev et al., 1985). This method, while probably effective in preventing new SRB from entering the reservoir, does nothing to kill or inhibit SRB already there.

Sulfide Inhibition

The inhibiting effect of sulfide on SRB primarily depends on the H_2S concentration and on the pH. Undissociated hydrogen sulfide (H_2S) was found to be the most toxic sulfide species for SRB (Speece, 1983; Parkin and Speece, 1983; Reis, 1991). H_2S will be highest in concentration at acidic pH values, as the pK_{a1} for H_2S is 7.0. Okabe (1992) reported a 50% inhibition of activity for *D. desulfuricans* grown on lactate at a total sulfide concentration of 500 ppm. McCartney and Oleskiewicz (1991) reported 50% inhibition of activity of a mixed mesophilic SRB consortium at 240 ppm total sulfide and 83 ppm H_2S . All authors reported that sulfide inhibition was reversible, as SRB growth and activity could be restored as soon as the sulfide concentration was lowered. However, the required presence of sulfide for this inhibition to occur rules it out as a primary souring control scheme.

Acetate Inhibition

Inhibition by acetate was reported to be significant for some SRB species at fairly low concentrations, with a K_i value (Monod kinetics with non-competitive inhibition) of 54 mg/L (Reis et al., 1990). This inhibition was especially effective at

low pH. Acetate inhibition appears to not play a major role in the system considered here, since acetate concentrations of over 600 ppm (concomitant with high levels of H_2S production) have been measured in produced water from several oil fields (Mueller, 1992).

Scavenging of H_2S

Hitzman (1994) reported that *Thiobacillus denitrificans* lithotrophically consumed any H_2S produced in experimental sand column and core systems, by using H_2S as an electron donor and NO_3^- as the electron acceptor. Chemical scavenging of H_2S has also been reported (Littman and McLean, 1987). Complete H_2S scavenging may be difficult, however, because of poor mixing in the formation. This might also have the negative effect of actually increasing the rate of sulfide production by removing the end product (H_2S), which is toxic to SRB in high concentrations.

Control of pH or E_h

SRB are found to remain active at pH values of 6 to 9 (Widdel, 1988). Since pH determines the concentration of dissociated or undissociated hydrogen sulfide, it will also determine the effectiveness of H_2S as an inhibitor. Changing the pH in an oil formation, however, may be very difficult because of carbonate and other buffer systems (Barth, 1991). SRB require a redox potential (E_h) of -100 mV or less (Postgate, 1984). Raising the E_h of the environment would be best accomplished by introducing oxygen. Because of problems with oxygen discussed in control via

aeration, and because of the huge environment involved, this option can probably also be eliminated.

Microbial Competition

Microbial control of sulfide production by SRB using *Thiobacillus denitrificans* has attracted considerable attention lately (McInerney et al., 1986; Montgomery et al., 1990). *T. denitrificans* is an autotroph and a facultative anaerobe which oxidizes sulfide to sulfate using oxygen or nitrate as the electron acceptor. The introduction of *T. denitrificans* into environments with SRB has the potential of controlling sulfide production as long as the nitrate concentration remains high. The application of this method focuses on controlling sulfide production at or near the water injection well in most oil reservoirs, since *T. denitrificans* grows only at mesophilic temperatures. A practical difficulty is efficiently inoculating *T. denitrificans* into the well-bore area.

A mutant of *T. denitrificans* (strain F) resistant to glutaraldehyde and sulfide was isolated by McInerney et al. (1986). This mutant strain would allow a combined microbial and biocidal (glutaraldehyde) treatment of SRB-contaminated industrial systems. Sublette and Sylvester (1987a, 1987b, 1987c) and Sublette (1987) have demonstrated that *T. denitrificans* can be readily cultured aerobically and anaerobically in batch and continuous reactors on gaseous H_2S under sulfide-limiting conditions. A microbial process for the removal of H_2S from gases has been

proposed based on mixing the gas with a culture of *T. denitrificans* (Sublette and Sylvester, 1987a).

Competition for substrate between SRB and denitrifying bacteria (DNB) has been reported recently (Hitzman and Sperl, 1994). Competitive removal of short-chain organic acids by DNB reduced the H_2S concentration seen in this system, as faster-growing DNB significantly reduced the concentration of carbon source available to SRB.

The competition for available electron donors between SRB and methanogenic or methane-producing bacteria (MPB) has also received considerable attention (Abram and Nedwell, 1978; Krisjansson et al., 1982; Yoda et al., 1987). SRB apparently have a higher affinity (lower K_s) for hydrogen and acetate relative to MPB. Yoda et al. (1987) reported that in an anaerobic fluidized bed, the methane production rate and MPB biomass decreased after several months of operation at low acetate concentration, while the sulfate reduction rate increased. MPB were able to form a biofilm faster than SRB at high acetate concentrations, presumably due to MPB's ability to adhere to surfaces better than SRB. Hilton and Oleszkiewicz (1989) reported that SRB are more sensitive than MPB to elevated total sulfide concentrations, while both are sensitive to elevated molecular H_2S concentrations. Thus, at high total sulfide concentrations and high pH (where less H_2S is present; $pK_a = 7.0$), the MPB should be able to outcompete the SRB for substrate.

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CHAPTER 2

MICROBIAL SOURING IN A POROUS MEDIA COLUMN.

Introduction

Very little is known about the souring process in an oil formation. Two scenarios have been proposed representing extreme cases. In the first case, souring is assumed to occur in the regions of the formation near the injection well. The extent of H_2S production is assumed to be limited by the injection water composition or by product inhibition (Taylor et al., 1992). In applications where a mixture of sea water and produced water is injected, this is a reasonable approach, since the injection water contains sulfate (the electron acceptor) from the sea water, organic acids (carbon and energy sources serving as electron donors) from the produced water, and other essential nutrients (nitrogen, phosphorus and trace metals). "Produced water" is the water separated from the oil-water mixture obtained from a production well and "formation water" is that present in a reservoir before water-flooding is initiated.

However, this scenario does not explain souring activity when injection water does not meet all the requirements for SRB growth (e.g., only sea water, with its low organic content, is used for injection). The second scenario is the case in which the injection water cannot support SRB growth. Souring is assumed to occur in a "mixing zone" between the injection and formation waters immediately behind the water-drive front (Ligthelm et al., 1991). Formation water contains organic acids

from degradation of hydrocarbons (Barth, 1991) or from thermal degradation of oxygen-containing compounds in the kerogen that is the precursor for crude oil (Subcasky, 1995). Thus, in the mixing zone, SRB would obtain all nutrients required for growth: sulfate from injection water, and organic acids and other nutrients from formation water. This scenario is reasonable in that it is again based on the assumption that souring will occur whenever all essential nutrients are available. This scenario is consistent with the ability of oil reservoir consortia to produce hydrogen sulfide over a wide range of temperatures and concentrations (Cochrane et al., 1988; Rosnes et al., 1990), and with the presence of SRB throughout the formation (Rosnes et al., 1991). However, due to reservoir heterogeneities (Barthel, 1991), a well-defined mixing zone is difficult to obtain. In addition, mixing is far more complex than the diffusion across a smooth front which has been proposed (Ligthelm, 1991).

The prevention and control of souring depends on when and where souring occurs in the formation, on the rate of H_2S production (or sulfate reduction), and on what levels the H_2S reaches. Thus, knowledge of rates and kinetics of souring in porous media is critical for the control of the souring phenomenon. In this study, microbial souring in porous media was investigated using microbial consortia obtained from oil reservoirs. The consortia were grown in anaerobic upflow fixed bed porous media reactors operated at a high temperature (thermophilic range) using the main organic acids found in reservoir waters as carbon and energy

sources. The terms "sulfate reduction," "sulfide production" and "souring" will be used interchangeably in this chapter.

David Chen worked with me in setting up and operating several of the porous media columns and Debbie Wine, a summer undergraduate research assistant, collected some of the data from two of the columns. Thanks are also due to Darla Goeres, who set up and operated a core reactor system (under my direction) as her undergraduate thesis project.

Experimental Approach

The first requirement in a laboratory study to control souring is, of course, to produce souring. This was performed using anaerobic techniques to grow mixed microbial consortia obtained from produced water in souring oil reservoirs. Most studies of souring and sulfate reduction have been done with a single species such as *Desulfovibrio desulfuricans*. Single species studies would not have been appropriate for this research since it is known that multiple species of sulfate reducers, and other anaerobic bacteria, are present or will become present in all water-injected oil reservoirs. In fact, no attempt was made to identify the species of SRB or any other microbes studied because such a study would have in all likelihood been a microbiological academic exercise, and less fruitful than an engineering approach (using consortia) to the control of microbial souring in an oil reservoir.

In producing and studying microbial souring in the lab, a familiarity with souring was achieved which would prove very useful in the long run. A great deal of information was gleaned concerning the time course of souring, substrate preferences, environmental requirements, perturbations from steady state, etc. Souring was studied in different reactor systems—batch reactors, chemostats and packed bed (or "porous media") columns. Columns were picked as the reactors of choice for the bulk of the experiments for several reasons: they provided a good environment for initial growth and for bacterial resiliency in long-term experiments, and they were appropriate for performing larger-scale experiments to explore new concepts, or concepts examined in small batch reactors, before attempting anything in the field. It was found to be possible to simulate a porous media column with a biofilm mathematical model, and data from column experiments could then be compared to the model predictions. Column experiments and modeling results served as the best predictors, it was felt, of what would happen in future field tests. Actual field tests were outside the scope of this research.

Materials and Methods

Reactor System—Column

The anaerobic upflow porous media reactor system used for this study was a packed bed (50 x 5.5 cm) column made of polycarbonate and equipped with six sampling ports (Figure 2.1). Four sampling ports were located at regular intervals on one side of the column (10, 20, 30 and 40 cm from the inlet), while two ports

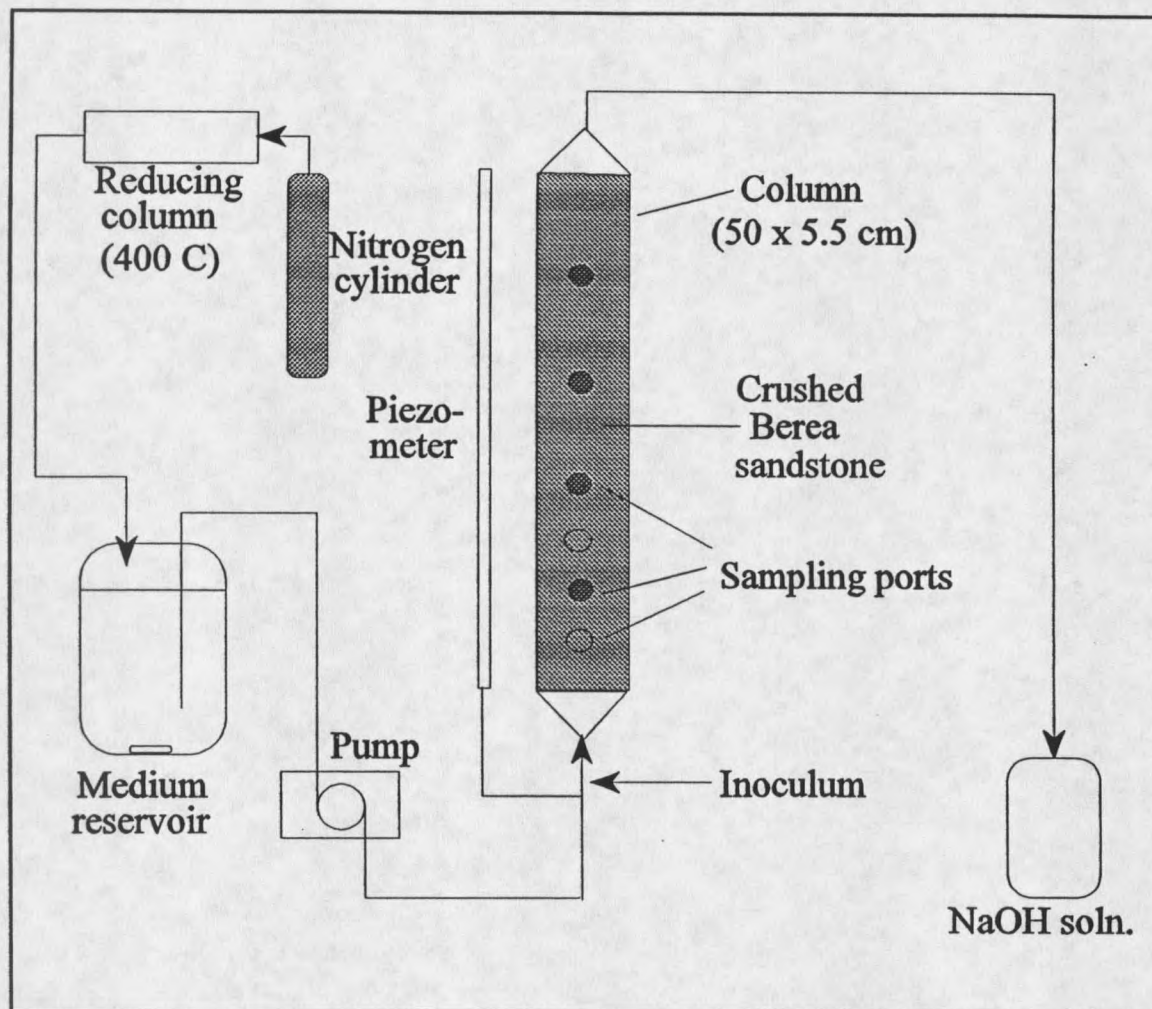


Figure 2.1. Schematic of the crushed Berea sandstone column reactor system.

were located on the opposite side of the column at five and 15 cm from the inlet. The column inlet and outlet were sampled directly through the tubing, for a total of eight sampling spots. Samples at each port were drawn from a position approximately one cm from the center of the 5.5 cm-ID column. Porous medium was contained in the reactor by brass gauge mesh over each end of the column. The flow was dispersed by a funnel at each end of the reactor column. Heavy-wall butyl rubber tubing (Cole-Parmer, Masterflex neoprene tubing #14 and #16) was used to minimize oxygen diffusion. Slow continuous nitrogen purging of the medium maintained anaerobic conditions. Traces of oxygen in the nitrogen feed gas were removed by a reducing column containing copper turnings maintained at 400°C. Sterility in the medium flask was maintained with 0.22 μm bacterial air vents (Gelman) at the gas inlet and outlet. A glass flow break separated the medium flask from the column inlet. Volatile H_2S in the effluent was trapped in a sodium hydroxide solution.

For two columns, a piezometer (water column) connected to the inlet was used to measure the reduction in intrinsic permeability in the column due to: plugging from bacterial growth and formation of precipitates, based on piezometric head change with a constant flow rate. On another column, a pressure gauge (0-100 psi) was used to determine the pressure at the inlet. In all cases, it was assumed that the column outlet was at atmospheric pressure; thus, the pressure reading equaled the pressure drop.

Crushed Berea sandstone, which is a standard material used by many oil companies for *ex situ* investigation of oil recovery, was used as the porous medium (Shaw et al., 1989). Solid Berea sandstone was obtained from the Idaho National Engineering Laboratory (INEL) and pulverized with a grinding wheel. The crushed sandstone contained sand particles ranging from 35 to 300 μm in diameter with over 50% of the particles residing in the 220- μm regime, thus providing a column diameter to grain diameter ratio of greater than 180, which is sufficient to ensure that the radial velocity variations due to wall effects were negligible (Schwartz and Smith, 1953). The reactor system (column and tubing) was sterilized at 121°C (17 psi) for 40 minutes before packing with sand. Sand particles were pretreated by heating at 400-500°C for four to five hours to remove organic material and bound water, washed again with distilled water and then autoclaved. The column was packed with autoclaved sand in a laminar flow hood equipped with UV light to eliminate microbial contamination. Sand particles were poured (from the top) into the column, which was partially prefilled with liquid medium. The pumping rate of the medium was controlled so that the liquid level above the sand bed after sand particles settled was one to two cm. Medium was pumped through the column after addition of sand particles was finished (bed height 49 to 50 cm) in order to tighten the bed and condition the column. The final bed height was approximately 49 cm. The porosity was calculated by dividing the volume of liquid medium used in packing the column (void volume) by the total packed column volume. The porosity of each sand bed packed in this manner was consistent at 0.50 ± 0.02 .

The packed bed columns were maintained at 60°C in an incubator (Stalil-Therm Incubator, Blue M Co.). High temperatures of >50°C have been found in Chevron's North Sea field as well as several other oil fields. The flow rate of the medium was maintained at 650 ml/day, which equals an average pore velocity of 55 cm/day. A similar flow regime is used by oil companies for water injection (Burger, 1993).

Reactor System--Core

The core reactor system consisted of two epoxy-covered, low permeability (600 mD) Berea sandstone cores from INEL (5 x 1 in.), arranged in parallel with gauges placed so that the pressure drop over each core could be measured (Figure 2.2). The core system was also maintained in a 60° incubator, and the same heavy-wall butyl rubber tubing and nitrogen purge system were employed. The flow rate of the medium was maintained at 185 ml/day. When combined with an assumed porosity of 0.40, this flow rate equals an average pore velocity of 91 cm/day, somewhat higher than that in the columns.

Bacterial Consortia and Cultivation

Samples of produced water from the Ninian North Sea oil field and the Kuparuk North Slope oil field were used as the microbial consortia in different experiments. Since production wells are the nearest accessible sites downstream from the point where oil souring occurs, produced waters should contain SRB and other microbes present in the reservoir, as well as nutrients, sulfate and sulfide.

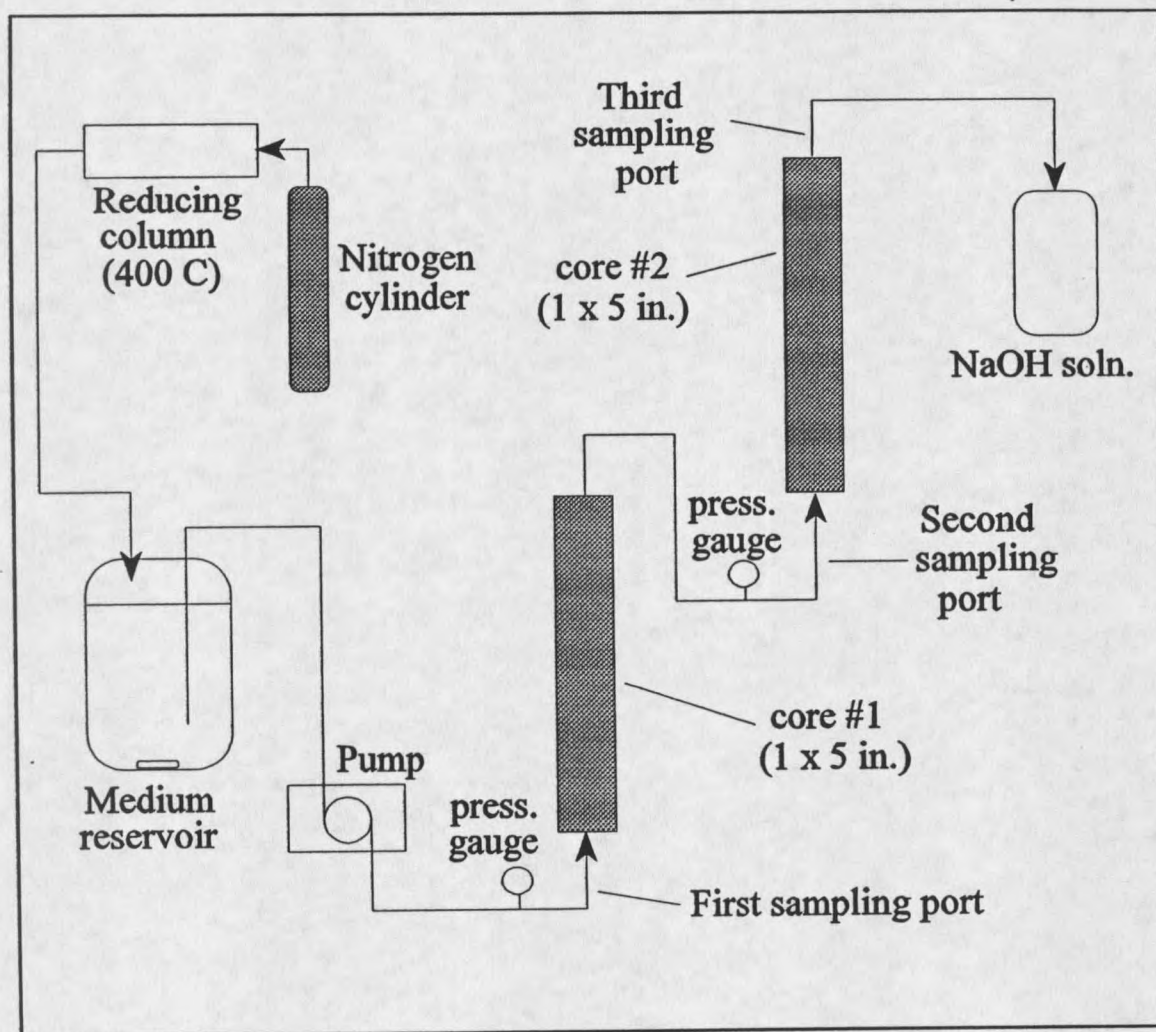


Figure 2.2. Schematic of the Berea sandstone core reactor system.

Produced waters were concentrated (100X) on-site by using a Pellicon filter under anaerobic conditions; samples were flash-frozen and stored at -70°C . Previous experiments indicated that frozen cultures maintained the same microbial activities as on-site produced waters before freezing. The microbial consortia contained mesophilic and thermophilic SRB, as indicated by sulfate reduction and H_2S production in batch cultures incubated anaerobically at 35 and 60°C (Mueller, 1994). The consortia also contained other species of anaerobic bacteria, as determined by MPN counts in appropriate media. Batch cultures used to inoculate the porous media reactors were prepared by inoculating one ml of frozen culture into a 50-ml vial (sealed and filled with purified N_2 gas in the head space) containing 30 ml of medium, inside an anaerobic chamber (model 12356, Coy Laboratory Products, MI). The vials were incubated at 60°C for seven to 14 days.

One vial was used to inoculate the porous media reactor at the inlet end. After inoculation, the column sat in "batch mode" (in which flow of liquid medium was stopped) for three days. Flow was resumed, and analysis for souring then began. The columns inoculated in this manner will be referred to as the "North Slope" column and the "North Sea" column, respectively. The North Slope column experiment was repeated--these runs will be referred to as "North Slope #1" and "North Slope #2." The core system was inoculated with a five-ml effluent sample from the North Slope column (#2) while it was actively souring. In contrast, a third column was inoculated by flowing one pore volume (approximately 600 ml) of freshly obtained produced water from the North Slope into the column (the

produced water was purged with nitrogen before and during introduction to the column). This column, whose bacterial consortium came from the same location as the North Slope column but was obtained at a different time, will be referred to as the "North Slope PW (produced water) column." A summary of these souring experiments is shown in Table 2.1. All were operated at 60°C.

Table 2.1. Summary and timetable of porous media experiments.

system	dates in operation	inoculum
North Slope #1 column	1/93 - 3/93	N. Slope batch vial
North Slope #2 column	6/93 - 7/94	N. Slope batch vial
North Sea column	5/93 - 1/95	N. Sea batch vial
North Slope PW column	4/94 - 2/95	N. Slope produced water
core system	7/93 - 4/94	N. Slope #2 column effluent

A high-salinity anaerobic medium suitable for recovery and growth of marine SRB (Pfennig et al., 1981) was used for the batch vials and the porous media reactors (Table 2.2). Five short-chain acids (formic acid, acetic acid, propionic acid, *n*-butyric acid and isobutyric acid) were used as the carbon sources since they were the major carbon sources found in the various produced water samples analyzed. A feed concentration of approximately 200 mg/L (varying slightly with each preparation) for each of the organic acids was used. The produced water contained significantly different concentrations compared to the medium (620 mg/L acetate, 74 mg/L propionate, seven mg/L *n*-butyrate, one mg/L isobutyrate and no

formate in the North Slope produced water, for example) but this was after the SRB activity had occurred, which would have probably produced acetate while consuming the other organic acids. The feed sulfate concentration was in excess at 300 mg S/L to avoid sulfate limitation. High NaCl and high magnesium concentrations simulated the high-salinity conditions in the oil reservoir. Trace elements and other essential nutrients were also contained in the medium (Pfennig et al., 1981). $\text{Na}_2\text{S}_2\text{O}_4$ and $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ were added in small amounts in order to maintain anaerobic conditions, in addition to purging with purified N_2 gas. The pH of the medium was 6.5-7.0. A neutral pH has been found in produced waters of several oil reservoirs.

Analytical Methods

Aqueous hydrogen sulfide was analyzed by the methylene blue method (Cline, 1969). Because of high sample dilutions used, the detection limit for H_2S was approximately five mg S/L. Sulfate was measured using a Dionex ion chromatograph (model AI-450; Dionex Co., San Francisco, CA) with a pulse electrochemical detector (model DX-300) using a Dionex Ionpac AS4A-SC column (2 mm). Both hydrogen sulfide and sulfate measurements were expressed in terms of sulfur content (S). Organic acids were measured using the ion chromatograph with a Dionex Ionpac AS10 column (4 mm). Cell numbers in suspension were determined by acridine orange direct counts (Hobbie et al., 1977). Standard curves and detailed procedures for all analytical methods are included in Appendix 4.

Table 2.2. Medium composition (per liter).

Carbon and energy sources	
formic acid	200 mg
acetic acid	200 mg
propionic acid	200 mg
isobutyric acid	200 mg
<i>n</i> -butyric acid	200 mg
Base medium	
Na ₂ SO ₄	300 mg S
NaCl	20 g
KCl	0.3 g
NH ₄ Cl	0.3 g
MgCl ₂ • 6 H ₂ O	3.0 g
KH ₂ PO ₄	0.2 g
CaCl ₂ • 2 H ₂ O	0.15 g
Trace elements	1 ml
Vitamin solution	1 ml
NaHCO ₃ solution	30 ml
Selenite (Na ₂ SeO ₃) solution	1 ml
Dithionite (Na ₂ S ₂ O ₄) solution	1 ml
Sulfide (Na ₂ S • 9 H ₂ O) solution	3 ml

Total biomass in different sections of the packed bed columns was measured by slicing the media in the column into 11 sections (each section 4-5 cm thick) at the end of the experiment (Chen et al., 1993). Each section was incubated at 100°C for four hours to evaporate water and volatile organic acids, and then heated in a 400-500°C oven for four hours to remove all organic material. The weight loss between these two steps was the total biomass.

Iron sulfide precipitate (FeS) was determined by measuring H₂S release upon acidification in the last column experiment (North Slope PW column). The

media from the column was removed in eight sections, each representing approximately five cm in length. A representative subsample of each section (about 10 g of wet porous media) was quickly removed and placed in a 125-ml Erlenmeyer flask containing 80-100 ml of distilled water and a magnetic stir bar. The flask containing this slurry was sparged with nitrogen for 15 minutes, then ten ml of 12 N HCl was added to the flask. The continuous N_2 sparge transported the H_2S gas, liberated from FeS by the addition of acid, to a second flask, where it was fixed in 1% ZnAc (100 ml). Sparging was maintained for another 20 minutes. The H_2S concentration in the ZnAc solution was determined by the colorimetric method previously used (Cline, 1969). It was found in earlier experiments that this flask trapped all the liberated H_2S and that a third flask also containing ZnAc was not necessary. Small Erlenmeyer flasks (125 ml) and short lengths of Masterflex neoprene tubing were employed to minimize the surface area available for H_2S to adsorb. This method was performed on a sample from each of the eight sections of the column, and is believed to be the most accurate one for determining iron sulfide concentrations in a porous medium (Nielsen, 1994).

After acidification and sparging, the sand in the first flask was removed and dried at 60°C for six hours. The quantity of sand in each sample was weighed to determine the concentration of iron sulfide (mg FeS(S)/g sand) for each section in the column.

Redox Potential Measurement

Reduction-oxidation potential (redox or E_h) was measured with microelectrodes (Microelectrodes, Inc., Londonderry, NH) connected to a pH- E_h meter (Cole-Parmer Chemcadet). The E_h microelectrode (Ag/AgCl) and reference microelectrode were encased in separate 18-ga. needles for insertion through the thick-walled butyl rubber stoppers at each sampling port in the column. The two microelectrodes were taped together for ease in handling and to minimize the distance between them. The microelectrodes were rinsed in ethanol to maintain sterility, inserted through the stopper into the column, allowed to reach steady state (up to two hours) and then rinsed and inserted into the next stopper. All E_h readings were taken on the same day while the column was at steady state. The microelectrodes were calibrated using pH 4 and pH 7 solutions saturated with quinhydrone at 25°C.

Results

A sample of the time course of souring observed in the columns will first be presented, since such a progression indicates the kinetics and ultimate level of H_2S production that can be expected when all the requirements for souring (SRB, sulfate, organic acids) are present. The remaining part of this section will present steady-state data, such as sulfide and sulfate gradients in the column, preferential utilization of substrates and biomass yields. Other steady-state results, such as E_h

readings, iron sulfide precipitation, permeability changes and calculated electron balances, will also be presented.

Time Course of Souring

Following a three-day batch-mode inoculation time for each column, flow of liquid medium through the column resumed at an average pore velocity of 55 cm/day. A fairly long lag phase of SRB growth followed, in which bacterial populations built up to the point of causing significant souring. Souring began in 7-14 days, as indicated by black iron sulfide deposits in the brown crushed Berea sandstone. As the souring expanded, iron sulfide deposits expanded radially and upward. Although patchy, this H_2S front (as evidenced by FeS precipitation) advanced upward in the North Slope PW column at a rate of about 0.75 cm/day (in contrast to the flow velocity of 55 cm/day). No H_2S was measured ahead of this front but H_2S was detected as the front reached each sampling port. This H_2S breakthrough was observed in all columns. The iron for sulfide precipitation came primarily from the crushed sandstone.

The time course of souring in the North Slope PW column is shown in Figure 2.3. Measurable H_2S was detected at the second sampling port (10 cm) nine days after flow resumed, and the level rapidly increased and progressed upward throughout the column. The sulfate concentration decreased simultaneously and to a greater degree than the H_2S increase (Figure 2.3), probably because some of

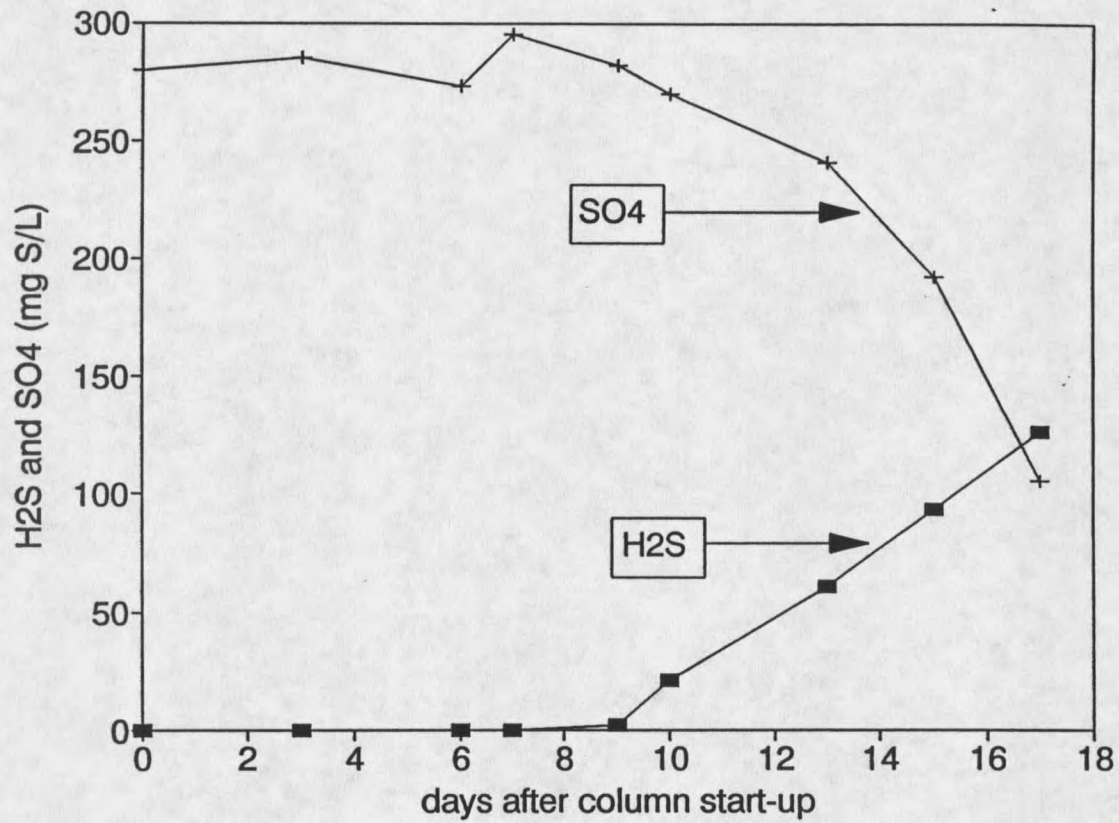


Figure 2.3. Timeline of H_2S and SO_4^{2-} concentrations in the North Slope PW column. Shown are single readings each day at distance = 10 cm.

the sulfate reduced was precipitated as iron sulfide as well as released into H_2S in the aqueous phase.

Cells were present in the effluent of the North Slope #1 column as early as four days after flow began (Figure 2.4). In this column, H_2S was not detected in the effluent until day 21. Consumption of organic acid substrates was noted at day 11, as formate, propionate, isobutyrate and *n*-butyrate concentrations all decreased in the effluent (Figure 2.5). Formate was rapidly depleted until its concentration reached zero at day 29. The other organic acids were consumed at slower rates until their concentrations, too, reached steady state at approximately day 40. Acetate concentration increased (Figure 2.5), indicating incomplete oxidation of other substrates. Both these phenomena were seen in single-substrate batch experiments (Mueller, 1994). Deviations from steady state seen with all the substrates on days 32 and 37 were due to temporary interruptions in souring activity (an unplanned stoppage and resumption of flow in the column).

Souring at Steady State

"Steady state" data were collected once concentrations of sulfide, sulfate and organic acids had stabilized throughout each column, and at least one week after any perturbations from steady state, such as plugged tubing (which happened fairly often due to the fine iron sulfide precipitate in the medium).

Sulfide and sulfate gradients. In the North Sea column (Figure 2.6), the H_2S concentration was over 100 mg S/L at the first sampling port (five cm) at steady

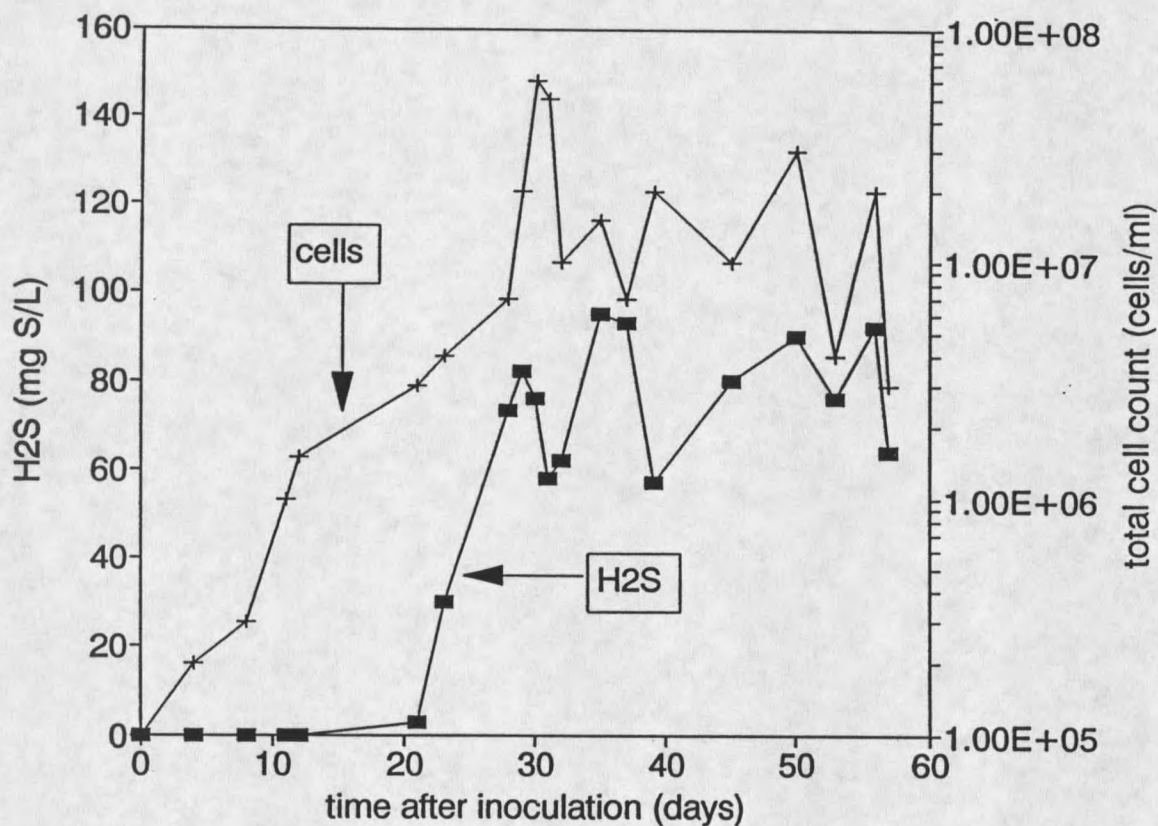


Figure 2.4. Timeline of H₂S concentration and total cell counts in the North Slope #1 column. Shown are single readings each day from the effluent (distance = 50 cm).

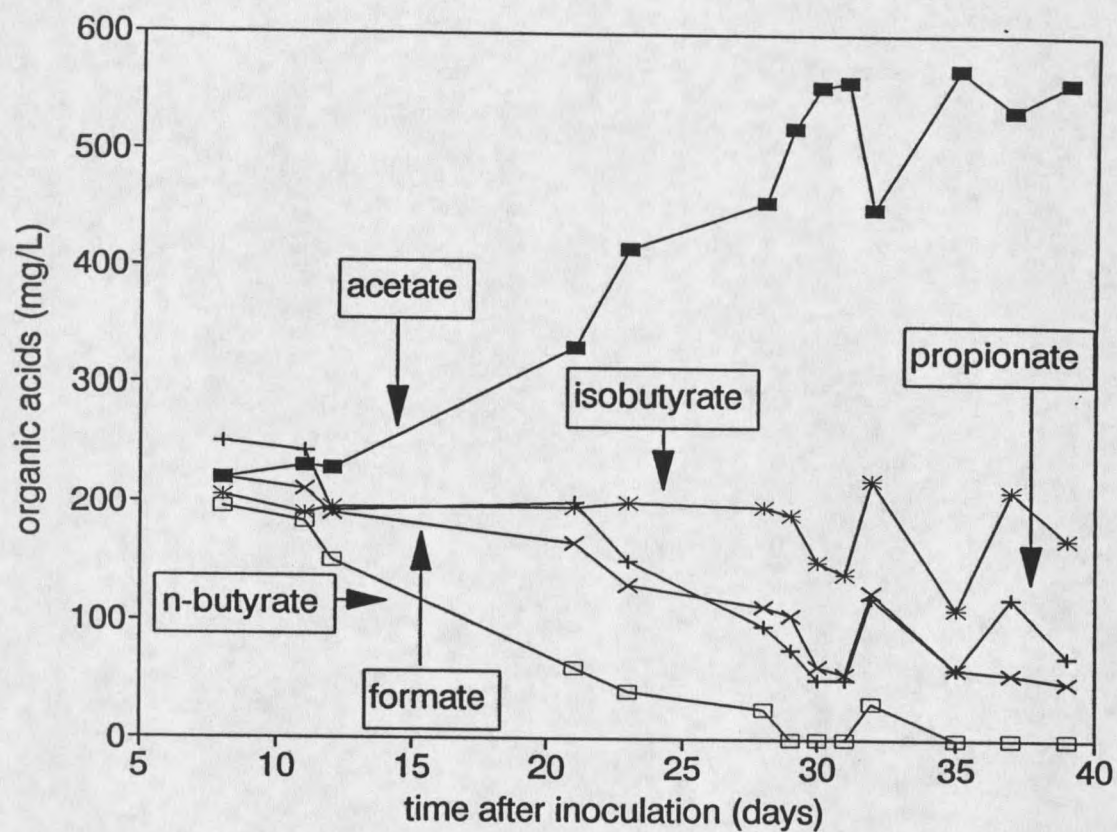


Figure 2.5. Timeline of organic acid concentrations in the North Slope #1 column. Shown are single readings each day in the effluent (distance = 50 cm).

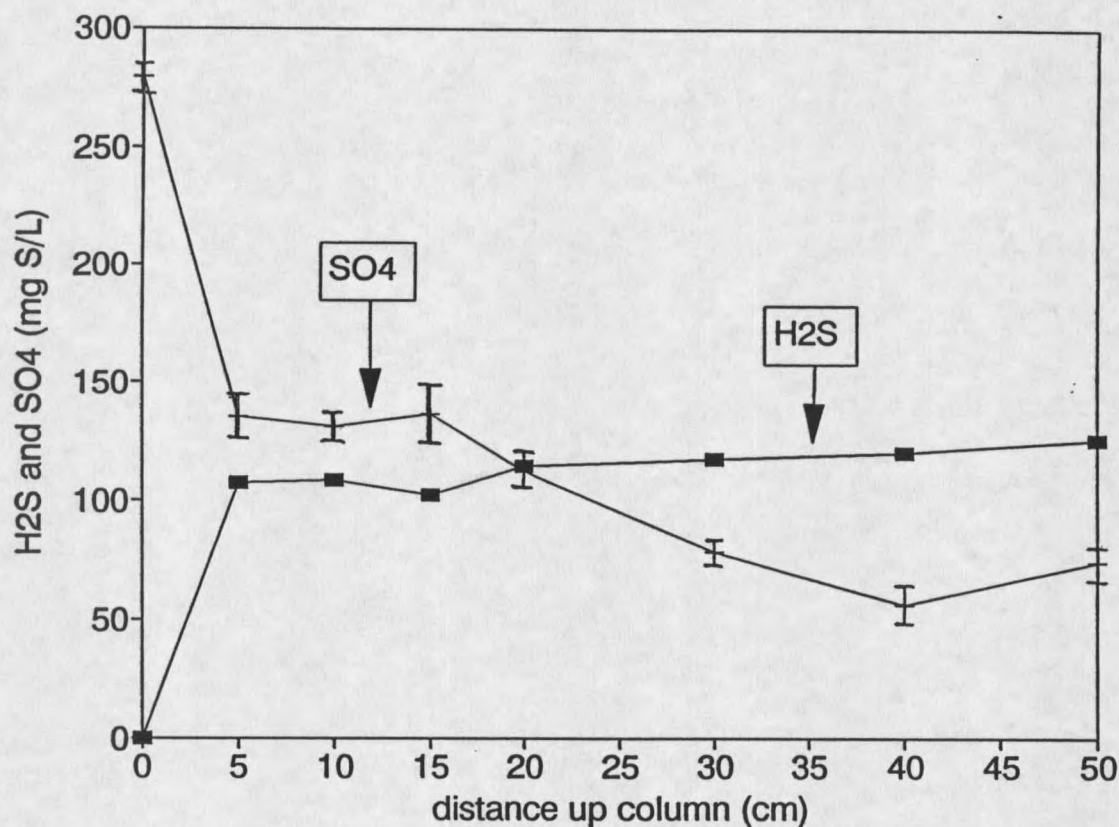


Figure 2.6. H_2S and SO_4^{2-} concentration gradients in the North Sea column at steady state. Shown are the average measurements over three days (mg S/L) with one standard error. Standard errors for H_2S concentration are not shown because they are very small (≤ 7 mg/L). Distance = 0 cm is the column inlet and distance = 50 cm is the column outlet.

state, and steadily increased to 125 mg S/L at the column outlet. Sulfate decreased simultaneously by almost 150 mg S/L at the first port and by about 200 mg S/L at the outlet. The greater change in sulfate concentration compared to sulfide (both were calculated based on S) was probably due to iron sulfide precipitating within the column. It was shown in an earlier experiment that sulfate concentrations did not change throughout the column in a sterile reactor system (Chen et al., 1993). This steady state data was taken in August 1993, three months after inoculation and over two months after the onset of souring.

Similar sulfide and sulfate data were obtained in the other three column experiments (Table 2.3). In the North Slope #1 column, the sulfide levels were slightly lower than in the North Sea column (Figure 2.6), and the sulfide gradient at the beginning of the column was not as steep (i.e., SRB activity appeared to be spread more evenly throughout the North Slope #1 column). Again, the amount of sulfate reduced was higher than the amount of sulfide measured. In the North Slope #2 experiment, sulfide levels were much higher than in the North Slope #1 or North Sea experiments, with very high activity at the beginning of the column (Table 2.3). The low sulfide levels at the end of the column show that this column was probably not yet at steady state when this data was taken. In the North Slope PW column, even higher sulfide concentrations (>200 mg S/L) were measured, again with about 90% of the effluent sulfide concentration seen by the first sampling port (Table 2.3). In the North Slope PW and North Slope #2 columns (up to 30 cm), the amount of sulfate reduced and the amount of sulfide measured were within

about 30 mg S/L, showing that these columns were very close to actual steady state with respect to both SRB activity and iron sulfide precipitation within the column.

Table 2.3. H_2S and SO_4^{2-} concentrations at steady state in the crushed Berea sandstone columns. Shown are the average measurements over three days (mg S/L) with one standard error. Distance = 0 is the column inlet and distance = 50 cm is the column outlet.

distance (cm)	H_2S (North Slope #1)	H_2S (North Slope #2)	H_2S (North Slope PW)	SO_4^{2-} (North Slope #1)	SO_4^{2-} (North Slope #2)	SO_4^{2-} (North Slope PW)
0	0	0	0	302 ± 12	242 ± 10	310 ± 4
5	N.M.	175 ± 7	195 ± 3	N.M.	47 ± 2	104 ± 3
10	46 ± 6	179 ± 4	217 ± 4	280 ± 18	45 ± 1	56 ± 1
15	N.M.	175 ± 11	206 ± 4	N.M.	36 ± 1	73 ± 3
20	87 ± 12	184 ± 6	229 ± 3	155 ± 47	36 ± 1	43 ± 2
30	72 ± 15	181 ± 3	223 ± 3	90 ± 30	29 ± 1	62 ± 2
40	93 ± 18	122 ± 9	229 ± 4	72 ± 12	44 ± 7	41 ± 4
50	86 ± 7	97 ± 7	225 ± 3	75 ± 14	62 ± 4	51 ± 3

* N.M. = not measured

Similar results were seen in the core system for sulfide and sulfate (Figure 2.7). Essentially all of the sulfate reduction occurred in the first core (no measurements could be taken within the core). The level of activity agrees closely with the North Slope #2 column, which served as the source of inoculum for the core system.

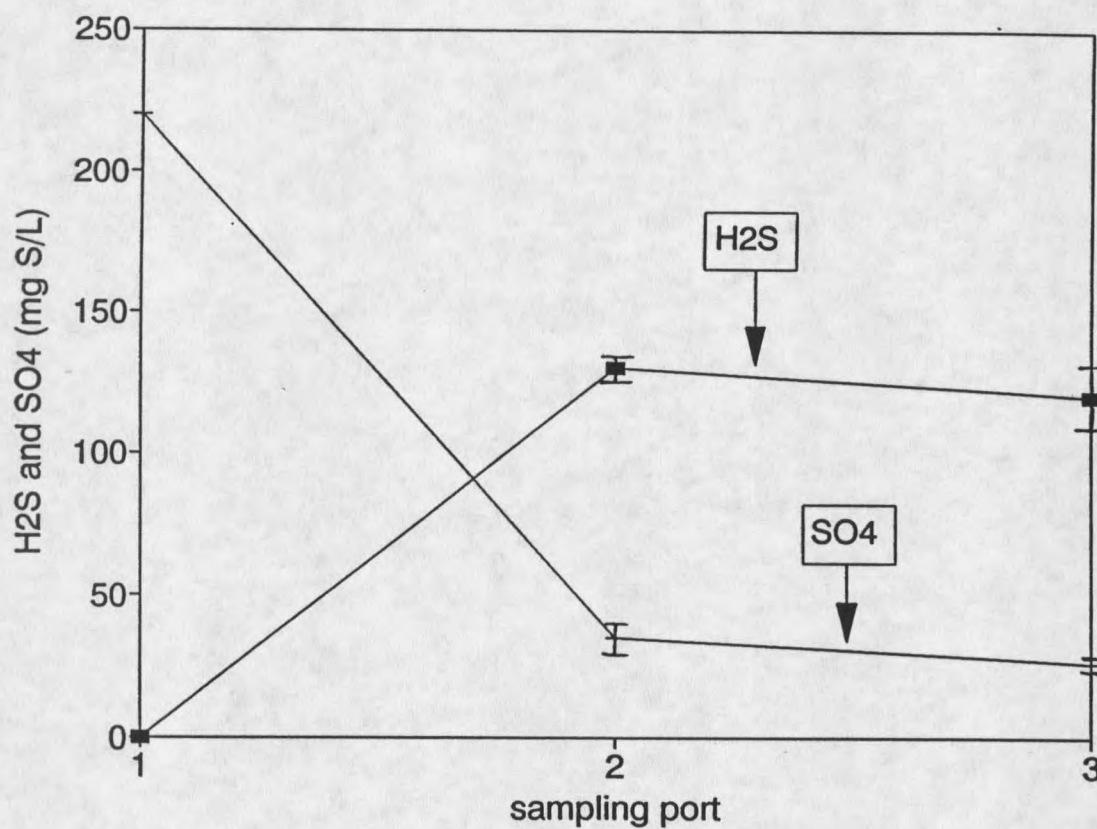


Figure 2.7. H_2S and SO_4^{2-} concentration gradients in the cores at steady state. Sampling port 1 is the inlet to core #1, port 2 is the inlet to core #2 and port 3 is the outlet from core #2.

Organic acid utilization. Formate and *n*-butyrate were the preferred substrates for sulfate reduction in the North Sea column (Figure 2.8), as they were completely or nearly completely consumed in the first five cm. Propionate and isobutyrate were consumed to significant degrees (over 50%) but at slower rates. Acetate was not consumed but rather produced by SRB and the concentration significantly increased throughout the column as *n*-butyrate and propionate were incompletely oxidized (Figure 2.8).

Similar substrate preferences and consumption rates were noted in the other experiments. Formate was the preferred substrate in the North Slope PW column, and second-preferred in the North Slope #1 and #2 columns (Table 2.4). *n*-Butyrate was the preferred substrate in both North Slope columns, and the second-most preferred carbon source in the PW column. Propionate was also significantly consumed in the North Slope #2 run (Table 2.5) but at much slower rates in the North Slope #1 and North Slope PW experiments. Isobutyrate was utilized at a lower rate than propionate in all of the column experiments (Table 2.5). As with all the substrates, however, most of the activity on isobutyrate occurred in the first five cm of the column. Acetate concentrations increased from about 200 mg/L at the column inlet to over 500 mg/L in both North Slope experiments (Table 2.6). In the North Slope PW column, only a small increase in acetate concentration was measured. The North Slope PW column data for acetate is reasonable because of the much lower consumption rates of *n*-butyrate and propionate (which produce acetate) compared to the other experiments. The large standard errors from the

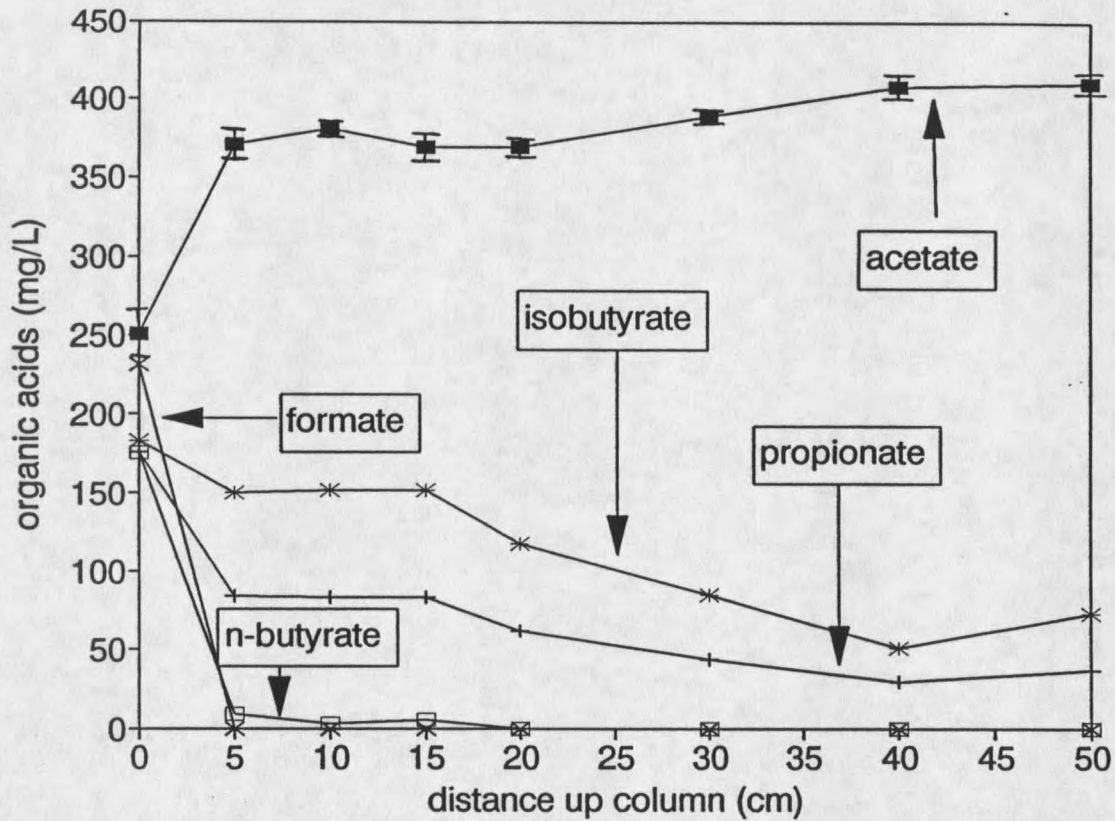


Figure 2.8. Organic acid concentration gradients in the North Sea column at steady state. Standard errors for substrates other than acetate are not shown because they are very small (≤ 6 mg/L).

North Slope #1 experiment, and the scattered acetate data from the North Slope #2 run, were due to problems with the ion chromatograph column at those times.

Table 2.4. Formate and *n*-butyrate concentrations (mg/L) at steady state.

distance (cm)	formate (North Slope #1)	formate (North Slope #2)	formate (North Slope PW)	<i>n</i> - butyrate (North Slope #1)	<i>n</i> - butyrate (North Slope #2)	<i>n</i> - butyrate (North Slope PW)
0	180 ± 6	238 ± 5	156 ± 5	180 ± 5	163 ± 5	160 ± 7
5	N.M.	18 ± 2	6 ± 3	N.M.	0	107 ± 4
10	173 ± 10	18 ± 2	0	0	0	86 ± 1
15	N.M.	14 ± 1	0	N.M.	0	94 ± 2
20	130 ± 23	14 ± 1	0	0	0	66 ± 2
30	120 ± 18	12 ± 1	0	0	0	77 ± 4
40	95 ± 16	16 ± 2	0	0	0	78 ± 1
50	115 ± 10	24 ± 3	0	0	0	79 ± 2

* N.M. = not measured

n-Butyrate was the preferred substrate in the core system (Figure 2.9), as it was for the North Slope #2 column (Table 2.4). Consumption of the other organic acids (Figure 2.9) and production of acetate was similar to the results from the North Slope columns.

Electron balances. When the inlet and outlet concentrations of the electron acceptor (sulfate in this case) and the electron donor (organic acids) are known, an electron balance may be calculated for sulfate reduction in the column. Does the

Table 2.5. Propionate and isobutyrate concentrations (mg/L) at steady state.

distance (cm)	prop. (North Slope #1)	prop. (North Slope #2)	prop. (North Slope PW)	isobut. (North Slope #1)	isobut. (North Slope #2)	isobut. (North Slope PW)
0	235 $\pm$ 5	194 $\pm$ 5	165 $\pm$ 5	248 $\pm$ 6	193 $\pm$ 5	182 $\pm$ 6
5	N.M.	26 $\pm$ 1	131 $\pm$ 3	N.M.	50 $\pm$ 2	87 $\pm$ 2
10	225 $\pm$ 16	24 $\pm$ 1	125 $\pm$ 2	242 $\pm$ 7	47 $\pm$ 1	63 $\pm$ 2
15	N.M.	22 $\pm$ 1	130 $\pm$ 2	N.M.	40 $\pm$ 1	72 $\pm$ 2
20	144 $\pm$ 27	21 $\pm$ 1	106 $\pm$ 1	205 $\pm$ 12	36 $\pm$ 1	45 $\pm$ 1
30	108 $\pm$ 26	19 $\pm$ 1	121 $\pm$ 2	185 $\pm$ 18	28 $\pm$ 1	55 $\pm$ 1
40	80 $\pm$ 22	24 $\pm$ 3	121 $\pm$ 1	110 $\pm$ 30	43 $\pm$ 10	54 $\pm$ 3
50	102 $\pm$ 18	31 $\pm$ 1	121 $\pm$ 1	170 $\pm$ 10	67 $\pm$ 5	58 $\pm$ 3

Table 2.6. Acetate concentrations (mg/L) at steady state.

distance (cm)	acetate (North Slope #1)	acetate (North Slope #2)	acetate (North Slope PW)
0	250 $\pm$ 15	223 $\pm$ 5	186 $\pm$ 3
5	N.M.	457 $\pm$ 7	217 $\pm$ 5
10	288 $\pm$ 29	572 $\pm$ 29	227 $\pm$ 5
15	N.M.	475 $\pm$ 13	224 $\pm$ 5
20	433 $\pm$ 60	506 $\pm$ 27	216 $\pm$ 3
30	504 $\pm$ 55	489 $\pm$ 1	225 $\pm$ 4
40	540 $\pm$ 35	523 $\pm$ 30	227 $\pm$ 2
50	548 $\pm$ 33	467 $\pm$ 14	222 $\pm$ 3

* N.M. = not measured

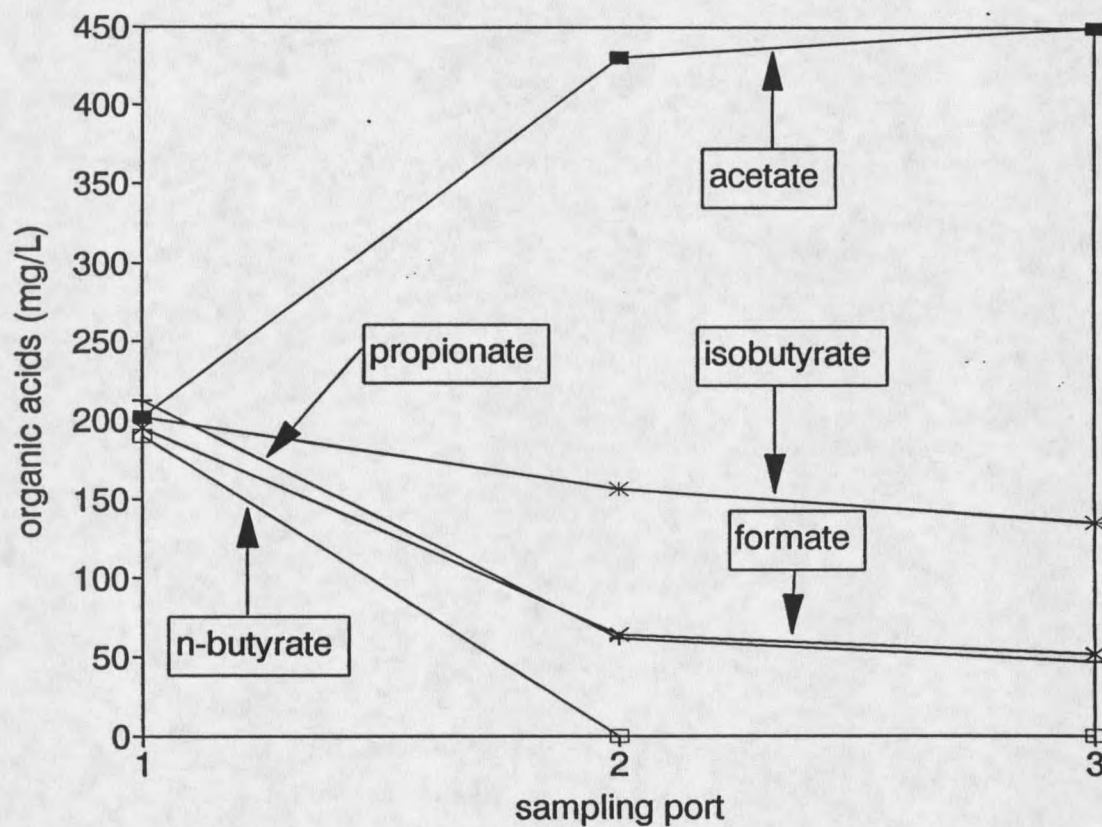
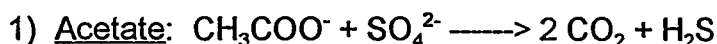
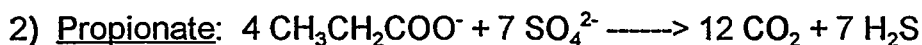


Figure 2.9. Organic acid concentration gradients in the cores at steady state. Standard errors are not shown because they are very small (≤ 10 mg/L).

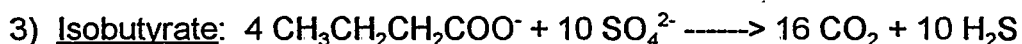
number of electrons donated by the organic acids in the oxidation to acetate and CO₂ match the number of electrons received by sulfate in the reduction to H₂S? The electron balance was based on the following reactions for each substrate (addition of H₂O, H⁺ and OH⁻ groups in the appropriate places will balance the reactions with respect to elements):



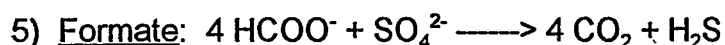
Eight electrons are transferred per mole of acetate in this reaction. Since there was a net production of acetate, however, the reverse reaction (though impossible metabolically) was used in the electron balance. It was assumed that the other four substrates were completely oxidized to CO₂, and the amounts incompletely oxidized were taken into account by including the net production of acetate in this reverse reaction.



Fourteen electrons are transferred per mole of propionate in sulfate reduction.



Twenty electrons are transferred per mole of isobutyrate.



Two electrons are transferred per mole of formate.

The electron balance for the North Sea column (influent versus effluent) is shown in Table 2.7. Only about 63% of the electrons donated by the organic acids can be accounted for in sulfate reduction. About 120% were accounted for in the

North Slope #1 experiment, 61% in the North Slope #2 column, and 114% in the North Slope PW column.

Table 2.7. Electron balance for souring in the North Sea column. The balance is based on steady state concentrations in the effluent versus the influent.

component	molecular weight	mg/L consumed	millimoles/L consumed	electrons/mole	electrons/ml
acetate	59	-180	-3.05	8	-24.4
propionate	73	138	1.89	14	26.5
isobutyrate	87	111	1.28	20	25.5
n-butyrate	87	174	2.00	20	40.0
formate	45	234	5.20	2	10.4
					sum = 78.0
sulfate	32	195	6.09	8	48.8

Electron balance = $48.8/78.0 = 62.6\%$

Biomass

Yields. Total cell counts were performed at each sampling port in the columns during souring steady state (Table 2.8). Cell counts at steady state were not performed in the North Slope PW column experiment. Higher numbers of cells (up to an order-of-magnitude) were detected in the aqueous phase withdrawn from sampling ports within the column than from the effluent (50 cm), probably due to some biofilm detachment when sampling within the column.

Table 2.8. Suspended cell counts (cells/ml) at steady state. Shown are averages (log base-ten) over three days.

distance (cm)	North Slope #1	North Slope #2	N. Sea column
0	0	0	0
5	N.M.	7.5	7.4
10	6.8	7.7	6.8
15	N.M.	7.6	7.4
20	6.9	7.6	7.3
30	7.0	7.3	7.4
40	7.1	7.1	7.5
50	6.6	6.6	6.4

* N.M. = not measured

Table 2.9. SRB yields based on differences in cell counts, sulfate concentrations and organic acid concentrations between the influent and effluent.

column	Y_{xs} (g dry biomass/ g total substrate)	Y_{xe} (g dry biomass/ g SO_4^{2-})
North Slope #1	0.0009	0.0010
North Slope #2	0.0006	0.0013
North Sea	0.0003	0.0010

Assuming that the cell counts were at steady state when data was taken means that cell detachment (which was measured as cells in suspension) equals cell growth. Using the cell counts and an average cell size of $0.30 \mu m^3$ (Mueller, 1992), active biomass quantities can be calculated. The product of cell count, cell

size, wet cell specific gravity of 1.09×10^{-12} g wet cell/ μm^3 (Doetsch and Cook, 1973) and the ratio of 0.22 g dry cell/g wet cell (Luria, 1960) provides the concentration of dry cell mass in suspension. Cell yields for SRB were quite low for the consortia in each experiment (Table 2.9), as are yields for most anaerobic bacteria. These yields were even lower than other yields reported in the literature, such as 0.028-0.041 g dry biomass/g lactate for *D. desulfuricans* (Okabe, 1992). The North Slope consortium had slightly higher yields than the North Sea consortium.

Destructive measurement. Biomass was also measured in the North Slope columns (#1 and #2) by another method. At the conclusion of each experiment, the column was dismantled and biomass was measured by sectioning the sandstone packing into lengths, drying at 100°C , then ashing at 400°C . In both columns, attached biomass (mg biomass per g sand) was found to be fairly consistent throughout the column (Figure 2.10) with no consistent trend in either experiment. Biomass was, however, approximately 50% higher and somewhat more consistent in North Slope #2. When coupled with the surface area available in the column, (based on the average media particle size), the biomass in the North Slope #2 column equals approximately a $4.0 \mu\text{m}$ biofilm. This method measured the total biomass, not just the active biomass in the column.

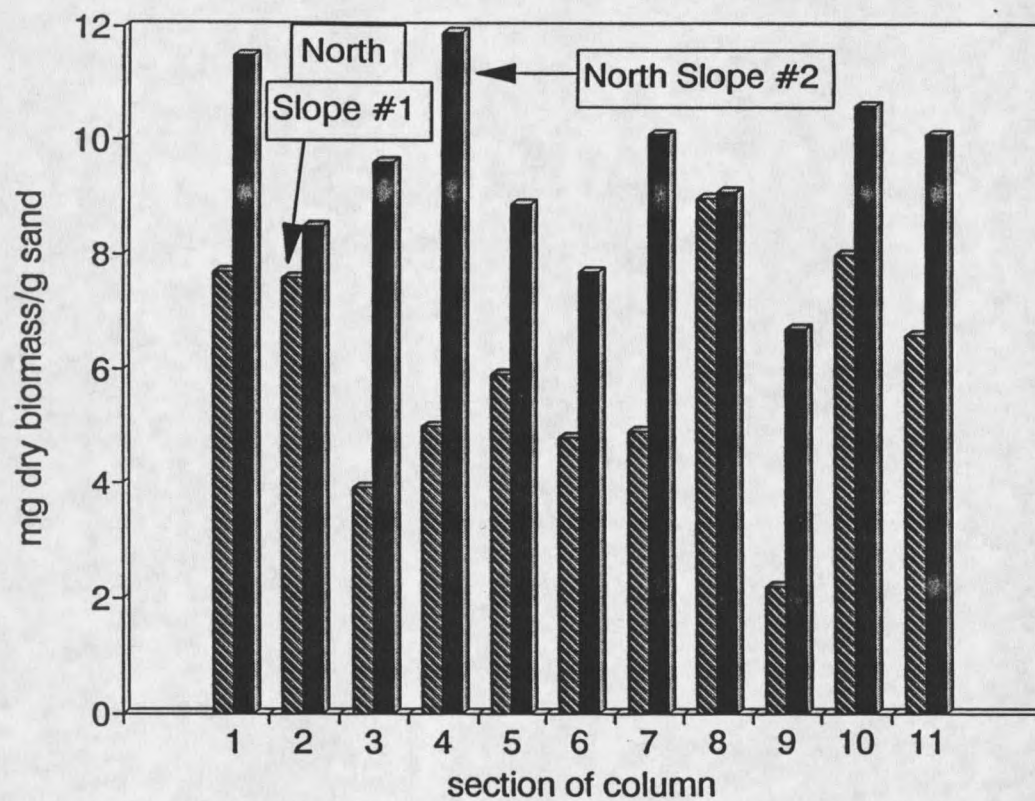


Figure 2.10. Biomass measurements by section in the North Slope #1 and #2 columns.

Iron Sulfide Precipitation

As previously mentioned, black precipitate due to iron sulfide formed in the columns upon H_2S production was initiated by SRB. During initial growth of SRB after the North Sea column was inoculated, this " H_2S front" proceeded up the column at a rate of about 0.75 cm/day. As the front reached each sampling port, H_2S was detected in the aqueous phase. No H_2S could be detected ahead of the front. Other researchers (Ligthelm et al., 1991) have predicted a retardation factor of up to 1000 impeding H_2S "flow" in a oil reservoir due to its reaction with indigenous minerals such as iron (the retardation factor of a particular component is the bulk water velocity divided by the velocity of that compound). With an average pore velocity of 55 cm/day, the retardation factor for H_2S in the North Sea column was about 75.

Acid-volatile sulfide (AVS) was measured in each section of the North Slope PW column at the conclusion of the experiment. Although the sand was much more solidly packed (nearly solid, like a core) at the bottom of the column at this point in time, the porous medium was a fairly uniform gray throughout the column, with no large color differences between the top and bottom of the column, or between radial positions. This observation is reinforced by the fairly uniform distribution of acid-volatile iron sulfide throughout the column (Figure 2.11). Calculations comparing the amount of iron sulfide probably deposited in the column in a month (based on differences between sulfate consumed and H_2S measured) and the amount of iron sulfide in the sand are in close agreement (Appendix 4).

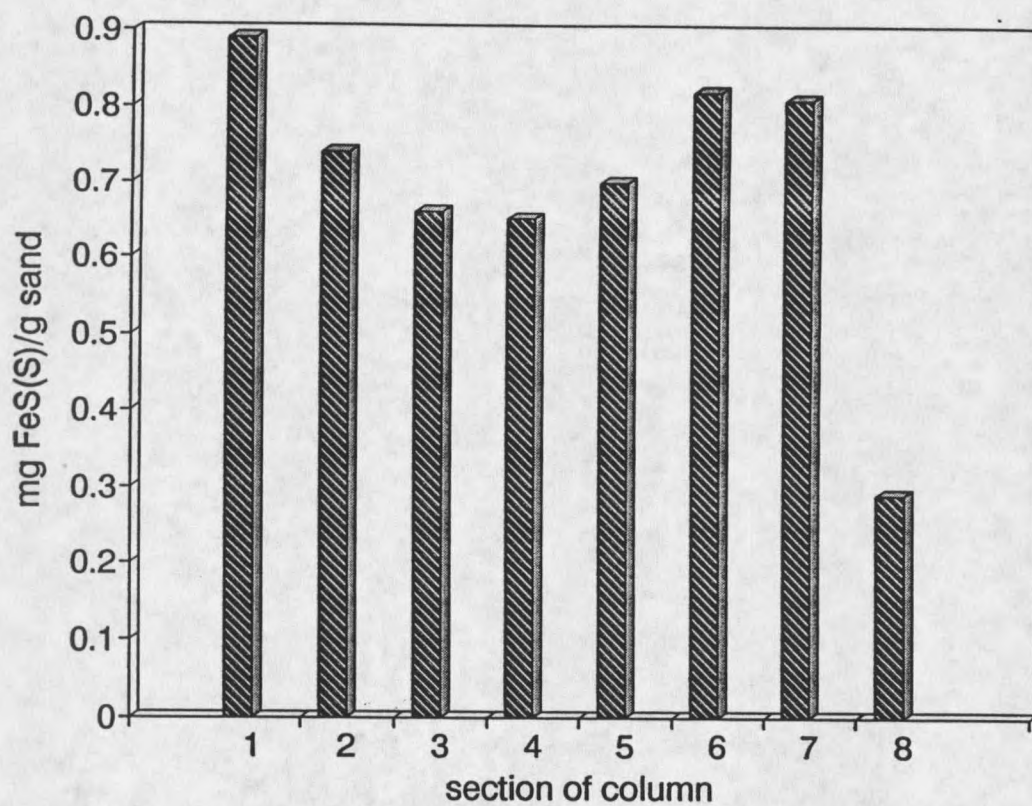


Figure 2.11. Iron sulfide measurements by section in the North Slope PW column.

Redox Potential

During active souring, the redox potential (E_h) of the anaerobic medium was reduced from about -100 mV to lower than -400 mV by the first sampling port (Figure 2.12). E_h was measured only in the North Slope #2 and North Sea columns, and was quite similar in both. This environment is well below the reducing threshold required for SRB growth of about -100 mV (Postgate, 1984). As expected, there was a strong negative correlation (R-squared value = 0.98) between the H_2S concentration and the redox potential at each point sampled in the North Slope #2 column (Figure 2.13).

Permeability

The Darcy permeability of the crushed Berea sandstone columns was calculated from the following equation (Freeze and Cherry, 1979):

$$v = - \frac{k}{\mu} \frac{dP}{dx} \approx \frac{k}{\mu} \frac{\Delta P}{\Delta x}$$

where:

v = flow velocity = volumetric flow rate/cross-sectional area (cm/hr)

k = Darcy permeability (mD)

μ = viscosity of fluid (pure water) = 3.13×10^{-4} lb/(ft-sec) at 60°C (Perry and Chilton, 1973)

$$\Delta P = \rho g \Delta h$$

ρ = density of fluid = 0.982 kg/L at 60°C (McCabe and Smith, 1976)

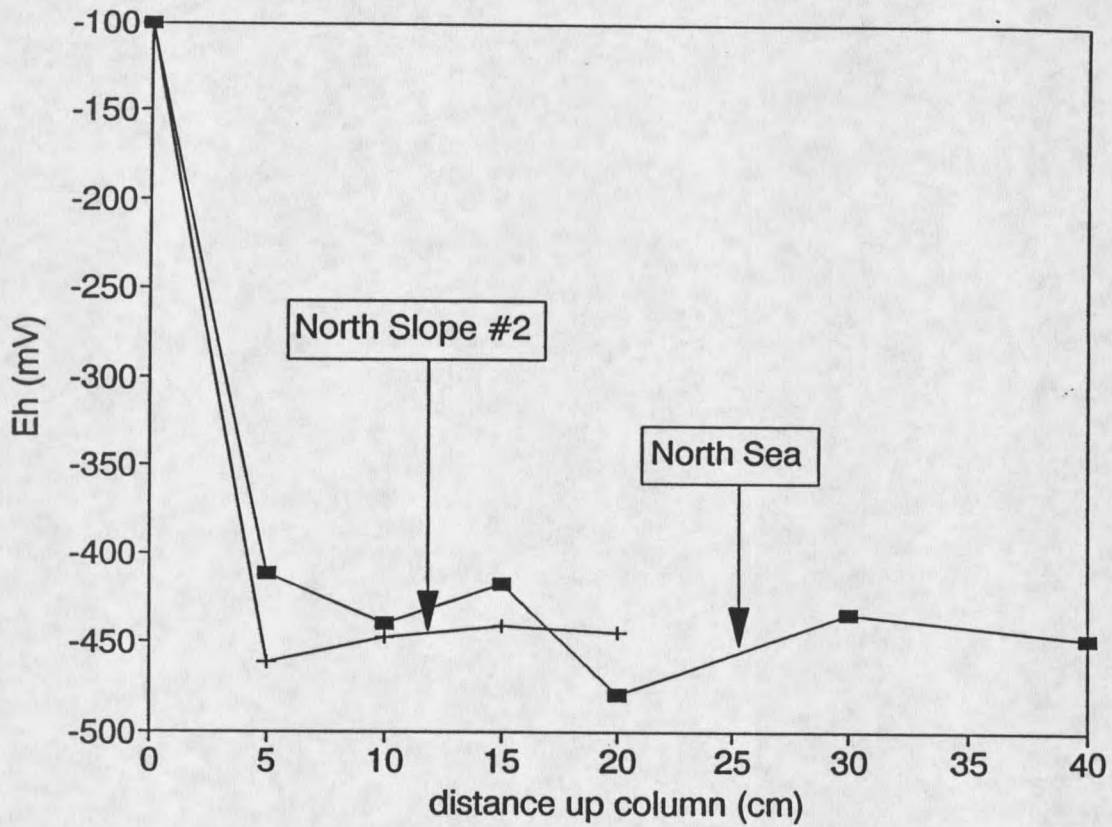


Figure 2.12. E_h gradients in the North Slope #2 and North Sea columns during souring steady state. Single readings at each distance over the course of one day were taken for each column.

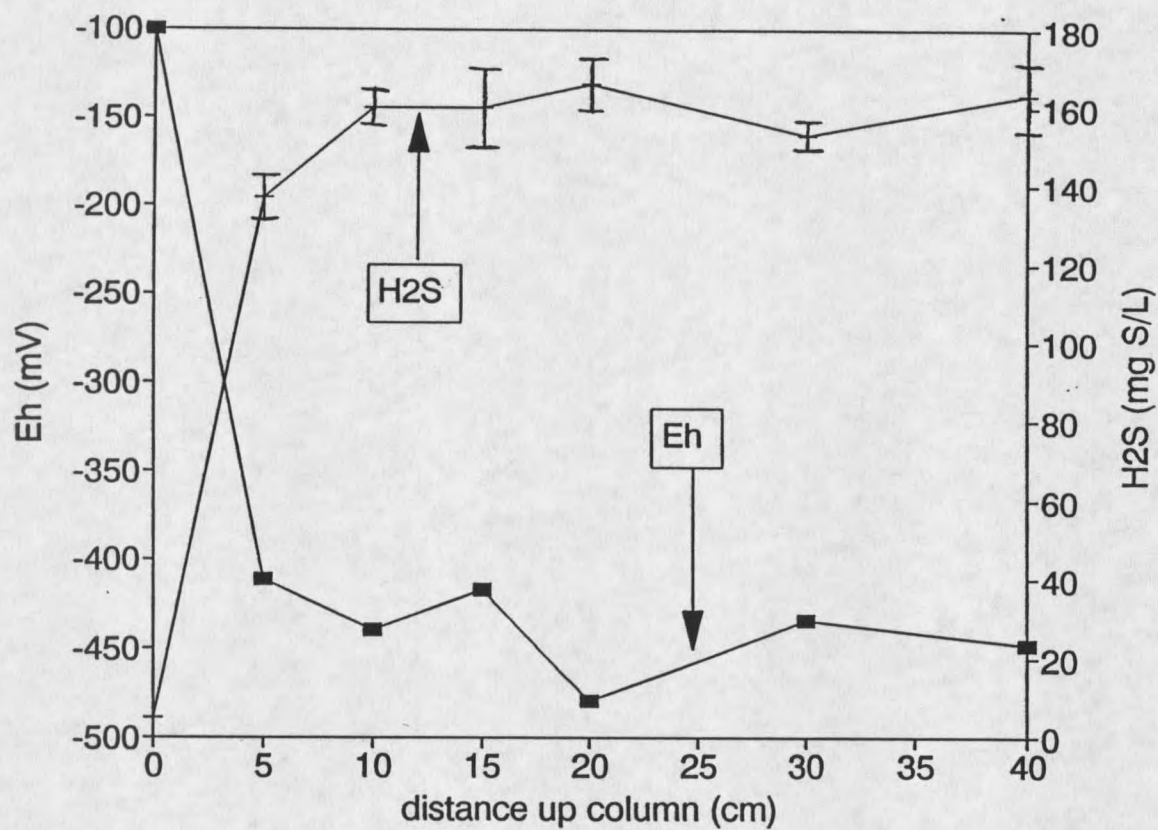


Figure 2.13. H₂S-E_h correlation in the North Slope #2 column during souring steady state.

$$g = 9.8 \text{ m/sec}^2$$

$$\Delta h = \text{piezometric head (cm H}_2\text{O)}$$

$$x = \text{length of column (cm)}$$

This equation can be rearranged to solve for k , the Darcy permeability:

$$k \approx \frac{\nu \mu \Delta x}{\rho g \Delta h}$$

With a constant volumetric flow rate (which was maintained by slight upward adjustments of the pump speed in these experiments) in a single column at a constant temperature, the only variable on the right-hand side of this equation is Δh , the piezometric head. Measuring Δh then allows direct calculation of k , the Darcy permeability. In the North Slope #2 and PW columns, piezometers were located at the column inlet to measure the pressure before inoculation and to monitor any changes. It was anticipated that biomass growth and/or iron sulfide precipitation would increase the pressure drop, meaning a loss in permeability. The pressure drops and calculated permeabilities are shown for these two systems in Table 2.10. Pressure drop data from the North Sea column was not useful because of the lack of sensitivity (0-100 psi).

Plugging was not very significant in the North Slope #2 column, as its permeability was reduced only about 30% to 620 mD (Table 2.10). The column was slightly more plugged than this at several times during the course of the experiment, as the piezometer level varied slightly, but the measured permeability decrease was never more than about 55%. Pressure drop was significant, however, in the PW

column (Table 2.10). The initial permeability of this column was slightly lower than the other columns because of increased settling during final packing (the top had to be hammered on to provide a leak-free fit). In fact, the initial permeability was halfway between that of the other columns and that of the cores. The pressure drop in the North Slope PW column increased steadily to the point that, after less than two months of operation, the pressure head exceeded the height of the piezometer, and the piezometer was subsequently disconnected. When this column was dismantled months later, the sand at the bottom of the column was hard-packed, core-like and impervious to flow. It appeared that the bulk of the flow at this time was through a 2-3 mm-diameter hole in the center of the column. The sand became progressively less packed up the column until loose sand was found at the top.

Table 2.10. Permeability changes in the columns during souring (k = permeability).

	North Slope #2	North Slope PW
Δh initial (cm H ₂ O)	8.1	10.2
k initial (mD)	920	750
Δh final (cm H ₂ O)	12	150+
k final (mD)	620	≤ 50
Δh^* (cm H ₂ O)	19	---
k^* (mD)	390	---

* at most plugged condition

Significant pressure drops were also noted in the core experiment (Table 2.11). In the core system, the calculated Darcy permeabilities were as low as 30

mD at the conclusion of the experiment. It has been suggested by some researchers (Costerton, 1994) that transport of most types of bacteria is impossible through porous media with a permeability below about 100 mD. Plugging in the first core was undoubtedly due to both biomass and iron sulfide. Plugging in the second core was probably due to iron sulfide alone, for two reasons: 1) cells could probably not be transported all the way through the first core after some time, and 2) nearly all of the SRB activity was seen in the first core. Therefore, based on the difference in pressure drops across the two cores (Table 2.11), it appears that iron sulfide may be a larger contributor to plugging than is SRB biomass.

Table 2.11. Permeability changes in the cores during souring.

	core #1	core #2
ΔP initial (psi)	0.4 ± 0.3	0.5 ± 0.3
ΔP final (psi)	3.0 ± 0.3	1.8 ± 0.3
k initial (mD)	600	560
k final (mD)	30	55

Discussion

Dynamics

Although SRB are routinely identified in oil field waters (Rozanova and Pivoravara, 1988; Rosnes et al., 1991; Voordouw et al., 1993), few papers have been published regarding the metabolic activity and H_2S production in porous media using native microbial consortia. This study investigated microbial souring

in porous media under simulated oil reservoir conditions (SRB, carbon sources, nutrients, porous media and temperature), with two different consortia and two methods of inoculation. The results indicate that souring initially occurred at the front part of the column (Figure 2.3) and progressively moved up the column. The majority of the souring occurred in the beginning of the column at steady state (Figure 2.6), irrespective of whether a small inoculum was introduced at the beginning (North Slope and North Sea experiments) or whether a large inoculum was delivered to the entire column (PW experiment). In the North Slope PW column it is unknown whether cells accompanied the produced water into the entire column and formed a biofilm, or whether the culture was "stuck" at the beginning of the column. Other experiments in this column (Appendix 1) support the latter scenario, in which case the two types of inoculation were probably no different as far as introducing cells into the column.

The movement of souring (as measured by H_2S) also indicated the adsorption, desorption and growth of SRB along the column, as bacterial cells were detected in the effluent only slightly ahead of the H_2S . Most of the substrates (organic acids and sulfate) were consumed in the front part of the column (first five cm) for both consortia and both types of inocula. Similar experimental trends (rates and location) of souring for both consortia indicate that abiotic parameters, as well as biological factors, may play an important role for souring in the column reactor system.

Souring in porous media is a process which combines biotransformation carried out by biofilms (and perhaps suspended cells) and transport phenomena. The accumulation of biofilm in porous media is the net result of microbial cell adsorption, desorption, attachment, detachment, growth on surfaces and filtration (Cunningham et al., 1990). Calculations were made to approximate the amount of biomass contained in the aqueous phase and the amount of biomass attached on the solid phase (sand) for the first six sections of the column, where the biotransformation primarily occurred. Data from Figure 2.12 and Table 2.8 were used for the calculations. The total biomass included biomass attached to the solid phase and bacterial cells in the remaining aqueous phase.

Results indicate that for the North Slope #1 column, the aqueous phase in the first six sections contained about 5×10^{-4} g dry biomass, while the corresponding solid phase contained about 6.5 g dry biomass (biofilm), as shown in Appendix 4. The difference is approximately four orders of magnitude and may be even larger, since the cell counts from within the column probably also include some biofilm cells. Although biofilm cells have been reported to contain a high amount of exopolysaccharides (Blenkinsopp and Costerton, 1991; Bryers, 1993), the overwhelming difference in biomass indicates the domination of activity by biofilm cells over suspended cells. Hence, the reduction of sulfate to H_2S in porous media is primarily carried out by SRB biofilm cells. The same conclusion was reached in an experiment using a single species SRB, *D. desulfuricans* (ATCC 5575), in a sea sand porous media column (Chen et al., 1993).

Electron Balance

Because of the incomplete electron balance for sulfate reduction in the North Sea and North Slope #2 experiments, it appears that microbiological processes in addition to sulfate reduction were occurring. Possibilities include:

- Fermentative organisms. These microbes could have consumed organic acids, probably producing hydrogen and CO_2 (Brock, 1991). No analysis was done for hydrogen and CO_2 in the columns, however.

- Methanogens. These microbes could have consumed hydrogen (from fermenters?) and CO_2 , producing methane (CH_4) and water. No analysis was done for methane. Also, it is known that methanogens can produce methane from formate and hydrogen, or from acetate and hydrogen (Widdel, 1988).

- Oxygen. It is possible that if the column were not completely anaerobic, some aerobic heterotrophs could have consumed organic acids. This is very unlikely to have happened while the column was at steady state, however, since the H_2S present would have acted as a very fast and effective oxygen scavenger (Widdel, 1988).

- Methanol. Methanol could have been another incomplete oxidation product along with acetate (Widdel, 1988). No analysis for methanol was performed.

- Improper calibration. If ion chromatograph (IC) standards for sulfate were too high (creating artificially low readings for sulfate) and/or standards for the organic acids were too low, this could explain the discrepancy seen. This is

unlikely for two reasons, however: 1) the balances were off by such a large amount (40%), and 2) the sulfate and organic acid influent readings were all close to what was theoretically added (300 mg/L and 200 mg/L each, respectively).

It is unknown why the electron balances for the North Slope #1 and PW columns were so different from the others. The fairly small deviations from 100% in these cases (20% and 14%, respectively) could be attributed to calibration error with the ion chromatograph.

Biomass

The yields reported for both consortia are fairly low. It may be that neither column was yet at steady state as far as suspended cells in the effluent, since more cells were possibly still being trapped in the sand and biofilm matrix in the column than were detaching into suspension. Yields (Table 2.9) are about an order-of-magnitude lower than the yields reported by Mueller in single-substrate batch experiments (1994). He reported yields of 0.0053-0.0101 g dry biomass/g substrate for North Slope and North Sea SRB on *n*-butyrate and formate, and yields of 0.0132-0.0413 g dry biomass/g S of sulfate. Yields observed here were based on the mass of suspended cells (detached, actively-growing biofilm cells, it was assumed) but it has been shown that the biofilm cell mass was approximately four orders of magnitude larger than this. If all these biofilm cells were active in sulfate reduction, the lower limit for yields would be four orders of magnitude higher than those shown in Table 2.9 (i.e., yields could approach 1.0).

In all four columns, the majority of the souring activity was in the first five cm (first 10%) of the column. This is somewhat peculiar in that an even distribution of biomass was found within the North Slope columns (Figure 2.10). It may be that most of the inactive biomass at the end of the column was merely "stuck" there due to produced exopolysaccharides (EPS). Or, it may be that the biomass at the beginning of the column was composed mostly of cells, while the biomass at the end of the column was mostly EPS. The ashing technique used for measuring biomass would not distinguish between these two types of biomass. Some souring activity was still occurring in the second half of the column, however, as shown by the steady consumption of secondary substrates like propionate and isobutyrate (Table 2.5).

Permeability Reduction

Souring studies of *D. desulfuricans* in sea sand porous media indicated that most of the substrate (lactate) was consumed in the first 20 cm of the 50-cm column, with the highest volumetric consumption rate observed within the first 10 cm (Chen et al., 1993). Taylor (1990), Taylor and Jaffe (1990a,b) and Taylor et al. (1990) investigated the permeability reduction in a sand porous medium caused by growth of sewage and sludge bacteria. They reported that most of the substrate (methanol) was consumed at the front part of the sand reactor, where biomass plugging occurred. They attributed the high permeability reduction and a thicker biofilm found at the front part of the sand column to high substrate utilization and

biofilm growth in the region.

Permeability reductions ranging from 80 to 99.9% for different porous media reactor systems, nutrient feeds and microbial compositions have been reported by numerous investigators (Geesey et al., 1987; MacLeod et al., 1988; Raiders et al., 1986; Raiders et al., 1989; Taylor et al., 1990). However, a 60% or less reduction in permeability was found in my experiments. One possible explanation is the very low growth rates and low yields of the consortia, which may have resulted in low cell density in the column during the experimental period. The maximum specific growth rate ($\mu_{\max}$) of the consortia was only 0.01-0.04/hr measured in a batch system at 60°C with the same carbon sources (Mueller, 1994). Another possibility is the fairly short-term duration (<100 days) and small amount of medium (<120 pore volumes) used in my experiments, as compared to the long-term operation (up to 356 days) and high flooding volumes (>2000 pore volumes) used by other investigators.

Effects of Initial Permeability

The effects of the initial permeability of the system may be seen by comparing the results of the North Slope #2 column and the core system. The type of porous medium, the liquid medium composition, the average pore velocity and the source of inoculum were very similar for both systems. The major difference was the initial permeability (about 920 mD in the column and about 600 mD in the core).

The souring activity in both systems was very similar. The same H₂S level

(over 100 ppm) and the same substrate preference were observed. In both systems, all of the *n*-butyrate was consumed, nearly all of the formate and propionate were consumed, some isobutyrate was utilized, and there was a large net production of acetate. Sulfate reduction did not go to 100% in either system, as sulfate and some organic substrate remained in the effluent.

The large difference in the two systems was the plugging caused by bacterial biomass and iron sulfide precipitation. In the higher permeability column (920 mD), the permeability was reduced by only 30%. In the core system, however, the permeability of the first core (600 mD) was reduced by 95% and that of the second core (560 mD) by 90%. This agrees with the results of the North Slope PW column, which had an initial permeability of 750 mD, and saw a permeability reduction of at least 93% during souring. This shows that the initial permeability will not affect the souring activity itself, but could have a dramatic effect on the permeability changes in the porous medium brought about by souring.

Conclusions

Despite the anaerobic requirements, active souring was fairly easy to achieve in a properly designed porous media column. Noticeable souring began seven to 20 days after inoculation of an actively growing batch culture at the inlet of the column, and was noted by measurement of H_2S and formation of black iron sulfide precipitate. The H_2S front was patchy and localized, and progressed up the column much slower (almost two orders of magnitude) than the liquid velocity. With

a minimum of flow interruptions, it took at least 40 days for each column to reach steady state with respect to souring activity.

Similar souring activity (H_2S concentrations and rates of production, and substrate preferences) were seen with consortia from both the North Slope and North Sea oil fields, and activity in the porous media systems was similar to that seen in batch experiments with suspended cells. The bulk of the activity was at the beginning of the columns with the experimental flow rates, which were similar to those seen in an oil field with water injection. This is consistent with other researchers' conclusions that when all of the ingredients necessary for souring are present (SRB, sulfate and organic substrate), souring will occur close to the injection well. Not all of the microbial activity seen in at least two of the columns was due to simple SRB activity, however, as an electron balance could not be closed.

Low cell yields were seen in all the columns, even lower than other researchers have reported for SRB and lower than those calculated for these consortia in batch experiments. An even distribution of biomass was found in the columns, an amount consistent with a thin anaerobic biofilm. An even distribution of iron sulfide was also found. These last two results contrast with the earlier result that most of the actual souring activity occurs at the beginning of the column. A low E_h was measured with both consortia during souring, well below the threshold required for SRB activity. A small permeability decrease was noted in one column experiment during souring, a much larger decrease was noted in a column which

had a lower initial permeability, and the largest permeability decrease was seen in a sandstone core system, which had the lowest initial permeability. In the core system, it appeared that plugging due to iron sulfide precipitation was at least as significant as that due to biomass formation.

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CHAPTER 3

NITRATE ADDITION TO A SOURING COLUMN

Introduction

The use of nitrate (NO_3^-) to control noxious odors caused by SRB in waste water treatment was recognized as early as 1929 (Allen). Nitrate treatment was believed to have only a transient effect on sulfide production, since any organic matter remaining after the nitrate was depleted could still be used to reduce sulfate to sulfide. However, long-term inhibition of sulfide production by nitrate addition, probably due to changes in the redox potential by the production of nitrite or nitrous oxide, has been observed (Jackson and McInerney, 1994; Jenneman et al., 1986). The relative importance of the individual nitrogen oxides in creating and maintaining oxidized conditions, thereby inhibiting SRB growth, has not been studied.

Another method for the microbial control of souring is the reoxidation of sulfide to sulfate, using nitrate as the electron acceptor, by a sulfide-resistant strain of *Thiobacillus denitrificans*, strain F (McInerney et al., 1992; Montgomery et al., 1989). In experiments using sandstone cores where SRB and strain F were both present, no H_2S was detected and no black precipitate (iron sulfide) was formed. The above studies were all done at mesophilic temperatures (30–40°C). There is no reported use of nitrate treatment to control souring in thermophilic reservoirs in the North Sea or the North Slope of Alaska.

It is well known that denitrification (reduction of nitrate) is thermodynamically the most favorable microbial process in an anaerobic environment, yielding more energy than either sulfate reduction or methane formation (Figure 3.1). Nitrate reduction is a series of reactions which may form one of several products, e.g., nitrite (NO_2^-), nitrogen gas (N_2) or ammonia (NH_3). Reduction of nitrate to nitrogen gas, partial reduction of nitrate to nitrite, and dissimilatory reduction of nitrate to ammonia have much more favorable Gibbs free energies than sulfate reduction (Table 3.1). Sulfate reduction is an energy-intensive process with a positive Gibbs free energy change, and is possible only under the proper environmental conditions. Thus, if both denitrifying bacteria (DNB) and sulfate-reducing bacteria (SRB) are present in a consortium, the DNB should dominate. If the two types of bacteria utilize a common substrate such as *n*-butyrate or acetate, the DNB should outcompete the SRB if both nitrate and sulfate are present in excess, which could limit or eliminate souring activity. This competitive removal of short-chain organic acids by DNB reduced the H_2S concentration in an experimental porous media system (Hitzman and Sperl, 1994).

Some species of sulfate reducers can also reduce nitrate, usually completely to ammonia (Widdel, 1988). Several researchers (Dalsgaard and Bak, 1994; Jenneman et al., 1986) have proposed that observed microbial activity on nitrate in experimental systems was due to SRB, not DNB. In either case, activity on nitrate would limit the production of H_2S , which was the goal of this research. To

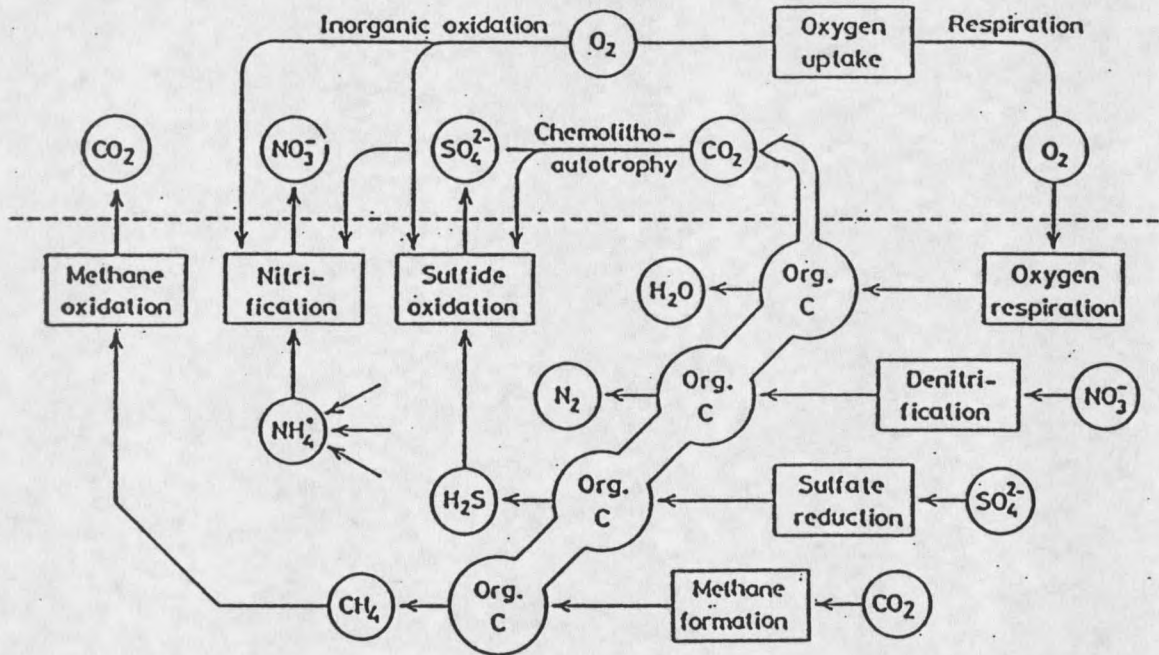


Figure 3.1. The hierarchy in anaerobic environments is determined by the availability of possible electron acceptors (Jorgenson, 1983). Inside a lake sediment, organic matter is oxidized to CO_2 through a vertical sequence of O_2 , NO_3^- and SO_4^{2-} respiration processes. After oxygen is depleted, nitrate and then sulfate will be utilized. If these electron acceptors become depleted deeper in the sediment, methane is produced.

avoid confusion, any activity on nitrite will be ascribed to "nitrate-reducing bacteria" (NRB), which may, in fact, be the same SRB which were described in Chapter 2.

Table 3.1. Free energy changes for various half-reactions (McCarty, 1972).

half-reaction		ΔG° (kJ/mol e ⁻)
$0.2 \text{ NO}_3^- + 1.2 \text{ H}^+ + \text{e}^-$	-----> $0.1 \text{ N}_2 + 0.6 \text{ H}_2\text{O}$	-71.66
$0.5 \text{ NO}_3^- + \text{H}^+ + \text{e}^-$	-----> $0.5 \text{ NO}_2^- + 0.5 \text{ H}_2\text{O}$	-39.43
$0.125 \text{ NO}_3^- + 1.25 \text{ H}^+ + \text{e}^-$	-----> $0.125 \text{ NH}_4^+ + 0.375 \text{ H}_2\text{O}$	-39.50
$0.125 \text{ SO}_4^{2-} + 1.19 \text{ H}^+ + \text{e}^-$	-----> $0.0625 \text{ H}_2\text{S} + 0.0625 \text{ HS}^- + 0.5 \text{ H}_2\text{O}$	21.28
$0.125 \text{ CO}_2 + \text{H}^+ + \text{e}^-$	-----> $0.125 \text{ CH}_4 + 0.25 \text{ H}_2\text{O}$	24.11

In the following experiments, the efficacy of nitrate addition to control microbial souring in a crushed sandstone column at thermophilic conditions was studied. Microbial consortia from thermophilic North Slope and North Sea oil reservoirs were grown in anaerobic upflow crushed sandstone reactors operated at high temperature under souring conditions, using the main organic acids found in reservoir waters as carbon and energy sources. The effects of adding and then removing nitrate on hydrogen sulfide production were studied.

Three crushed Berea sandstone columns were operated in nitrate reduction experiments. Darla Goeres continued her work with the Berea sandstone core

system under my direction by adding nitrate after all the souring data had been obtained, and also assisted with the columns.

Materials and Methods

Reactor System

The same anaerobic upflow porous media reactor systems used in the souring experiments were used for this study. Nitrate was added to the medium in the form of sodium nitrate. When nitrate was added, the same levels of sulfate and organic acids were maintained in the medium.

Bacterial Consortia and Cultivation

It was assumed that the consortium present in each column during souring would also reduce nitrate. No additional strains or consortia of bacteria were added to the columns to achieve nitrate reduction. Several experiments were reinoculated with the initial consortium of bacteria, which will be noted when applicable.

Analytical Methods

Nitrate concentrations were measured simultaneously with sulfate analysis, using the Dionex ion chromatograph (model AI-450; Dionex Co., San Francisco, CA) with a pulse electrochemical detector (model DX 300) and Dionex Ionpac AS4A-SC column (2 mm). Nitrate was expressed in terms of nitrogen content (N). Nitrite was analyzed using a Hach test kit (cat. no. 20596) with a spectrophotometer (Milton Roy, Spectronic 601) at 546 nm and nitrite measurements were also

expressed in terms of nitrogen content (N). Ammonia was analyzed using a Hach test kit (cat. no. 12524) with a spectrophotometer at 400 nm and was also expressed in terms of nitrogen content (N). Standard curves and detailed analytical procedures are included in Appendix 4. Organic acid and sulfide concentrations, and total cell counts, were analyzed by the methods described in Chapter 2. E_h was again determined by using microelectrodes inserted into the column.

Results

Effects of nitrate addition on actively souring microbial biofilms were demonstrated in experiments utilizing the North Slope and North Sea microbial consortia, operated side-by-side in identical reactor systems. Souring data from the North Sea consortium were similar to the North Slope results, as was shown in Chapter 2 and, as will be shown here, both consortia were similarly affected by the addition of nitrate. Nitrate was added at the conclusion of souring experiments in the North Slope #2 column, the North Sea column and the core system, shortly after each set of data shown in Chapter 2 was obtained. This souring data will also be shown here to demonstrate the effectiveness of nitrate in controlling souring with these two consortia. Results will also be shown for nitrate addition to the North Slope PW column, which was inoculated with produced water from the North Slope. A second experiment was done later in the North Sea column with propionate as the sole substrate for nitrate reduction. In total, five experiments (four with columns, one with cores) were run to study nitrate reduction with the two consortia.

Inhibition of Souring

Sulfide production and sulfate reduction. H_2S production was dramatically reduced after the addition of nitrate to the North Sea and North Slope columns (Table 3.2). In both cases, over 100 mg S/L of H_2S was rapidly reduced to zero shortly after nitrate reduction started (because of the high sample dilutions used, the detection limit for H_2S was approximately five mg S/L). In the North Sea column, 50 mg N/L of nitrate (varying slightly with each medium preparation) was added to the medium one week after H_2S concentrations reached steady state, and about three months after the initial inoculation of the reactor. One week after nitrate addition, H_2S concentrations remained at the same high level, so the column was reinoculated with an actively growing batch vial containing the original North Sea consortium. About ten days after this inoculation, the disappearance of nitrate in the column was first noted. Within three more days, essentially all the nitrate was consumed, a stoichiometrically equivalent amount of nitrite was detected in the column, and the H_2S concentrations throughout the column were below the detection limit. The residence time in the column was about 20 hours.

In the North Slope #2 column, repeated nitrate addition and reinoculation did not change H_2S concentrations in the column. Only after reinoculation of the column with freshly obtained North Slope produced water (about six months after nitrate was first added) were nitrate reduction and inhibition of souring seen. Subsequently, the time course of souring inhibition was similar to that seen in the

North Sea column: once nitrate disappearance and nitrite appearance were noted, disappearance of H_2S from the column followed in nearly plug-flow fashion.

Table 3.2. H_2S concentrations at steady state in the crushed Berea sandstone columns, before and after nitrate addition. Shown are the average measurements over three days (mg S/L) with one standard error. Distance = 0 cm is the column inlet and distance = 50 cm is the column outlet. "With NO_3^- " readings were taken about one month after the addition of nitrate and subsequent appearance of nitrite.

distance (cm)	North Sea before NO_3^-	North Sea with NO_3^-	North Slope #2 before NO_3^-	North Slope #2 with NO_3^-
0	0	0	0	0
5	107 ± 3	0	175 ± 7	0
10	108 ± 3	0	179 ± 4	0
15	102 ± 5	0	175 ± 11	0
20	114 ± 3	0	184 ± 6	0
30	117 ± 5	0	181 ± 3	0
40	120 ± 7	0	122 ± 9	0
50	125 ± 3	0	97 ± 7	0

Sulfate concentrations measured in both columns (Table 3.3) agree with the H_2S data and the hypothesis that souring was completely inhibited. Approximately 200 mg S/L of sulfate was consumed under souring conditions at steady state in each column, with 125 mg S/L of H_2S produced in the North Sea column and 180 mg S/L of H_2S in the North Slope #2 column. However, after nitrate addition, there was no significant consumption of sulfate in either column ($p=0.05$), even though it was still available at high concentrations (Table 3.3).

Table 3.3. SO_4^{2-} concentrations at steady state in the porous media columns.

distance (cm)	North Sea before NO_3^-	North Sea with NO_3^-	North Slope #2 before NO_3^-	North Slope #2 with NO_3^-
0	279 ± 7	290 ± 7	242 ± 10	314 ± 6
5	135 ± 10	287 ± 7	47 ± 2	308 ± 7
10	131 ± 6	286 ± 5	45 ± 1	316 ± 7
15	136 ± 13	282 ± 5	36 ± 1	308 ± 9
20	112 ± 6	288 ± 4	36 ± 1	309 ± 4
30	78 ± 4	283 ± 3	29 ± 1	311 ± 2
40	56 ± 6	283 ± 5	44 ± 7	307 ± 3
50	74 ± 5	286 ± 7	62 ± 4	305 ± 4

Substrate utilization. Substrate consumption patterns in each column (Table 3.4) changed as the column shifted from sulfate reduction to nitrate reduction. Before nitrate addition, formate and *n*-butyrate were the preferred substrates for North Sea SRB, and *n*-butyrate was also completely consumed in the North Slope #2 column. There was a net production of acetate in both cases due, it is believed, to incomplete oxidation of propionate and *n*-butyrate and the lack of acetate utilization by SRB. Both of these phenomena were seen in single-substrate batch experiments (Mueller, 1994). After nitrate addition, small amounts of acetate, isobutyrate, *n*-butyrate and formate were consumed in the North Sea column, and acetate and formate were consumed in the North Slope #2 column. It is believed that these were the substrates supplying energy for nitrate reduction. The other carbon sources were not significantly consumed ($p=0.05$) after nitrate addition. A

smaller amount of total substrate was consumed after nitrate addition for two reasons: a lower level of electron acceptor available and consumed (50 mg N/L of NO_3^- vs. 200 mg S/L of SO_4^{2-}), and a lower requirement of electrons (eight electrons per mole for sulfate reduction vs. two electrons per mole for nitrate reduction to nitrite).

Table 3.4. Substrate concentrations at steady state in the porous media columns.

A. North Sea column

substrate	influent before NO_3^-	effluent before NO_3^-	influent with NO_3^-	effluent with NO_3^-
formate	232 ± 1	0	202 ± 2	191 ± 2
acetate	251 ± 14	410 ± 7	218 ± 2	189 ± 2
propionate	176 ± 1	37 ± 3	220 ± 2	227 ± 7
isobutyrate	183 ± 1	73 ± 3	231 ± 4	190 ± 7
n-butyrate	175 ± 1	0	204 ± 2	194 ± 3

B. North Slope #2 column

substrate	influent before NO_3^-	effluent before NO_3^-	influent with NO_3^-	effluent with NO_3^-
formate	238 ± 5	24 ± 3	190 ± 1	181 ± 1
acetate	223 ± 5	467 ± 14	164 ± 5	147 ± 1
propionate	194 ± 5	31 ± 1	157 ± 3	158 ± 1
isobutyrate	193 ± 5	67 ± 5	142 ± 1	147 ± 1
n-butyrate	163 ± 5	0	147 ± 3	139 ± 3

Nitrate reduction. Nitrate reduction occurred primarily in the first five cm of the column and stopped at nitrite (Figure 3.2). In both columns, the concentration of nitrate decreased from approximately 50 mg N/L in the influent to about five mg N/L by the first sampling port. As during sulfate reduction, therefore, most of the microbial activity occurred at the front part of the column. In the North Sea column, about 80% of the nitrate was recovered as nitrite at the first port, but 100% of the North Slope nitrate was converted to nitrite. In both columns, no significant nitrate reduction was seen after five cm but nitrite concentrations gradually decreased throughout the column. Other possible products of nitrate reduction, such as nitrous oxide, nitrogen gas and ammonia, were not determined in the analysis.

Nitrate Level Required for Inhibition

About 50 mg N/L of NO_3^- , which was biologically converted to 40-50 mg N/L of NO_2^- , completely inhibited souring when added to an actively souring column (Table 3.2). This scenario was maintained for over two months while this level of nitrate was continuously fed to both columns. When the nitrate level in the North Sea column was lowered to five mg N/L, souring was completely inhibited for another 20 days. When nitrate was entirely removed from the influent medium, however, SRB activity resumed, as indicated by the high concentrations of H_2S once again measured in the aqueous phase (Figure 3.3), and by the upward progression of black precipitate in the column (the sandstone had gradually returned to its natural brown color while souring had been suppressed for three

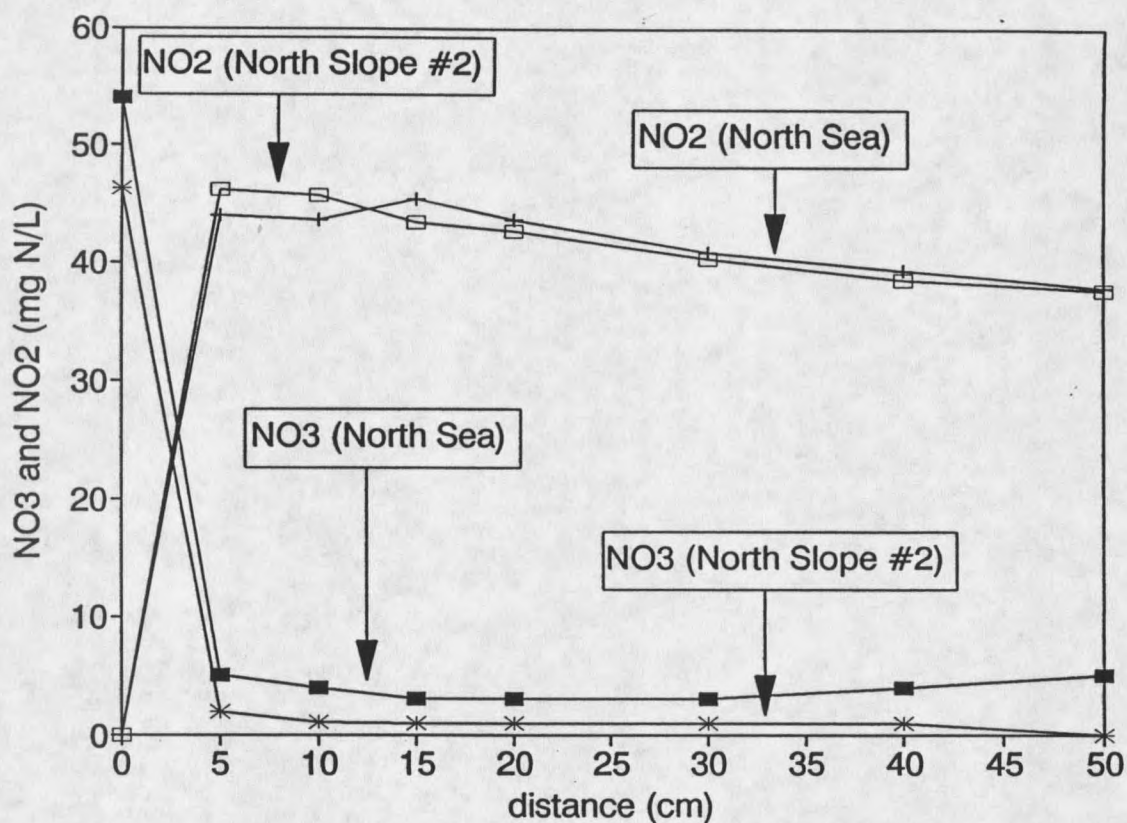


Figure 3.2. NO_3^- and NO_2^- concentration gradients in the North Sea and North Slope #2 columns at steady state. Shown are the average measurements over three days (mg N/L). Standard errors are not shown because they were very small (≤ 2 mg/L). Distance = 0 cm is the column inlet and distance = 50 cm is the column outlet.

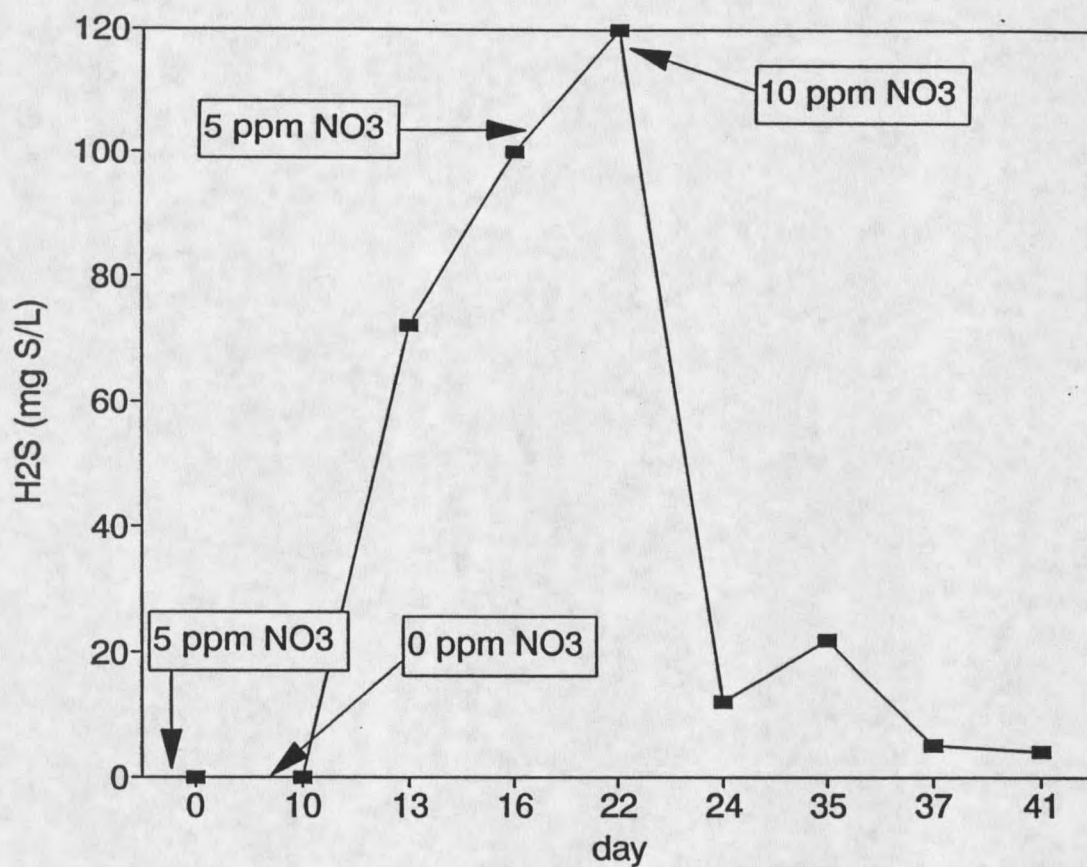


Figure 3.3. H₂S concentration in the North Sea column with different nitrate concentrations in the influent. Shown are single readings each day at distance = 5 cm.

months). When five mg N/L of nitrate was again added to the medium, nitrate was reduced to nitrite but souring was not inhibited, as the H_2S concentration remained above 100 mg S/L. When the influent nitrate level was increased to 10 mg N/L, souring was suppressed.

This last scenario was repeated by entirely removing the nitrate, then adding five mg N/L and then 10 mg N/L to the medium (data not shown). The same results were seen: souring returned in the absence of nitrate, it was not inhibited by five mg N/L of nitrate but it was completely inhibited by 10 mg N/L (and the subsequent nitrite production) and inhibition was maintained with five mg N/L. Since about 80% of the nitrate added was recovered as nitrite in the North Sea column, this suggests that approximately eight mg N/L of NO_2^- was needed to completely inhibit active souring, and that about four mg N/L of NO_2^- maintained this inhibition.

Biomass

Amount. Total cell counts were performed at each sampling port in the columns at steady state during sulfate reduction and nitrate reduction (Table 3.5). After nitrate addition, fewer cells were detected in the North Sea column. In the North Slope #2 column, about the same number of cells (possibly even higher in the effluent) were seen after nitrate addition as compared to during souring. Cell yields for SRB (sulfate-reducing bacteria) and NRB (nitrate-reducing bacteria), calculated using the same conversions from cell counts to dry biomass as shown in Chapter 2, were quite low for the consortia in both columns (Table 3.6). Under souring

conditions, the North Slope #2 consortium had slightly higher yields than the North Sea consortium. When nitrate was present, cell yields for both consortia were comparable except for a lower Y_{xe} for the North Sea, which means that fewer cells per mass of nitrate consumed were produced.

Table 3.5. Suspended cell counts (cells/ml) at steady state. Shown are averages (log base-ten) over three days.

distance (cm)	North Sea before NO_3^-	North Sea with NO_3^-	North Slope #2 before NO_3^-	North Slope with NO_3^-
0	0	0	0	0
5	7.4	6.9	7.5	7.7
10	6.8	8.0	7.7	7.6
15	7.4	6.5	7.6	7.9
20	7.3	7.0	7.6	7.2
30	7.4	6.7	7.3	7.0
40	7.5	6.9	7.1	7.1
50	6.4	5.6	6.6	7.0

The attached biomass in the North Slope #2 experiment, shown in Chapter 2, was calculated after nitrate reduction had occurred in the column. It is probably representative of the biomass present during souring, however, and not necessarily that during nitrate reduction. This column was allowed to sour, then nitrate was added, then nitrate was removed and the column was allowed to sour again before the experiment concluded and the column was cut into sections.

Table 3.6. Cell yields during souring and nitrate reduction for both consortia.

	North Sea	North Slope #2
Y_{xs} (SRB) ^a	0.0003	0.0006
Y_{xe} (SRB) ^b	0.0010	0.0013
Y_{xs} (NRB) ^c	0.0004	0.0004
Y_{xe} (NRB) ^d	0.0006	0.0013

^a = g dry biomass/g total substrate during souring

^b = g dry biomass/g SO_4^{2-} (S) during souring

^c = g dry biomass/g total substrate during nitrate reduction

^d = g dry biomass/g NO_3^- (N) during nitrate reduction

Appearance. The morphology of the cells in suspension, which originated from unknown mixed populations, changed dramatically in both columns when the environment switched from souring to nitrate reduction. During souring at 60°C, about 75% of the cells were thick rods (with a surface area of $0.9 \mu\text{m}^2$), another 20% were small cocci (with a surface area of $0.3 \mu\text{m}^2$) and the remaining five percent were large filaments (with a surface area of $12 \mu\text{m}^2$), as calculated by Mueller (1992). At steady state during nitrate reduction the appearance of the cells in suspension had changed dramatically, due to either a shift in population or a change in morphology. About 60% of the cells were small cocci, about 30% were filaments and the remainder were rod-shaped bacteria.

Chemical/Physical Phenomena

Iron sulfide. As previously mentioned, a black precipitate of iron sulfide formed in the column when H_2S was produced by SRB. During the initial growth of

SRB in the column, this iron sulfide precipitate proceeded upwards through the column at a rate of about 0.75 cm/day. As the precipitate reached each sampling port, H_2S was detected in the aqueous phase. No H_2S could be detected ahead of the front. After nitrate was added and souring was suppressed, the iron sulfide precipitate gradually disappeared and the sand returned to its original brown color. When nitrate was removed from the North Sea column after a period of three months, the sand was sufficiently light-colored to see the H_2S precipitate move up the column after six days. This time the velocity of the front was much faster, about eight cm/day.

E_h . The redox potential (E_h) of the system changed dramatically during and after souring. During active souring, the E_h of the liquid medium decreased from about -100 mV in the influent to less than -400 mV in the effluent in both columns. After nitrate addition, however, the E_h of the North Sea column rose to above zero (Figure 3.4). In this case, the lowest E_h (about -100 mV) was observed at the beginning of the column, where several mg S/L of H_2S was still present in the aqueous phase (Figure 3.5), probably due to dissolution of iron sulfide.

Other Reactor Systems

Core. Fifty mg N/L of nitrate was added to the medium supplying the Berea sandstone core system after the data described in Chapter 2 was obtained. After several weeks, no nitrate reduction was observed, so the core was reinoculated with an actively growing vial of NRB from the North Slope consortium. This also

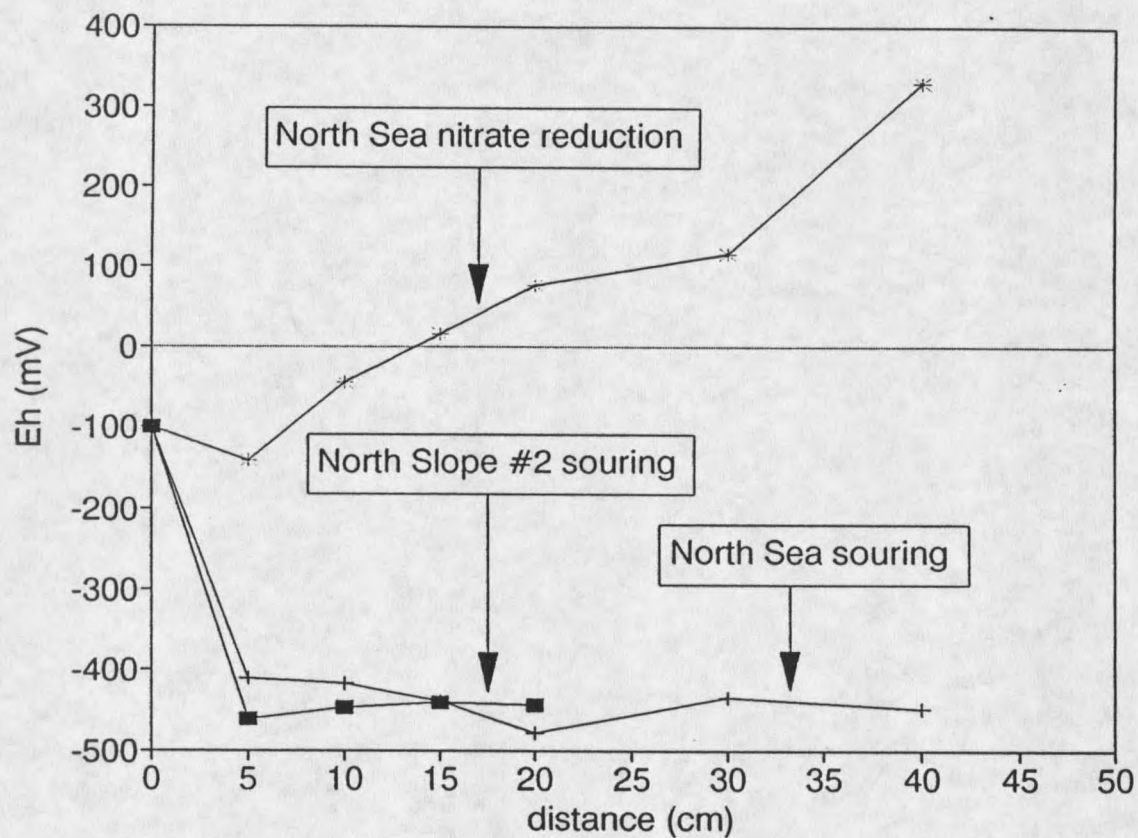


Figure 3.4. E_h gradients in the North Slope #2 and North Sea columns during souring, and in the North Sea column after nitrate addition. Single readings at each distance over the course of one day were taken for each column.

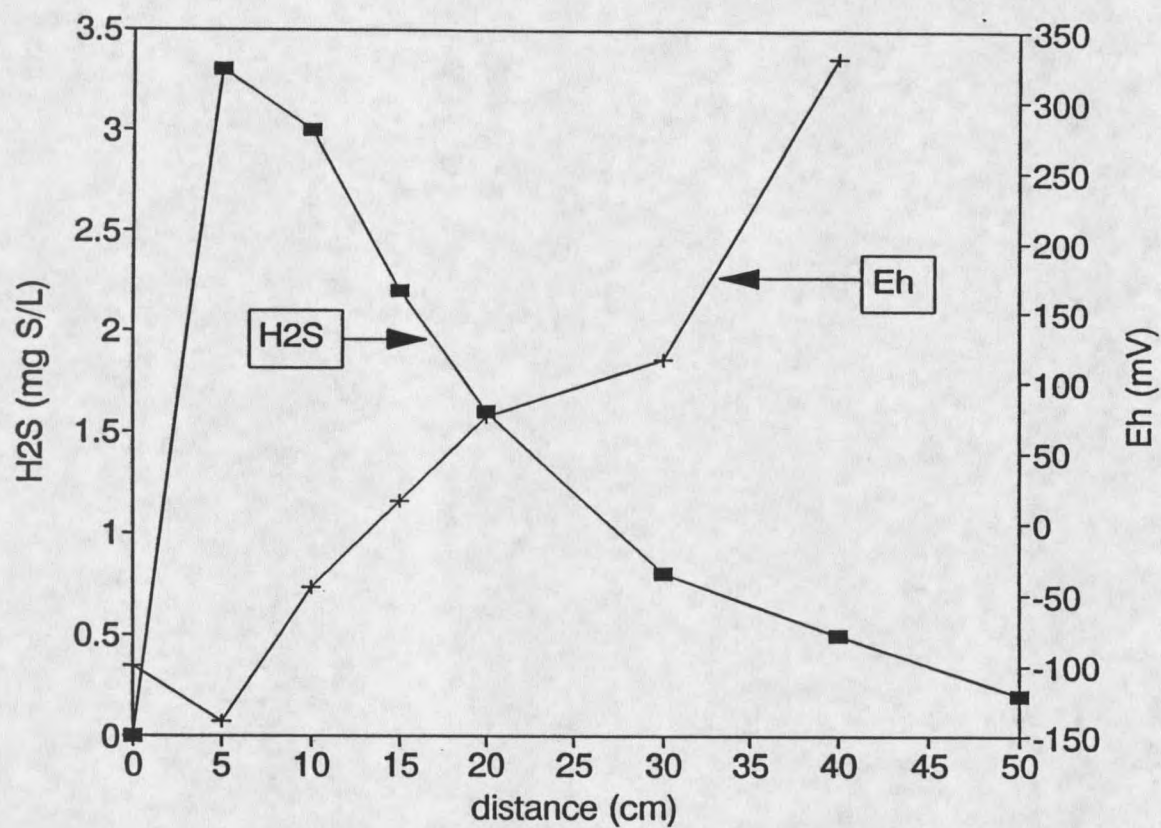


Figure 3.5 H_2S - E_h correlation in the North Sea column after nitrate addition.

failed to initiate nitrate reduction in the cores. No nitrate reduction had been attained at this time in the North Slope #2 column (from which the core inoculum had come), so these results are consistent. Only after the North Slope #2 column was reinoculated with fresh produced water was nitrate reduction seen in this column.

North Slope PW column. As with the other columns, 50 mg N/L of NO_3^- was added to the North Slope PW column, along with the standard levels of sulfate and organic acids. In this column, however, nitrate reduction did not cause immediate and complete cessation of souring. A timeline of concentrations measured in the effluent after nitrate addition began (Table 3.7) shows that souring was suppressed by over 60%, as the H_2S concentration decreased from about 160 mg S/L before nitrate addition (data not shown) to about 60 mg S/L. All of the nitrate was consumed in the column but the nitrite concentration remained at zero.

Nitrate reduction in the North Slope PW column can be better studied by examining the data at the second sampling port (10 cm). At this point, nearly all of the nitrate was already reduced and some of it was measured as nitrite (Table 3.7). The H_2S concentration at this point was rapidly reduced to below the detection limit, in contrast to nearly 200 mg S/L without nitrate (data not shown). The H_2S measured in the effluent was produced farther up the column, by SRB normally less active. Nitrite concentrations measured at port 1 varied widely from day-to-day (Table 3.7) but were sufficient to inhibit souring. The difference between the nitrate supplied and the nitrite produced was measured as ammonia nitrogen (in

approximately the proper stoichiometric amount) in several spot checks (data not shown). A steady state was never achieved with nitrate reduction in the North Slope PW column, as the H_2S and NO_2^- concentrations varied significantly in the first 20 cm.

Table 3.7. Concentrations at different distances in the North Slope PW column, after addition of 50 mg N/L of NO_3^- .

date	H_2S (50 cm)	NO_3^- (50 cm)	NO_2^- (50 cm)	H_2S (10 cm)	NO_3^- (10 cm)	NO_2^- (10 cm)
5/18/94	100	0	0	92	4	0
5/26	N.M.	0	0	68	0	0
6/6	N.M.	0	0	N.M.	2	28
6/7	N.M.	0	0	N.M.	1	11
6/8	19	0	0	0	0	3
6/9	22	0	0	0	1	4
6/10	22	0	0	0	1	0
6/16	35	0	0	0	2	31
6/17	40	0	0	0	1	31
6/20	65	0	0	0	0	0
6/22	54	0	0	0	0	41
6/30	54	0	0	8	2	7
7/1	67	0	0	13	2	1

* N.M. = not measured

North Sea column (with propionate). In the final column experiment, 100 mg N/L of NO_3^- and 200 mg/L of propionate (as the sole carbon source) were fed

into the North Sea column, once souring had been re-established. The objective was to determine whether NRB would utilize propionate, since they previously had consumed only acetate and isobutyrate when a mixture of five organic acids was present. A higher level of NO_3^- was added (100 mg/L vs. 50 mg/L) so that a larger amount of substrate could be consumed. Results from this experiment show some interesting similarities and differences compared to previous experiments. Similar results are that: 1) all or nearly all of the microbial activity occurred in the first five cm; 2) all of the nitrate was consumed and nitrite was produced; and 3) this nitrite completely eliminated H_2S production. The differences from previous experiments are significant. All of the propionate was consumed by NRB in the first five cm (Table 3.8), which was the first observation of propionate usage during nitrate reduction in these experiments. It is impossible to determine from the data whether propionate was completely oxidized by NRB to CO_2 , or whether it was incompletely oxidized to acetate (as in sulfate reduction), with the acetate subsequently taken to CO_2 .

Two more differences occur in the level of nitrite in the column. Only 20-25% of the 100 mg N/L of nitrate was measured as nitrite at the first sampling port (Table 3.8), in contrast to the 80-100% seen in the other experiments. This lower level of nitrite was still sufficient to completely inhibit souring (Table 3.8). The bulk of the nitrate must have been converted to nitrogen gas, since there was no ammonia production in the column (Table 3.8). Small decreases in ammonia concentration were probably due to the nitrogen requirement for cell synthesis. Nitrite

concentration increased slightly throughout the column, the opposite of previous results.

Table 3.8. Steady state concentrations in the North Sea column after nitrate addition, with propionate as the sole substrate.

distance (cm)	H ₂ S (mg S/L)	SO ₄ ²⁻ (mg S/L)	NO ₃ ⁻ (mg N/L)	NO ₂ ⁻ (mg N/L)	NH ₃ (mg N/L)	acetate (mg/L)	prop. (mg/L)
0	0	107	103	0	103	6	208
5	0	111	1	22.8	96	0	0
10	0	113	0	24.0	99	0	0
15	0	109	0	25.1	98	0	0
20	0	117	0	26.3	99	0	0
30	0	119	0	30.8	96	0	0
40	0	115	0	31.2	95	3	0
50	0	117	0	35.8	92	0	0

Before the experiment started, it was thought that perhaps SRB would consume propionate at the beginning of the column, thereby producing acetate, the preferred substrate for NRB. The column would then have a souring zone (probably small with fairly low levels of H₂S), followed by a nitrate-reducing zone with no souring. This did not happen, however, as the NRB directly utilized propionate at the beginning of the column.

Discussion

Addition of nitrate to a souring column can completely inhibit souring and nitrite will usually be present in nearly stoichiometric proportions to the nitrate added. Halting nitrate addition allows souring to resume, even after souring has been completely inhibited for over three months. This, along with data to be presented in Chapter 4, indicates that SRB are being inhibited and not killed by the nitrate/nitrite.

Consortia Comparison

Several similarities were seen between the North Sea and North Slope consortia. In both columns, the majority of the souring, and then the nitrate reducing activity, was in the first five cm (first 10%) of the column. Other similarities between the two columns were the effect of NO_2^- on both consortia, the yields during souring and nitrate reduction, the substrate preference during souring (formate and *n*-butyrate) and nitrate reduction (acetate), the decrease in NO_2^- concentration seen throughout the columns, and the change in cell morphology between souring and nitrate reduction.

Several differences were also seen between the North Sea and North Slope consortia. Nitrate reduction was much easier to start in the North Sea column, although once it was started in the North Slope column, it seemed to behave very similarly. Slightly different substrates also seemed to be preferred. The North

Slope SRB did not consume as much formate as the North Sea SRB, and the North Slope NRB did not consume as much isobutyrate as the North Sea NRB.

Electron Balances

Electron balances, similar to those calculated during souring in Chapter 2, were performed with data from the columns during nitrate reduction. Concentrations between the influent and the first sampling port were compared, instead of between the influent and the effluent, for two reasons: 1) most of the activity occurred before the first port, and 2) doing so avoided the complication of having to account for some nitrite reduction after the first port, which may be biological or chemical.

An electron balance on the North Sea column, where acetate and isobutyrate served as the electron donors and nitrate as the electron acceptor, was 87% (87% of the electrons donated were accepted by nitrate). This seems very good, given the small amounts of substrate utilized compared to the possible measurement error. An electron balance over the North Slope #2 column, however, was over 300% off, probably due to the variable organic acid data. For the North Sea column using propionate as the sole carbon source, 78% of the electrons were accounted for in nitrate reduction, which is a very satisfactory result.

Partial versus Complete Denitrification

From a thermodynamic standpoint, the most favorable reaction for nitrate is to be reduced to nitrogen gas (Table 3.1), followed in order by reduction to nitrite

or reduction to ammonia. It is unclear why, in the majority of the systems used, the nitrite produced from nitrate did not proceed to nitrogen gas or, if it did, proceeded very slowly (a maximum of 20% was reduced to N_2 in about 20 hours, the residence time of the column). It may take time for the nitrite reductase enzyme to develop in the NRB, longer than the three months run in nitrate-reducing conditions in these experiments. Effects such as temperature, pH or the initial consortium present also may play a role in which type of nitrate reduction occurs.

Other Experimental Phenomena

Yields. The yields reported for both consortia are fairly low. It may be that neither column was yet at steady state with respect to suspended cells in the effluent, since more cells were possibly still being trapped in the sand and biofilm matrix in the column than were detaching into suspension. These yields (Table 3.6) are about an order-of-magnitude lower than the yields reported by Mueller in single-substrate batch experiments (1994). He reported yields of 0.0053-0.0101 g dry biomass/g substrate for North Slope and North Sea SRB on *n*-butyrate and formate, and yields of 0.0132-0.0413 g dry biomass/g S of sulfate. No yields for the NRB from either consortium were calculated in batch experiments. The yields for NRB in the column may also be slightly in error because it was assumed that they had the same average cell size as SRB. The majority of the SRB were rods, while the majority of NRB were cocci (smaller than rods), and a considerable minority were filaments (larger than rods).

Iron sulfide. Once nitrate was removed from the columns, iron sulfide precipitation returned very quickly. One possible reason for this order-of-magnitude increase is that due to wash-out caused by many pore volumes of water flow, less iron was available in the column to react with H_2S and precipitate it as iron sulfide (it is known that unbound iron was initially present in the sand). The more likely explanation is that an SRB biofilm was now distributed throughout the column, so that H_2S could be generated throughout the column after the inhibitor was removed, instead of H_2S being generated only at the bottom of the column and slowly flowing upward (and being retarded through reaction).

Nitrate reduction in the medium. Another result which must be discussed is the phenomena of nitrate reduction in the medium versus in the column. On several occasions during nitrate addition (in all columns), the anaerobic medium became contaminated and nitrite was detected at the column inlet. During these periods, sulfate reduction remained at zero but further nitrate reduction also went to zero. Any unreacted nitrate in the medium remained unreacted throughout the column. This suggests that nitrite is not only inhibitory to SRB but also to NRB. It may be that previously observed nitrate reduction occurred so rapidly in the beginning of the column that the produced nitrite did not have time to inhibit the NRB (the columns approximated plug-flow reactors, as will be shown in Appendix 1). When nitrite was already present in the influent, however (probably produced by a mesophilic species of NRB, since the medium was at room temperature), thermophilic nitrate reduction could not occur in the column. Whenever

contamination was observed, fresh medium was prepared and nitrate reduction in the column soon returned. Sulfate reduction was inhibited at all times.

Comparison to Previous Work

Several mechanisms can possibly explain the cessation of H_2S production seen in these experiments. The first is that H_2S may be used as an electron donor by nitrate-reducing bacteria, as shown by McInerney et al. (1992) and explored further by Hitzman et al. (1994). This is very unlikely, as no SO_4^{2-} was consumed by either consortium after nitrate was added (Table 3.2). If there had been sulfate consumption concomitant with no sulfide appearance, this could mean that souring was not completely inhibited, but that the produced H_2S was merely being consumed biologically, or that it was chemically reacting with another species in the column. Both these scenarios can be ruled out based on the sulfate data.

Successful competition for electron donor (organic acids) by NRB is another explanation for the cessation of H_2S production and SO_4^{2-} reduction. Results show that large quantities of the preferred substrates for SRB (formate and *n*-butyrate) were still available after nitrate addition (Table 3.3). "Competitive removal" of organic acids was suggested by Hitzman (1994) as the reason for cessation of souring after nitrate addition, but that was definitely not the case in these studies.

It is also possible that the microbial activity on nitrate was due to SRB, not DNB, as has been suggested by several researchers (Dalsgaard and Bak, 1994; Jenneman et al., 1986). Some SRB preferentially use nitrate when both sulfate and

nitrate are present because of the greater energy available from nitrate, but the end product is usually NH_3 , not NO_2^- . In fact, Hitzman measured no NO_2^- in his system. Further, Dalsgaard and Bak noted that when both sulfate and nitrate were present, only SO_4^{2-} was reduced, except when the sulfate was at very low concentrations. When the sulfate concentration was greater than 16 mg S/L (as it was here), Dalsgaard and Bak noted no NO_3^- reduction. This work also noted that H_2S inhibited growth on NO_3^- (four mg/L of H_2S inhibited nitrate reduction by 50%). In the systems studied here, over 100 mg S/L of H_2S was present when nitrate reduction began. It is possible that SRB system reduced NO_3^- to NO_2^- , without proceeding to NH_3 . If this was SRB activity it was either a species different from those performing sulfate reduction (since this was simultaneously inhibited), or the same species underwent dramatic physiological changes, since the cell morphology changed so drastically between souring and nitrate reduction.

A fourth possible mechanism is the inhibition of SRB by nitrous oxide (N_2O). Jenneman et al. (1986) measured NO_3^- , NO_2^- and N_2O after nitrate addition inhibited sulfate reduction and suggested that N_2O had a "cytotoxic effect" on SRB by raising the E_h . E_h did rise in this system after nitrate addition (Figure 3.4) but it is doubtful that this was due to the presence of nitrous oxide. At the beginning of the column, where the bulk of the SRB activity occurred and where the greatest inhibition was thus required, nearly all of the nitrate added to both columns was measured as nitrite. This leaves very little nitrogen to be converted to nitrous oxide. It is also doubtful whether the nitrite directly affected the E_h , as Jenneman et al. state

"addition of large amounts of nitrite to a reduced medium did not affect the redox state." Data from Mueller (1994) shows that addition of NO_2^- (as little as five mg N/L) to souring batch vials increased the E_h from -390 to -30. However, this may not mean that nitrite directly affected the E_h . Nitrite may have reacted with H_2S in the system, thereby increasing the E_h . This would also explain the higher E_h seen with low H_2S concentrations after nitrate addition in the North Sea column (Figure 3.5).

The last and most plausible mechanism is that nitrite directly inhibited SRB activity, probably due to its reaction with cellular iron or on such cellular sites as enzymes, enzyme cofactors, nucleic acids and cellular membranes (National Academy of Sciences, 1981). Detailed microbiological studies would be needed to verify this hypothesis and determine the exact mechanism, but all the evidence certainly points to direct inhibition of SRB by nitrite. Experiments involving nitrite addition and its possible mechanisms will be discussed in Chapter 4.

Many researchers have shown that nitrate will inhibit sulfate reduction in some manner and several have done this work in porous media systems, but most have required much higher concentrations and lower temperatures than used in my studies. McInerney et al. (1992) found that *Thiobacillus denitrificans* strain F (which oxidizes H_2S to SO_4^{2-} and reduces NO_3^- to N_2), when combined with an increased hydraulic residence time of the system, will lower the H_2S concentration up to 98% at 30°C. However, they added about 560 mg N/L of NO_3^- . Jenneman et al., in batch studies, inhibited H_2S production for over six months but required 826 mg N/L

of NO_3^- (1986). 280 mg N/L only temporarily inhibited H_2S production. Montgomery (1990) reported another requirement of *T. denitrificans* strain F that clearly differentiates it from the behavior in this work: "Temperatures in excess of 40°C are totally inhibitory to growth." Columns here were operated at 60°C .

Another difference from previous research is that all reported microbial activity was done by native mixed consortia. Studies of nitrate addition by Hitzman (1994), McInerney (1992), Jenneman (1986), and Montgomery (1990) were all done with added *T. denitrificans*, either as part of two-species cultures or to supplement a native microbial population. In the North Slope column, the column had to be reinoculated to achieve nitrate reduction but this was done using a microbial population from the same source. The North Sea column was also reinoculated with another batch vial grown with the initial North Sea consortia—it is not known whether this is what was effective in initiating nitrate reduction or whether the original consortia had not yet started to grow.

Conclusions

A concentration of ten mg N/L nitrate was required to completely inhibit active souring in crushed Berea sandstone columns but could be lowered to five mg N/L to maintain inhibition. Nitrite was produced at a level 80-100% of the nitrate added and the nitrite concentration decreased slowly throughout the column. As to be shown more conclusively in Chapter 4, nitrite appears to be the active inhibitory agent for SRB and the same degree of nitrite production was seen with

both microbial consortia tested. As with souring, most of the microbial activity occurred in the first 10% of the column. Data indicates that nitrite also inhibits NRB activity.

Of the substrates studied, acetate was preferred by NRB, with isobutyrate consumed to a lesser degree. Propionate was also consumed by NRB in one consortium in the absence of acetate and isobutyrate. Cell yields for NRB were comparable to those for SRB but a very different cell morphology was observed under nitrate-reducing conditions. The electron transfer during nitrate reduction can be accounted for in a balance between the amounts of substrate and nitrate consumed. It was also shown that in at least one system, nitrite was further reduced to ammonia and only partial inhibition of souring occurred. When souring was inhibited, iron sulfide precipitates slowly disappeared but appeared rapidly (much faster than during initial souring) when the nitrate was removed. It was also shown that either souring or nitrate-reducing activity could be initiated in the column after long absences (without the reinoculation of bacteria) simply by the removal or addition of nitrate. With both consortia, the levels of nitrite production and sulfide inhibition remained unchanged after up to three months of continuous nitrate addition.

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CHAPTER 4

NITRITE ADDITION TO A SOURING COLUMN

Introduction

It has been shown (McInerney et al., 1992; Hitzman and Sperl, 1994) that addition of nitrate (NO_3^-) to a souring environment may reduce H_2S production by sulfate-reducing bacteria (SRB). In the experimental system studied by McInerney et al., it was found that introduction of *Thiobacillus denitrificans* (strain F) and an increased hydraulic retention time were important factors in suppressing souring (up to 98% reduction of the original H_2S concentration). Recent work in our lab suggests that nitrite (NO_2^-) produced from nitrate reduction serves as the inhibitory agent for SRB growth and subsequent H_2S production, as shown in Chapter 3. In laboratory experiments, indigenous mixed microbial populations from both the North Sea and North Slope oilfields were stimulated through the addition of nitrate to eliminate souring.

However, there may be situations when promoting nitrate-reducing activity to produce nitrite will not effectively inhibit SRB activity. At certain temperatures and pH values, nitrate-reducing bacteria (NRB) may not be present, may be unable to utilize nitrate to produce nitrite, or may continue the denitrification process from nitrite on to nitrogen gas or ammonia (neither of which inhibit sulfate reduction). Nitrate addition may also fail to control souring due to transport limitations in supplying the correct nutrients to nitrate-reducing bacteria. Such transport

requirements and microbial interactions are often poorly understood or characterized in an underground oil reservoir; consequently, a less complicated system to control microbial souring may be desired or required in many instances.

A simpler approach would be direct addition of nitrite as an inhibitory agent. The antimicrobial effects of nitrite were first recognized in the late 1920's and nitrite is currently added to many cured meat products to inhibit a wide variety of organisms (National Academy of Sciences, 1981). Nitrite especially inhibits anaerobic and aerobic spore-forming bacteria, and nitrite effects are generally greater under anaerobic conditions. Direct data on the toxicity of nitrite is limited but it is known to be mutagenic in microbial systems, while not being a direct carcinogen.

In this study, inhibition of microbial souring in porous media was studied by the direct addition of nitrite. A microbial consortium obtained from an oil reservoir was grown in an anaerobic upflow porous media reactor operated at high temperature under souring conditions, using the main organic acids found in reservoir waters as carbon and energy sources. The inhibitory action of nitrite was compared to control achieved by conventional biocide treatment using glutaraldehyde.

Glutaraldehyde (glutaric dialdehyde) is a highly toxic, corrosive chemical often used as a biocide in oilfield applications. Commercial glutaraldehyde solutions normally consist of free aldehyde and impurities of polymers, but only the free aldehyde groups have high biocidal activity. In test systems studied by

Ruseska et al. (1982), 100 ppm glutaraldehyde was somewhat effective in lowering H_2S production by sessile organisms (an undefined mixed population from an oilfield), as SRB counts were reduced by about 70% after 20 days of daily treatment. Glutaraldehyde, which is normally added in slugs or pulses to injection water in an oilfield, was added to Ruseska's system for six hours a day.

In an experiment by Grab and Theis (1993), 100 ppm glutaraldehyde was very effective against planktonic SRB (a pure culture of *Desulfovibrio desulfuricans*), and equally effective against sessile organisms (a three-log reduction in bacterial numbers). It was found in another lab experiment by these authors that 220 ppm almost completely killed a mixed-population biofilm from the North Slope, and that the presence of H_2S had no effect on glutaraldehyde efficacy. When used in a field study, 100 ppm glutaraldehyde was very effective in reducing SRB cell counts, Grab and Theis reported.

Eagar et al. (1988) experimented with pulses of glutaraldehyde at different concentrations, testing their effectiveness against *Pseudomonas fluorescens*. They found that the minimum "cidal" concentration (MCC) of glutaraldehyde for planktonic organisms was less than 25 ppm, the MCC for dispersed cells (taken from the biofilm) was between 25 and 50 ppm, and the MCC for sessile cells was between 100 and 200 ppm. The authors noted that organisms did recover between pulses.

An experiment was performed to determine glutaraldehyde's effectiveness in reducing H_2S concentrations. Its effectiveness, and the recovery of SRB after

removal of the inhibitory substance, were compared to parallel experiments using nitrite.

Materials and Methods

Reactor System

The same anaerobic upflow porous media reactor system described in Chapter 2 was used for this study. The successful method employed to suppress souring was addition of a nitrite stock solution through a Masterflex peristaltic pump. The solution was prepared by adding potassium nitrite (Sigma) to Nanopure™ water in a 100-ml stoppered vessel, then autoclaving. A 24-hour timer (Fisher Scientific, cat. no. 06-662-12) was used to control the nitrite addition rate by adding a solution of 1200 mg nitrogen as nitrite per liter (hereafter referred to as "mg N/L of NO_2^- ") for a specified number of minutes at four-hour intervals. Two schemes of nitrite addition with the peristaltic pump were used--pulse addition and continuous addition.

Pulse addition of nitrite was achieved by operating the nitrite pump for five minutes (at a rate of 11 ml/hr) at four-hour intervals. This resulted in a time-averaged concentration of 10 mg N/L of NO_2^- over the four-hour time period but a pulse of 480 mg N/L over the first five minutes at the column inlet. Continuous addition of nitrite (10, 12 and 14 mg N/L) was achieved by inserting a 250-ml flask for the mixing of the nitrite stock solution and the anaerobic medium directly ahead of the column inlet. This system, which provided a mixing time of almost ten hours,

avoided the problem of nitrite reacting with the medium over extended periods of time (as in the first unsuccessful method for nitrite addition), yet provided enough time to dampen the nitrite pulse. Nitrite concentrations using this continuous addition method were very consistent over time throughout the column, with no observable pulse at the first sampling port located five cm up the column (data not shown). Continuous addition of nitrite to the Berea sandstone core system was achieved by adding potassium nitrite to the medium. Continuous addition was used in the glutaraldehyde experiments, as glutaraldehyde (50% w/w, Aldrich) was thawed and added directly to the medium supplying the porous media column.

Bacterial Consortia and Cultivation

Produced water from the Ninian North Sea oil field was used as the microbial consortium. The same inoculation procedure and liquid medium (containing sulfate and five organic acids) as described in Chapter 2 were used in these experiments.

Analytical Methods

Aqueous hydrogen sulfide, sulfate, nitrite and organic acids were analyzed by the methods described in Chapters 2 and 3. E_h was measured by the methods described in Chapter 2.

Results

A column inoculated with the North Sea mixed consortium was allowed to sour, then nitrite was added, either continuously at varying concentrations or in

pulses. Later, glutaraldehyde was continuously added to compare its effectiveness in inhibiting souring to that of nitrite.

Nitrite Addition

Column experiments. It was seen in Chapter 3 that as little as 10 mg N/L of NO_2^- produced *in situ* by nitrate-reducing bacteria can completely inhibit SRB actively growing in a column. It was observed in the North Sea column that a pulse of nitrite (480 mg N/L but an average of only 10 mg N/L over a four-hour time interval) achieved the same degree of souring suppression as 10 mg N/L of nitrite produced by NRB in the experiments detailed in Chapter 3.

Sulfate reduction was lowered to zero by the pulsed nitrite addition (Figure 4.1), as a statistically insignificant amount of H_2S was produced at steady state. There was also no consumption of organic acids (data not shown), since there was no biological activity.

When 10 mg N/L of NO_2^- was added continuously, however, souring activity was not completely suppressed. As much as 15 mg S/L of H_2S was seen in the column (Figure 4.1). While this is 90% less H_2S than seen during active souring without nitrite, it is more than seen during pulse addition, which delivered the same amount of nitrite on a four-hour basis. The H_2S concentration decreased throughout the column in this experiment, as did the nitrite concentration (Figure 4.2).

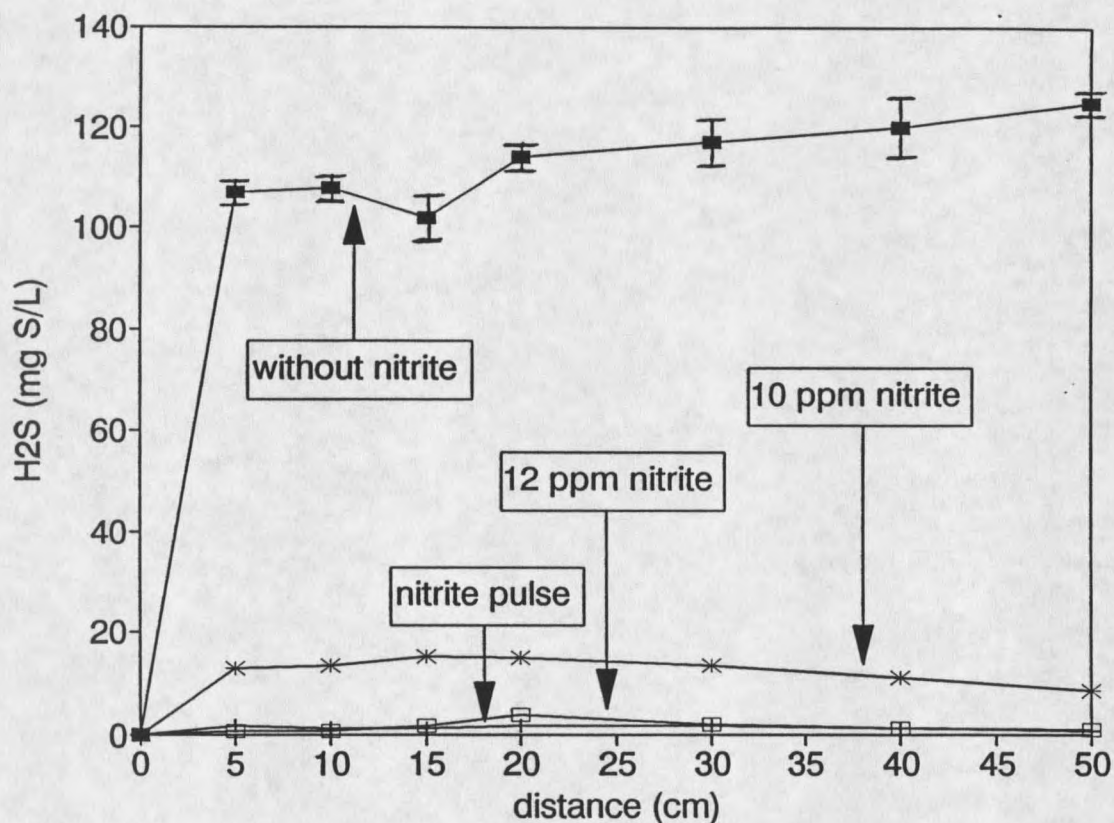


Figure 4.1. H₂S concentrations throughout the North Sea column at steady state with different levels and methods of nitrite addition. Shown are the average measurements over three days (mg S/L) with one standard error. Standard errors after nitrite addition are not shown because they were very small (≤ 1 mg/L). Distance = 0 cm is the column inlet and distance = 50 cm is the column outlet.

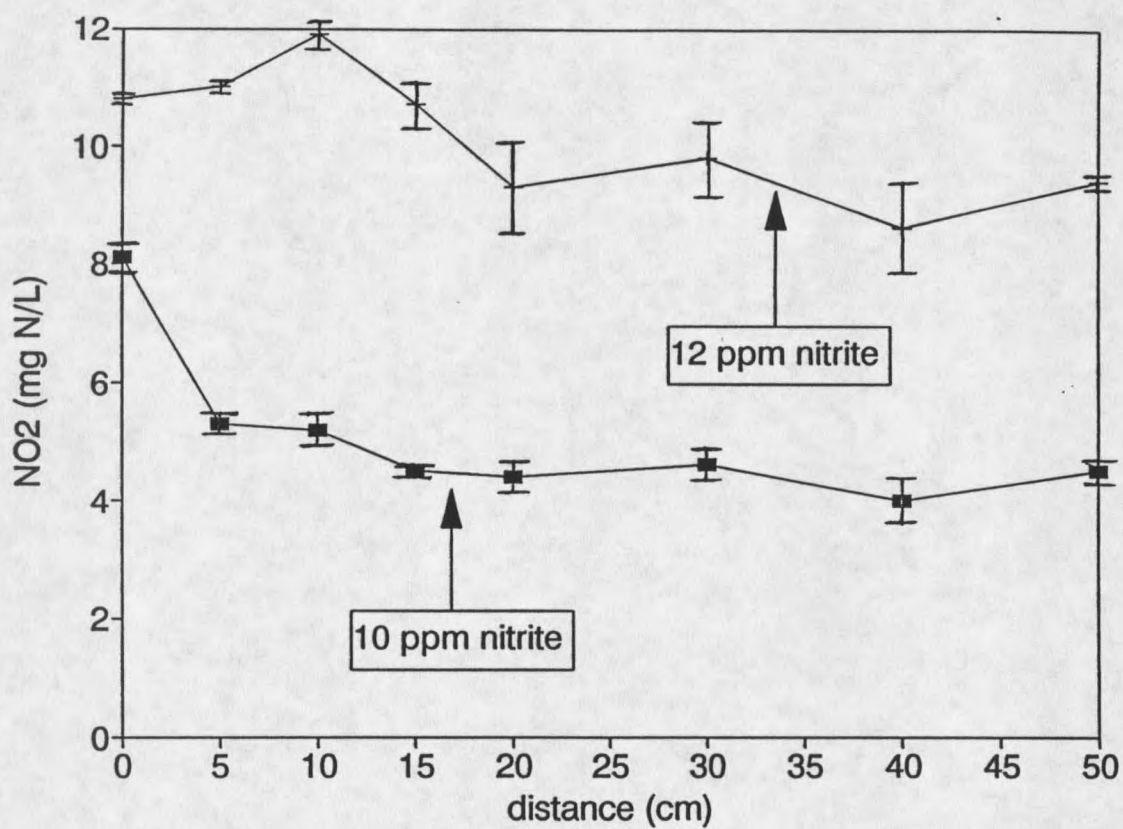


Figure 4.2. Nitrite concentration profiles throughout the North Sea column at steady state during souring inhibition with nitrite. Shown are the average measurements over three days (mg/L) with one standard error.

Organic acid profiles during the continuous addition of 10 mg N/L of NO_2^- , as seen in Table 4.1, show the dramatic decrease in substrate consumption compared to the actively souring case. *n*-Butyrate and formate were still the preferred substrates for SRB during nitrite addition but at much lower consumption rates than during active souring. An electron balance based on this lower quantity of substrates used for sulfate reduction predicts the consumption of 22 mg S/L of SO_4^{2-} and the subsequent production of 22 mg S/L of H_2S . Previous work (Chapter 2) indicated that only about 60% of the substrate electrons consumed in this reactor system could be accounted for by sulfate reduction. Thus, only about 60% of 22 mg/L (13 mg/L) would be expected, which agrees very well with the 15 mg S/L of H_2S measured in the column.

Table 4.1. Organic acid concentrations in the North Sea column at steady state after addition of 10 mg N/L NO_2^- . Shown are the means and standard errors from four samples.

	<u>influent</u> <u>with NO_2^-</u>	<u>effluent</u> <u>with NO_2^-</u>	<u>influent</u> <u>before NO_2^-</u>	<u>effluent</u> <u>before NO_2^-</u>
acetate	143 $\pm$ 2	158 $\pm$ 2	251 $\pm$ 14	410 $\pm$ 7
propionate	147 $\pm$ 4	137 $\pm$ 5	176 $\pm$ 1	37 $\pm$ 3
isobutyrate	146 $\pm$ 4	148 $\pm$ 5	183 $\pm$ 1	73 $\pm$ 3
<i>n</i> -butyrate	152 $\pm$ 4	132 $\pm$ 4	175 $\pm$ 1	0
formate	144 $\pm$ 9	119 $\pm$ 7	232 $\pm$ 1	0

When the continuous addition of NO_2^- was raised to 12 mg N/L, the H_2S concentration was lowered to less than five mg S/L. This reduced sulfate reduction

rate had two effects: lower H_2S concentrations in the column (Figure 4.1) and a smaller decrease in NO_2^- concentration throughout the column (Figure 4.2).

When the continuous addition of NO_2^- was raised to 14 mg N/L, H_2S concentration throughout the column was less than two mg S/L (data not shown), the same result as pulse injection of nitrite. At nitrite addition rates of 12 mg N/L and 14 mg N/L, organic acid concentrations were not measured, since there was little or no sulfate reduction to consume these substrates.

Measurements of reduction-oxidation potential (E_h) in the column are shown in Figure 4.3. SRB require an E_h value of about -100 mV to survive and become active (Postgate, 1984). When H_2S is produced, this further lowers the E_h of the environment. The anaerobic medium used in the column contains small amounts of $\text{Na}_2\text{S} \cdot 9 \text{H}_2\text{O}$ and other reducing agents, and has an E_h reading of about -100 mV. The E_h of the souring system dropped to below -400 mV in regions where high concentrations of H_2S were present (Figure 4.3), as E_h is negatively correlated (R-squared value = 0.98) with the H_2S concentration (shown in Figure 4.1). The same profile of E_h vs. distance in the column occurred while 10 mg N/L of NO_2^- was added (Figure 4.3), with the E_h being about 100 mV higher while souring was inhibited.

Core experiments. At the conclusion of the Berea sandstone core experiment, nitrate was added but no nitrate reduction was seen, as shown in Chapter 3. Nitrite was then added to the medium to determine its effectiveness in inhibiting souring in this system. One day after adding seven mg N/L of NO_2^- (in the form of potassium nitrite) to the medium, the H_2S concentration dropped to zero

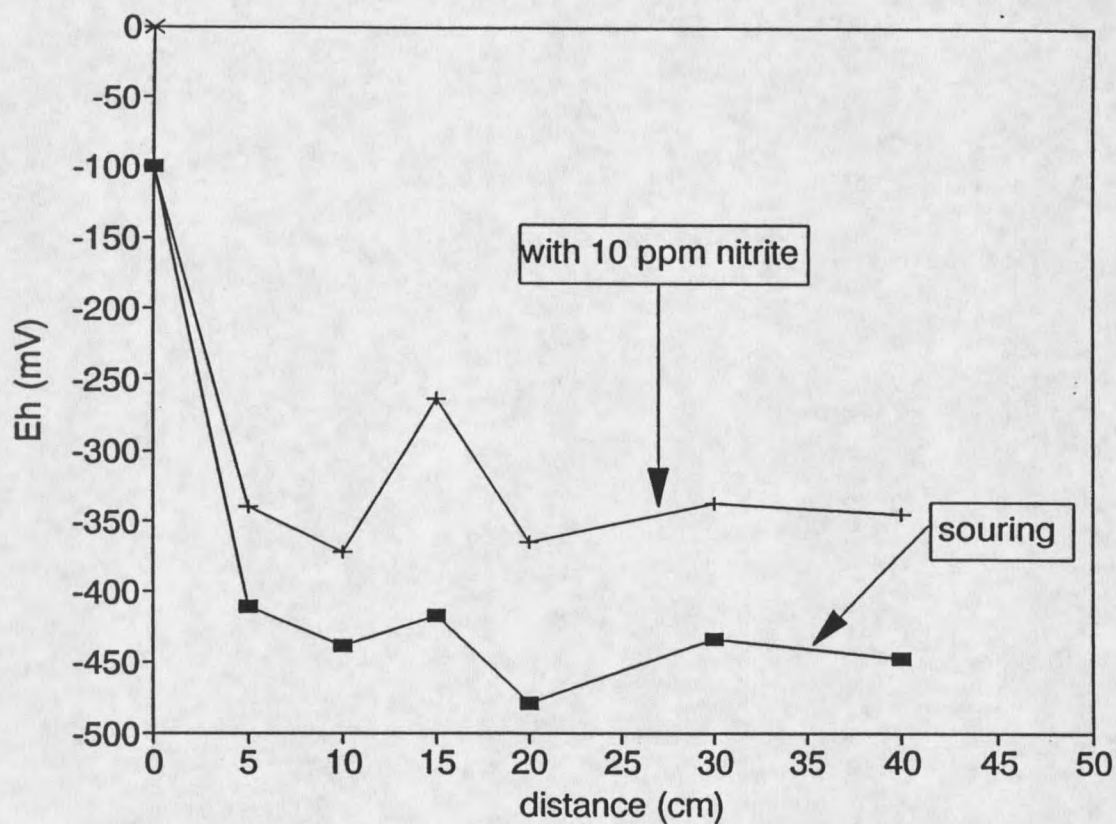


Figure 4.3. Redox potential (E_h) throughout the North Sea column at steady state, before and after addition of 10 mg N/L nitrite. Single readings at each distance over the course of one day were taken for each condition.

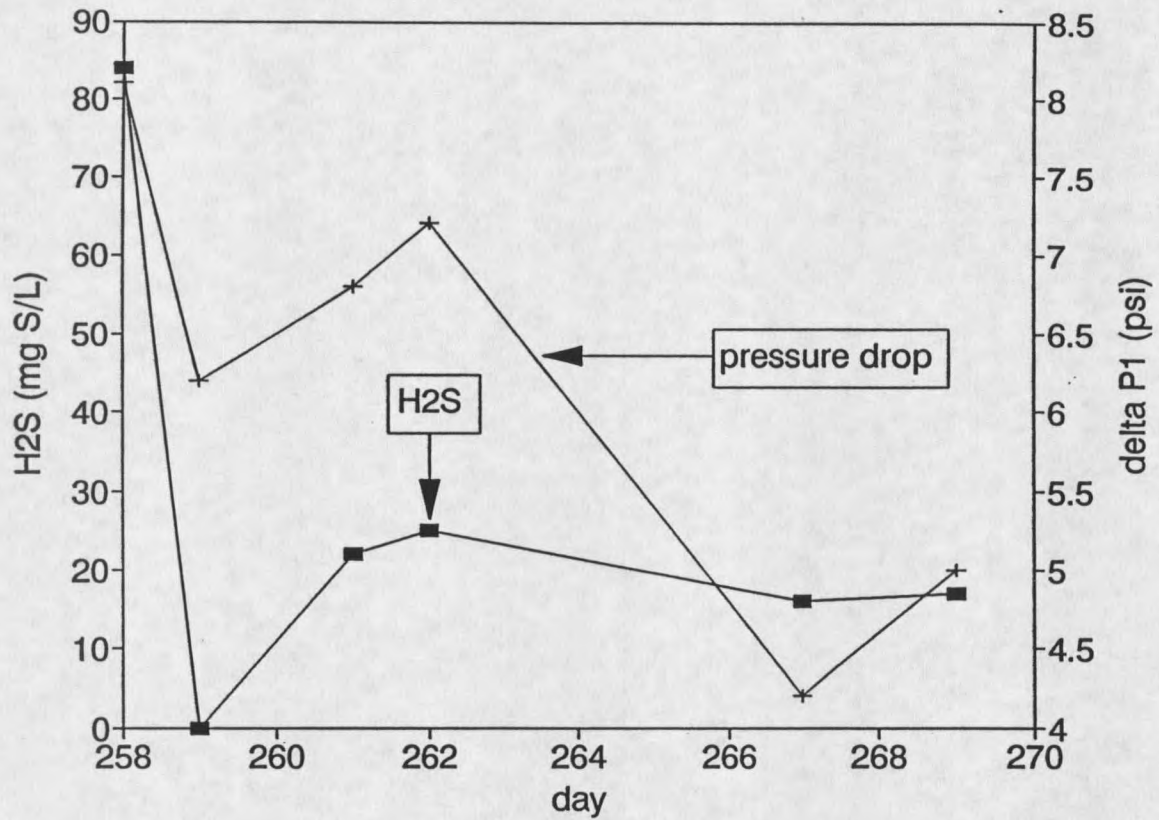


Figure 4.4. H_2S concentration and pressure drop in the first core after addition of seven mg N/L nitrite.

Figure 4.4). SRB recovered slightly in the following days while NO_2^- was continuously added, but the H_2S concentration remained around 20 mg S/L, 25% of the pre-nitrite level.

Nitrite addition also had a significant effect on the pressure drop across the first core. Pressure drop decreased from about eight psi to 6-7 psi after several days, and dropped further to 4-5 psi after another week of nitrite addition (Figure 4.4), indicating a doubling of permeability in the first core with nitrite addition.

Glutaraldehyde Addition

Initially, glutaraldehyde was added to the souring column at a level of only 10 ppm, with the idea that it may be more effective than seen in other studies due to the known thin biofilm ($4\ \mu\text{m}$) in this system. However, after five days, H_2S production was not inhibited anywhere in the column (data not shown). Glutaraldehyde concentration could not be measured with a colorimetric method (Hajdu and Friedrich, 1975) due to interferences from the medium, but it is unlikely that it decreased below 10 ppm due to reaction with H_2S or another species in the column (Grab and Theis, 1993). Consequently, the influent glutaraldehyde concentration was increased to 100 ppm. An immediate effect on H_2S production was seen at the first sampling port, while a delayed, more gradual inhibition effect was seen at the last sampling port (Figure 4.5). Glutaraldehyde was added to the column for two weeks, then the medium was replaced with fresh, glutaraldehyde-free medium (also without nitrate or nitrite). At the second sampling port, H_2S

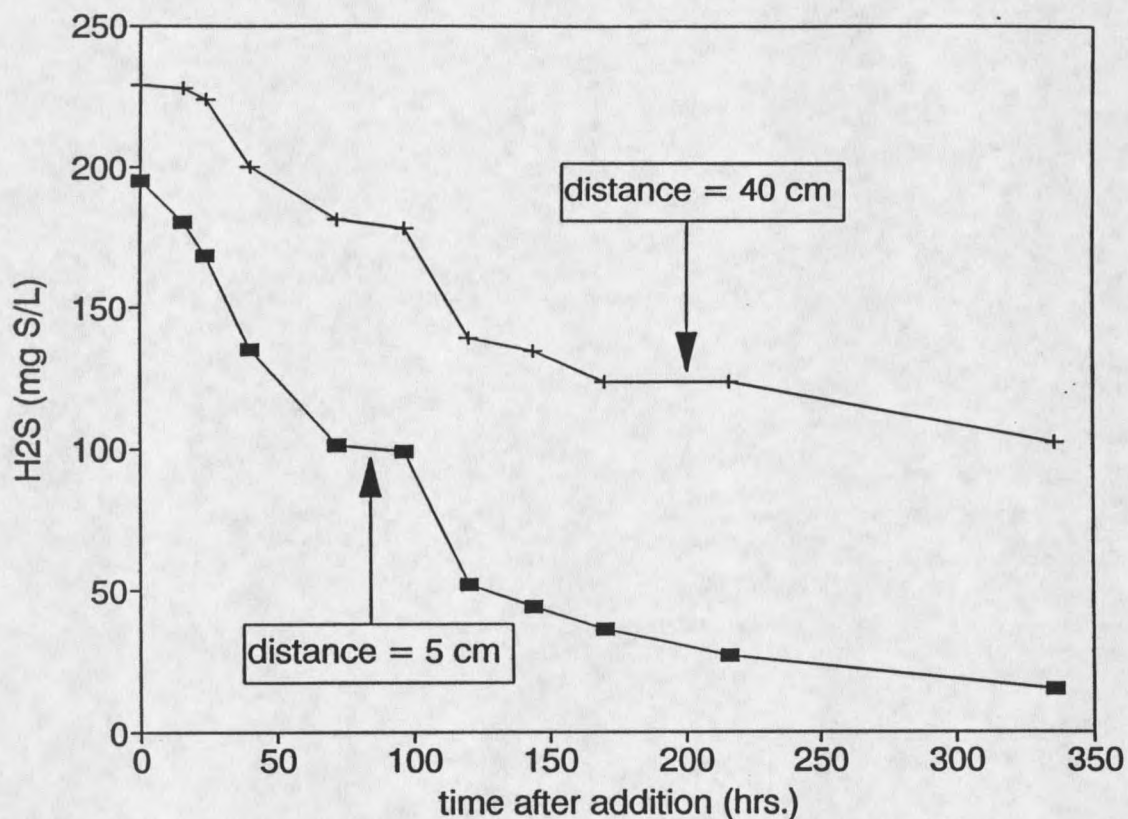


Figure 4.5. H₂S concentration in the North Slope PW column after addition of 100 ppm glutaraldehyde. Shown are single readings at each time interval at distances of 5 and 40 cm.

production immediately resumed at a rate equal to the initial SRB growth in the column (Figure 4.6). After two weeks, the column had recovered to approximately the same souring steady state seen before glutaraldehyde addition, with 140 mg S/L of H_2S at the first sampling port.

At this point, continuous addition of 50 mg N/L of nitrite was begun. At the first sampling port, H_2S production was reduced to less than five mg S/L within one day, a much quicker and more effective inhibition than with glutaraldehyde (Figure 4.7). Nitrite addition was stopped after three weeks and subsequent recovery at the second sampling port was much slower compared to glutaraldehyde (Figure 4.6). After six days of partial inhibition, H_2S production rapidly increased to the levels seen before nitrite or glutaraldehyde addition.

Discussion

Amount of Nitrite Required for Inhibition

In the column reactor, it was shown that both a pulse of 480 mg N/L of NO_2^- and continuous addition of 12 mg N/L of NO_2^- or greater completely stopped active H_2S production (99% or greater inhibition compared to the actively souring case without nitrite), and maintained this inhibition. Continuous addition of 10 mg N/L of NO_2^- significantly inhibited H_2S production, lowering it by about 90%, but did not completely inhibit souring. In the case of pulse addition, the actual nitrite concentration reaching the microorganisms in the column was significantly less than 480 mg N/L (as seen in Figure 4.8), due to diffusion, mechanical dispersion and

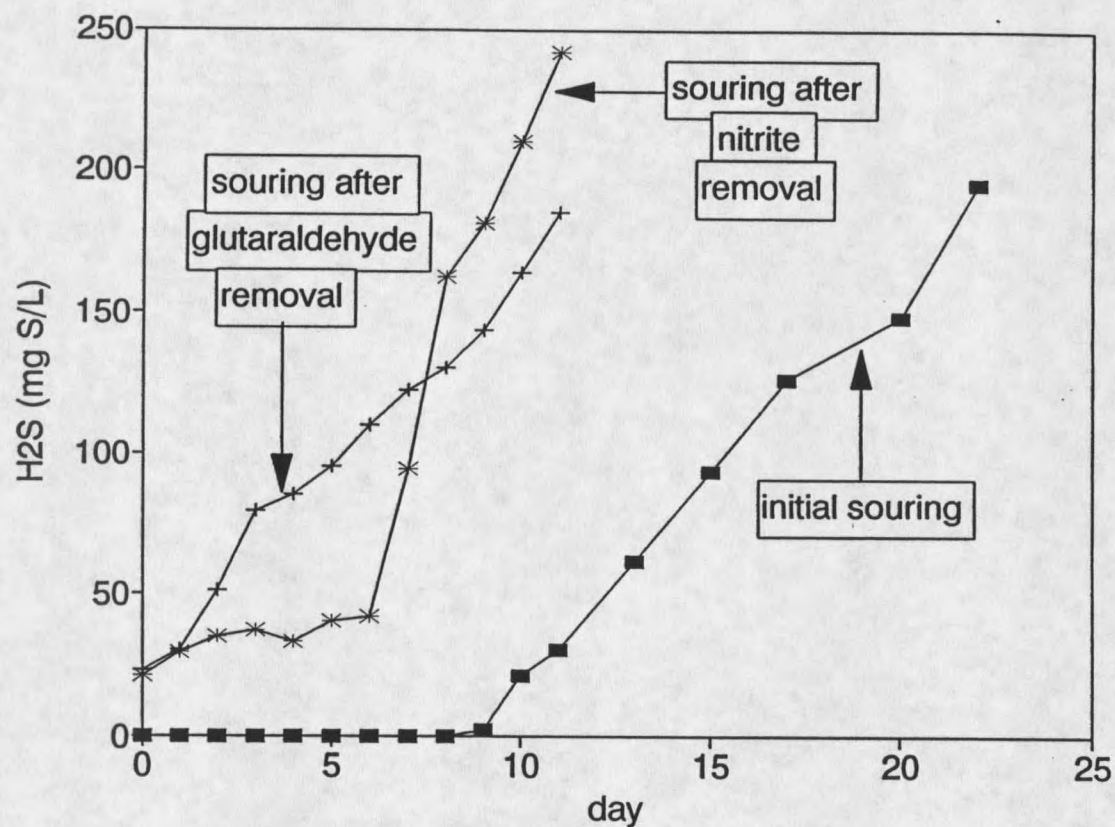


Figure 4.6. H₂S concentrations in the North Slope PW column at distance = 5 cm: initial souring, souring after glutaraldehyde removal and souring after nitrite removal. Single readings were measured each day.

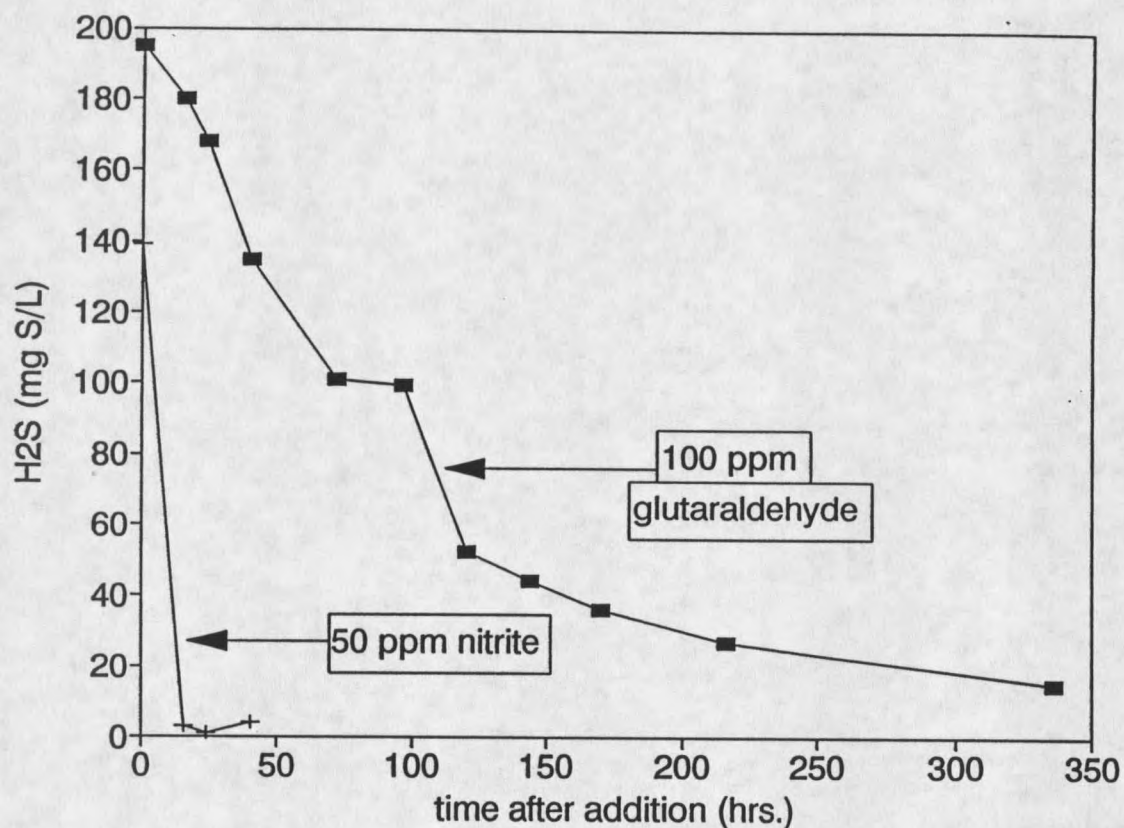


Figure 4.7. H₂S concentrations in the North Slope PW column at distance = 5 cm, after addition of 100 ppm glutaraldehyde and after addition of 50 ppm (mg N/L) nitrite. Shown are single readings at each time interval.

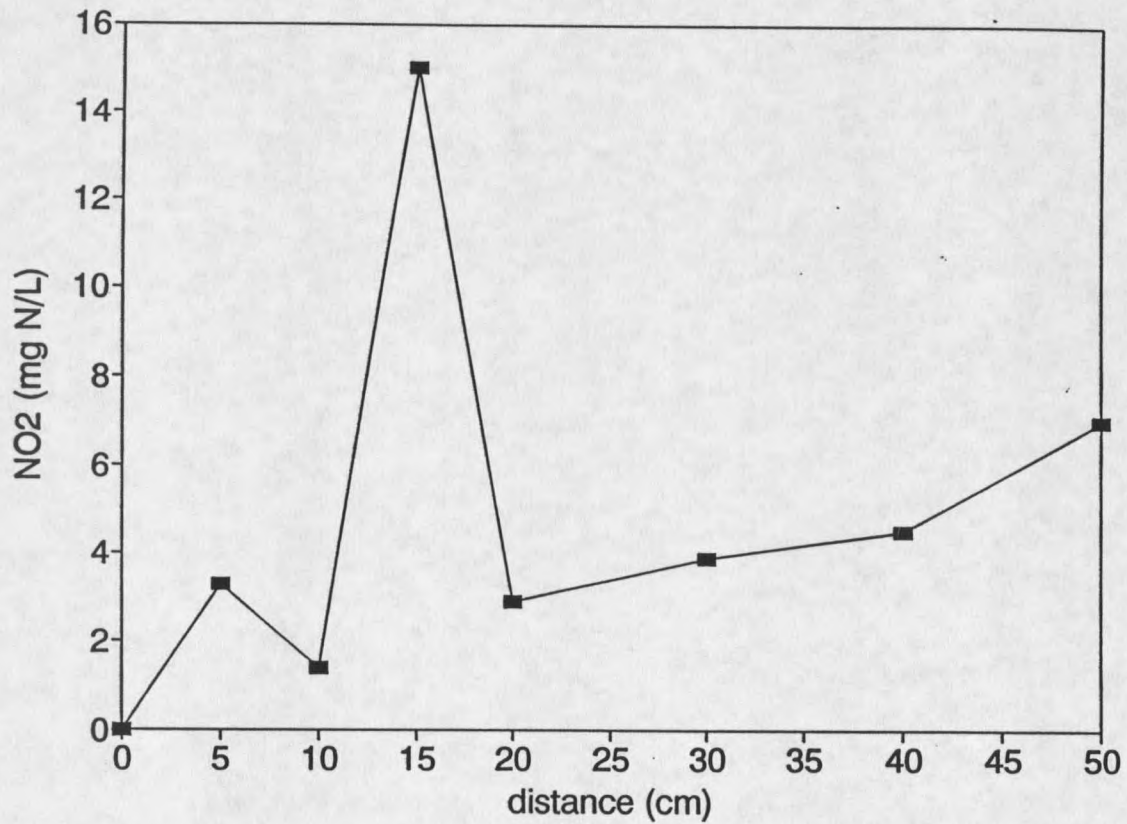


Figure 4.8. A snapshot of nitrite concentration in the North Slope PW column, sampled six hours after pulse addition to the influent. Duplicate samples were taken but the standard errors are not shown because they were very small (≤ 0.2 mg/L).

possibly reaction. This dispersion effect would be greater at distances farther up the column, as the nitrite pulse disperses and increases in length (distance) but decreases in concentration. The majority of souring activity (i.e., the highest gradient of H_2S production and substrate consumption) was seen at the beginning of the column in this and other studies, as detailed in Chapter 2. Consequently, pulse addition, in which the concentration of inhibitory agent is highest at the beginning of the column, would be most effective.

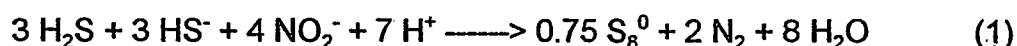
Identical columns were used to study inhibition of souring by adding nitrate to the influent medium and allowing indigenous nitrate-reducing organisms to produce nitrite (Chapter 3). In separate experiments with both the North Sea consortium (used in this nitrite inhibition study) and a North Slope consortium from the Kuparuk field, ten mg N/L of NO_3^- produced approximately eight mg N/L of NO_2^- (the other two mg N/L were unaccounted for, either due to fast reaction with other species or formation of other nitrogen products such as N_2 gas, which were not measured). With both consortia, the eight mg N/L of NO_2^- measured in the column was sufficient to completely inhibit souring. It is unclear why when nitrite was added directly to the North Sea column instead of being biologically produced, approximately 50% more nitrite (12 mg N/L vs. eight mg N/L) was required.

H_2S versus NO_2^- Concentrations

The small amount of residual H_2S (one to two mg S/L) in the column during souring inhibition (Figure 4.1) may come from dissolution of iron sulfide, a black

precipitate. H_2S produced during souring precipitates with iron from the medium and from the sandstone. This precipitation reaction is reversible, becoming a dissolution reaction after H_2S production is suppressed, as evidenced by a slow return of blackened column media to its original brown color.

A proposed mechanism for the disappearance of nitrite is its reaction with H_2S :



A high concentration of H_2S would be available for reaction when nitrite is first introduced, and during inhibition of souring, H_2S would be available from the aforementioned dissolution reaction. The simultaneous disappearance of H_2S and NO_2^- in the column appeared to match the stoichiometry from (1) but the reaction could not be duplicated in batch experiments under conditions identical to the column (60°C , high-salinity produced water, pH 7 and 9). Therefore, the slow disappearance of NO_2^- in the column (Figure 4.2) was probably not due to this reaction. Nitrite may have reacted with other species in the column or continued along the denitrification pathway to N_2 gas, although this step is ordinarily very fast. A yellow precipitate was found in the column effluent during nitrite addition but analysis for elemental sulfur was not performed.

Redox Potential

The E_h of the liquid in the column during souring was very low due to soluble H_2S , well below the -100 mV threshold required for growth of SRB (Postgate, 1984).

The E_h in the column was lowered further as SRB activity farther up the column increased the H_2S concentration. Inhibition of 90% of the souring activity by addition of 10 mg N/L of NO_2^- raised the E_h by approximately 100 mV throughout the column but did not raise it close to the level where SRB activity would be inhibited. At approximately -350 mV (Figure 4.3), the environment in the column was still very reducing and hospitable for growth of SRB. Therefore, the action of the nitrite does not appear to change the E_h of the environment so that SRB cannot grow, but rather, it must be the nitrite itself which inhibits the growth of sulfate-reducing bacteria. The somewhat-higher E_h after nitrite addition was probably due to the lower concentration of aqueous H_2S .

It is unclear why nitrite addition only slightly raised the E_h while inhibiting souring, but nitrate addition raised the E_h to positive values, as shown in Chapter 3. A positive E_h would be sufficient to halt SRB activity in and of itself. Other researchers (Jenneman et al., 1986) have suggested that N_2O produced in nitrate reduction has a cytotoxic effect on SRB by raising the E_h . Perhaps N_2O was produced after nitrate addition in these column studies, while it was not produced after nitrite addition.

Mechanism for Nitrite Inhibition

Nitrite has not been previously described as a strong inhibitor of SRB by other researchers. Postgate (1984) has compiled an extensive list of SRB inhibitors, including several nitro compounds, but nitrite is not one of these. U.S.

Patent No. 4,681,687 to Mouche et al. (1987) describes experiments in which alkali metal nitrites added in a concentration of 50 ppm inhibit sulfide production in flue gas desulfurization system sludges, but this patent explicitly states that 25 ppm NO_2^- and below was not inhibitory. Their weight basis is apparently based on the molecular weight of NO_2^- (46) and not on the molecular weight of N (14), as here. The 50 ppm NO_2^- stated by Mouche corresponds to 15.2 mg N/L of NO_2^- in this system, and 25 ppm NO_2^- equals 7.6 mg N/L, so it appears that Mouche's 25 ppm was at the bottom of the effective range found in this study.

Nitrite has long been known to inhibit a wide variety of microorganisms, leading to its widespread use in the food industry (National Academy of Sciences, 1981). It is difficult to determine the mechanism by which nitrite inhibits microorganisms or spore outgrowth since the nitrite ion is capable of a variety of reactions. These possible mechanisms described in the literature are:

1. *Nitrite reacts with other components during heating to form an inhibitory substance.* This mechanism was unlikely in these studies for two reasons: 1) very high temperatures were never reached (the reactors were maintained at 60°C) and: 2) significant concentrations of nitrite were measured throughout the column. If nitrite were reacting and producing another inhibitory substance, this substance would have to be even more inhibitory than nitrite, since most of the nitrite remains unreacted and the concentration of this new substance would be very small.

2. *Nitrite reacts with thiols to form nitrosothiols, which react with a spore membrane component, thus interfering with metabolic and/or transport steps.*

Thiols are not known to be present in these reactor systems, so this mechanism is unlikely.

The two most likely explanations are:

3. *Nitrite acts as either an oxidant or reductant on cellular sites such as enzymes, enzyme cofactors, nucleic acids and cellular membranes.* This is a definite possibility for the inhibition of SRB seen in these studies, but would require detailed microbiological studies beyond the scope of these experiments.

4. *Nitrite reacts with cellular iron.* Proving or disproving this hypothesis is also beyond the scope of these experiments.

Comparison of Nitrite to Glutaraldehyde

It was shown that 50 mg-N/L of NO_2^- inhibited H_2S production in the column much quicker and to a greater degree than did 100 ppm glutaraldehyde. Nitrite almost immediately inhibited H_2S production by at least 95% throughout the column, while glutaraldehyde inhibited souring by about 90% after two weeks at the beginning of the column and by about 50% at the end of the column (Figure 4.5). The low level of H_2S in the column (less than five mg S/L) after nitrite addition was probably due to dissolution of iron sulfide.

H_2S production recovered quickly after removal of glutaraldehyde, increasing at a rate equal to that after the column was initially inoculated (Figure 4.6). This suggests that glutaraldehyde did not have any long-term inhibition effects but instead lowered the SRB population. After glutaraldehyde removal, SRB apparently

multiplied at their original growth rate and simultaneously produced H_2S . In fact, previous studies of biofilm regrowth after disinfection with another biocide, chlorine, show that growth rates doubled or tripled after removal of the chlorine, even with repeated applications (Characklis, 1980).

Nitrite, on the other hand, continued to inhibit H_2S production by over 75% for six days after nitrite was removed. When H_2S began to increase at day six, it increased much more rapidly than either the initial growth curve or the glutaraldehyde regrowth curve. These two phenomena suggest that SRB were not killed by nitrite but were still inhibited after nitrite was removed, and the original number of cells were present and able to produce H_2S once the inhibitory effect stopped.

Raw material costs of glutaraldehyde and nitrite, which can be added with similar methods to either laboratory systems or a field application, are comparable. The cost of 50% glutaraldehyde is \$1.84/lb and the cost of sodium nitrite is \$0.74/lb, when buying in bulk. Converting these to an active-ingredient weight basis shows that the cost of one ppm glutaraldehyde equals the cost of one ppm (one mg N/L) nitrite.

Effect of Nitrite in the Core Reactors

When nitrite was added to the liquid medium supplying the core reactor, the H_2S concentration dropped to zero but then increased somewhat (Figure 4.4). It was later discovered that nitrite concentrations in the medium steadily decreased

due to reaction with H_2S or some other species. In later experiments in the column, therefore, nitrite was kept as a separate solution and added immediately before the column inlet. This means that in the core, however, the NO_2^- concentration was probably lower than seven mg N/L after some time, leading to the partial recovery of souring (Figure 4.4).

The decrease in pressure drop after nitrite addition means that the permeability of the first core increased, demonstrating that nitrite not only addresses the H_2S problem in souring but also reduces plugging. There was no decrease in pressure drop across the second core, possibly because all the nitrite had reacted before reaching it.

Conclusions

Continuous addition of 10-12 mg N/L nitrite completely inhibits souring in a porous media column, a concentration which appears to be slightly higher than the level of nitrate (subsequently reduced to nitrite) required to achieve the same degree of inhibition. Pulses of nitrite were seen to be more effective than the same amount of nitrite added continuously. Some nitrite was consumed in the column, probably due to chemical reaction or to biological denitrification. Nitrite raised the E_h in the column but not to inhibitory levels for SRB; rather, it appears to be some type of more specific inhibitor. Nitrite addition was observed to reduce H_2S production in a sandstone core and to also increase the permeability.

Nitrite was found to be more effective at inhibiting souring than was

glutaraldehyde. Recovery of SRB was also delayed much longer with nitrite. From the data presented in this chapter and from the data in Chapter 3 demonstrating SRB recovery after three months of nitrate addition, it appears that glutaraldehyde kills SRB while nitrite provides a long-term inhibition without killing. The costs of glutaraldehyde and nitrite are approximately equal on an active-weight basis, meaning that nitrite addition would be much more cost-effective for controlling souring.

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CHAPTER 5**A MATHEMATICAL MODEL OF SULFATE-REDUCING
AND NITRATE-REDUCING BACTERIAL ACTIVITY
IN A POROUS MEDIA COLUMN**Introduction

In this research, inhibition of microbial souring in porous media was studied by direct addition of nitrate or nitrite to laboratory-scale columns. The purpose of this chapter is to report use of a computer solution to a mathematical model to attempt to describe the activity of sulfate-reducing bacteria (SRB) and nitrate-reducing bacteria (NRB) in a column using parameters from batch studies and from the literature. The mathematical model was used as a tool to identify phenomena necessary to account for the experimentally observed activity: inhibition of SRB both by nitrite produced by NRB and by nitrite directly added. Model predictions at steady state and during the transition from souring to souring inhibition will be compared to porous media column data. The model was also used to identify which parameters were particularly important in determining the system behavior.

Several models have been proposed recently to explain phenomena associated with microbial growth in porous media columns. Most of these explore permeability changes, such as the studies by Torbati et al. (1986) and Taylor et al. (1990), which include extensive discussions of permeability reduction as a function of the amount of biomass in the system and the pore size distribution of the solid

media. In a model proposed by Taylor and Jaffe (1990), changes in column dispersivity arising from biofilm growth were investigated.

Probably the closest comparison to the work described in this chapter is another study done by Taylor (1990). His model focuses on biomass production, but also describes the transport of substrate in porous media and accounts for the transport, growth and decay of biomass in suspension and in a biofilm. Changes in the porosity, permeability and dispersivity are predicted. The Taylor model predictions were compared to experimental data, and profiles for substrate concentration, biomass concentration and biofilm thickness were calculated. The Taylor model did not specifically consider microbial souring and souring inhibition due to nitrate/nitrite.

Bryan Warwood performed the initial modeling work on BAM to simulate souring and nitrate reduction. The model he had developed was then refined and expanded to include unsteady-state conditions and addition of nitrite.

Materials and Methods

Model Overview

BAM (Biofilm Accumulation Model) attempts to describe the growth of a biofilm in contact with a perfectly mixed bulk liquid phase (i.e., a Continuous Flow Stirred Tank Reactor, or CFSTR). BAM was developed using the formulation outlined by Wanner in his development of the BIOSIM model (Wanner, 1989; Wanner and Gujer, 1986). The governing principle of BAM is the conservation of

mass. Conservation of energy and momentum are not considered relevant in this application, thus they are not taken into account. Two major assumptions are made: the properties of the film change only in the direction perpendicular to the film, and the biomass is treated as a continuum. The model assumes that the density of particulates in the film is constant; therefore, particulate volume fractions are appropriate measures of population dynamics.

Model Components

The model defines a "unit" as a system of two compartments, bulk liquid (water) and biofilm. A series of units can be chained together to simulate a pipe or column. The bulk compartment contains species in suspension: any dissolved substrates (oxygen, glucose, sulfate, etc.) and particulates (microorganisms, inert materials, etc.). The bulk is considered well-mixed; there are no concentration gradients in the bulk liquid. Within a given unit, dissolved species in the bulk liquid can diffuse into a biofilm according to Fick's law; the driving force is the concentration gradient between the bulk liquid and the biofilm.

In the biofilm compartment, particulates are "immobile", i.e., they are attached to the surface or each other, while substrates are assumed to be in solution in the aqueous phase of the film. The model assumes that the only way particulates can move in the transverse direction is by displacement resulting from growth, while the soluble components can diffuse in the transverse direction only.

In other words, there is no axial movement or displacement of materials in the film compartment.

Model Column Geometry

Modeling of a porous media column with BAM requires multiple sequential units in which the outlet of one is the input to the next, analogous to a series of CFSTR's. The surface area-to-volume ratio in each unit was set to reflect the actual ratio present in the experimental porous media column.

Eight units were used for BAM modeling of the column. Eight CFSTR's in series were representative of the seven sampling sites available in the experimental column plus one additional unit. The 50-cm experimental column had sampling ports at 5, 10, 15, 20, 30, 40 and 50 cm. An additional computational unit was added by subdividing the first five-cm sampling section into two 2.5-cm computational units. This subdivision was added due to the high amount of microbial activity occurring within the first five cm of the experimental column. Increasing the number of units in the region of high microbial activity provided more information on the transition region.

The surface area of the porous media in the model reactor was calculated based on an average spherical sand particle diameter of 220 microns. The column porosity was based on the measured void volume (0.50) of the experimental reactor column. The characteristics of the eight computational units are shown in Table 5.1.

Model Equations

Biofilm compartment. Within the biofilm, the concentrations of cells, electron acceptor (sulfate or nitrate), and growth substrates (organic acids) are analyzed in the model. The concentrations of cells inside the biofilm are expressed in terms of volume fractions. Volume fractions are converted to mass units through the intrinsic cell density, ρ_x . The balance on cells is

$$\frac{\partial X}{\partial t} = \mu X - \frac{\partial}{\partial z} (vX) \quad (1)$$

where the terms represent (from left to right) accumulation, growth and advection.

Table 5.1. Model dimensional parameters for the 50-cm crushed Berea sandstone column.

unit	length (cm)	distance from inlet (cm)	surface area (m ²)	liquid volume x 10 ⁵ (m ³)
1	2.5	2.5	0.81	2.97
2	2.5	5.0	0.81	2.97
3	5.0	10	1.62	5.94
4	5.0	15	1.62	5.94
5	5.0	20	1.62	5.94
6	10	30	3.24	11.9
7	10	40	3.24	11.9
8	10	50	3.24	11.9

The substrate concentration dependence of microbial growth is modeled by a "double-Monod" kinetics form, which is dependent on the concentration of both

the substrate (C_s) and the electron acceptor (C_e). Non-competitive inhibition kinetics are also built into the model. In this type of inhibition, the inhibitor has affinity toward the inhibitor site, while the substrate has affinity toward the enzyme-inhibitor complex. Therefore, inhibition affects growth at all substrate concentrations. The inhibition term is multiplied by the standard double-Monod kinetic expression, so the kinetic expression for the growth rate μ is thus of the form

$$\mu = \frac{\mu_{\max} C_s}{K_s + C_s} \frac{C_e}{K_e + C_e} \frac{K_i}{1 + K_i} \quad (2)$$

Inhibition effects are seen for H_2S in the SRB model, and for H_2S (acting against SRB) and for NO_2^- (acting against SRB and NRB) in the NRB model. When both H_2S and NO_2^- are present, two inhibition terms are required.

For SRB growing on propionate, two growth expressions were used. The first employed double-Monod kinetics with inhibition, while a second, faster growth expression was dependent on the presence of *n*-butyrate and formate, as well as being dependent on sulfate and propionate. The growth term in this case is of the form

$$\mu = \frac{\mu_{\max} C_{s1}}{K_{s1} + C_{s1}} \frac{C_{s2}}{K_{s2} + C_{s2}} \frac{C_{s3}}{K_{s3} + C_{s3}} \frac{C_e}{K_e + C_e} \frac{K_i}{1 + K_i} \quad (3)$$

where propionate, *n*-butyrate and formate are substrates 1, 2 and 3, respectively.

As cell volume is created in the biofilm due to growth, cells displace their neighbors away from the substratum. The advective velocity must satisfy

$$\frac{\partial v}{\partial z} = \frac{\sum \mu_i \epsilon_i}{\sum \epsilon_i} \quad (4)$$

subject to the boundary condition

$$v = 0 \quad \text{at } z = 0 \quad \text{for } t > 0. \quad (5)$$

Growth and detachment processes change the thickness of the biofilm according to

$$\frac{dL_f}{dt} = v|_{z=L_f} - k_d L_f^2 \quad (6)$$

with the initial condition

$$L_f = L_f^0 \quad \text{at } t = 0. \quad (7)$$

The concentration of growth-limiting substrate in the biofilm is described by a reaction-diffusion equation

$$\frac{\partial C_s}{\partial t} = D_s \nabla^2 C_s - \frac{\mu}{Y_{xs}} \rho_x \quad (8)$$

Molecular diffusion is the only transport mechanism for dissolved species within the biofilm. Reduction in the effective diffusion coefficient inside the biofilm is allowed through the parameter γ . The initial condition is

$$C_s = C_s^i \text{ at } t = 0 \text{ for } 0 \leq z \leq L_f^o \quad (9)$$

Boundary conditions on Equation (8) impose a matching flux condition at the biofilm-bulk fluid interface

$$\tau \frac{\partial C_s}{\partial z} \Big|_{z=L_f} = \frac{C_s^* - C_s|_{z=L_f}}{L_L} \text{ for } t \geq 0 \quad (10)$$

and a no-flux condition at the substratum

$$\frac{\partial C_s}{\partial z} \Big|_{z=0} = 0 \text{ for } t \geq 0. \quad (11)$$

Similar equations to (8) through (11) apply to the electron acceptors sulfate and nitrate.

Bulk fluid compartment. Differential material balances on the bulk fluid compartment account for flow in and out of the column, bulk phase reactions, and the net reaction arising from the biofilm. The bulk fluid is considered to be the fluid residing within that specific unit of the column. The balance on cells is

$$\frac{dX_a^*}{dt} = \mu X_a^* + k_d e_a|_{z=L_f} \rho_x L_f^2 \frac{A}{V} - \frac{Q}{V} X_a^*. \quad (12)$$

From left to right, the terms in this equation account for accumulation, bulk phase growth, biofilm detachment of cells and flow out of the reactor. The reactor bulk fluid is initially free of particulates:

$$X_a^* = X_f^* = 0 \text{ at } t = 0. \quad (13)$$

Substrate is continually supplied in the influent, but is consumed by cells in the bulk fluid and biofilm, giving

$$\frac{dC_s^*}{dt} = \frac{Q}{V} (C_s^I - C_s^*) - \mu X^* - D_s \tau \left. \frac{dC_s}{dz} \right|_{z=L_f} \frac{A}{V} \quad (14)$$

with

$$C_s^* = C_s^I \text{ at } t = 0. \quad (15)$$

Equations analogous to (14) and (15) for electron acceptor concentration in the bulk fluid are included in the model.

These equations are solved numerically by a finite-difference method using a tridiagonal technique and a variable time step.

Nomenclature. (i) Latin characters. Latin characters are defined as follows: A , biofilm surface area; C_e , concentration of electron acceptor; C_s , concentration of substrate; D_s , diffusion coefficient of substrate in water; k_d , detachment rate coefficient; K_e , Monod half-saturation coefficient for electron acceptor; K_i , Monod inhibition constant; K_s , Monod half-saturation coefficient for substrate; L_f , biofilm thickness; Q , volumetric flow rate; t , time; v , cell advective velocity; V , reactor volume; X , bulk concentration of cells; Y_{xs} , yield coefficient of biomass on substrate; z , distance coordinate normal to the substratum.

(ii) Greek characters: μ , local specific growth rate; $\mu_{\max}$, maximum specific growth rate; ρ_x , cell intrinsic density; τ , biofilm-bulk fluid effective diffusivity ratio.

(iii) Superscripts: 0 , initial value; * , bulk fluid value.

Experimental Column Studies

The experimental column studies were performed in the manner described in previous chapters. A microbial consortium obtained from an oil reservoir (the Ninian field in the North Sea) was grown in an anaerobic upflow porous media reactor operated at 60°C, using the main organic acids found in reservoir waters as carbon and energy sources. Sulfate added at concentrations similar to those in seawater allowed souring to occur. Nitrate, when introduced to inhibit souring, was added to the medium in the form of sodium nitrate. During nitrite addition, a potassium nitrite solution was added to the medium immediately ahead of the column inlet via a peristaltic pump. A timeline of the North Sea experiments is shown in Figure 5.1.

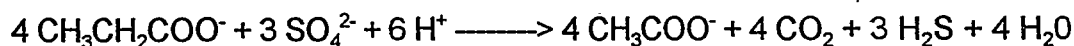
Results

Four scenarios in a porous media column were modeled using BAM: 1) sulfate reduction (souring) at steady state; 2) nitrate reduction (and consequently, inhibition of souring) at steady state; 3) nitrate reduction at non-steady state (the transition from souring to nitrate reduction); and 4) nitrite addition (and consequently, inhibition of souring) at steady state.

Souring at Steady State

The following assumptions were necessary to complete BAM for the description of souring:

1. *Cell yields and intrinsic kinetics for SRB in the column utilizing formate and n-butyrate are equal to those obtained in batch cultures using the North Sea consortium.* Batch results were obtained using the same medium and same environmental conditions (initial pH 7, 60°C) at which the column was operated.
2. *Isobutyrate yields and kinetics are the same as those for n-butyrate.* The yield and kinetics for isobutyrate were not determined in batch culture, so the values for n-butyrate were used as an initial estimate.
3. *The cell yields and intrinsic kinetics for SRB in the column utilizing propionate are equal to those obtained in batch cultures using the North Slope consortium.* Yields and kinetics on propionate were not determined with the North Sea consortium but they were determined using a consortium from the North Slope field at Kuparuk, Alaska. The two consortia behaved very similarly in duplicate columns, as shown in Chapter 2.
4. *There is no consumption of acetate by SRB in the column.* This agrees with the results of batch cultures and also with the net increase in acetate concentration in the column.
5. *Propionate is incompletely oxidized to acetate and CO₂.* This agrees with the results from batch studies and is supported by the literature on SRB (Pfennig et al., 1981). The reaction of propionate is as follows:



The only reaction of formate possible is complete oxidation as follows:



North Sea Column Timeline

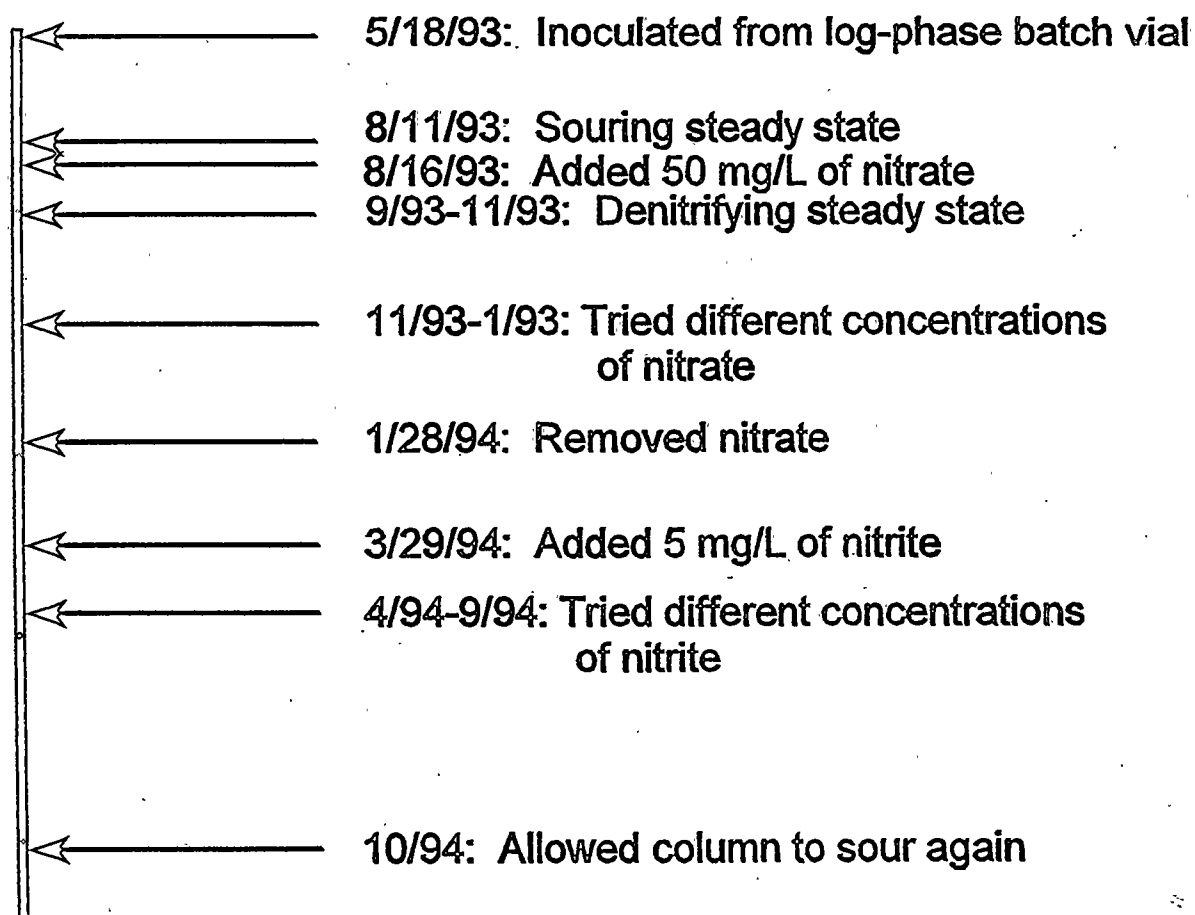
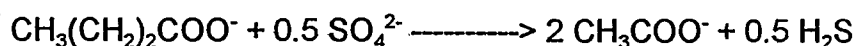


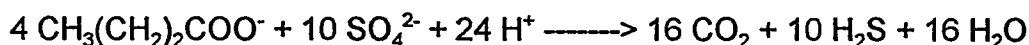
Figure 5.1. North Sea column experimental timeline.

SRB cell yields are so low that the cell synthesis reaction may be neglected for each substrate.

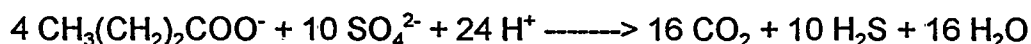
6. *n*-Butyrate is partially oxidized, with half going to acetate and the other half to CO₂. These reactions, which are in accordance with the batch experiment data (Mueller, 1994), are as follows:



and



7. *Isobutyrate* is completely oxidized to CO₂. This reaction was not determined in batch studies but is also supported by Pfennig et al. (1981). The stoichiometry is:



8. The diffusion coefficients (Perry and Chilton, 1973) and biofilm densities (Doetsch and Cook, 1973; Luria, 1960) are based on literature values. The biofilm density for all cells equals 2.35×10^5 g dry cell/m³.

9. The inhibition constant for H₂S acting on SRB activity is 251 mg/L (Okabe, 1992).

10. Non-competitive inhibition kinetics are used in BAM. For nitrite, both non-competitive and uncompetitive inhibition qualitatively match the experimental data (Mueller, 1994).

11. All kinetics are double-Monod in reaction order.

12. The column surface area, volume and porosity are based on measured values (Chen et al., 1994).

13. The volumetric flow rate is 27 cm³/hr. This equals a superficial velocity of

about two ft/day, which was used in column experiments and is in the range of normal oilfield velocities (Burger, 1993).

14. *The biofilm thickness is 4.0 μm and is constant throughout the column.* This was based on destructive biomass measurements from a similar column.

Results of the BAM simulation using the above parameters are shown in Figures 5.2a, 5.3a and 5.4a. In each figure, the model output is compared to data measured at souring steady state during August 1993. The model predicted that all of the SRB activity, as shown by SO_4^{2-} consumption and H_2S production, will occur in the first five cm of the column (Figure 5.2a). It also predicted that all the sulfate in the influent will be utilized in this region. Although most of the souring activity actually did occur in the first five cm, the extent of sulfate utilization was significantly less than predicted by the model.

The model correctly predicted that nearly all of the *n*-butyrate was consumed in the first five cm (Figure 5.3a). The model also correctly predicted formate consumption (Figure 5.4a), the other favorable substrate for SRB growth, until the system ran out of sulfate. The fit to isobutyrate concentration data, however, was poor as the model failed to show the slow utilization of isobutyrate throughout the column (Figure 5.3a). The model overpredicted the SRB consumption of propionate (Figure 5.4a), which was also utilized at a slow rate. The model predictions for acetate concentration in the column (Figure 5.4a), which account for acetate initially present plus the acetate created from the metabolism of *n*-butyrate and propionate, err due to the overpredicted propionate usage. In summary, the

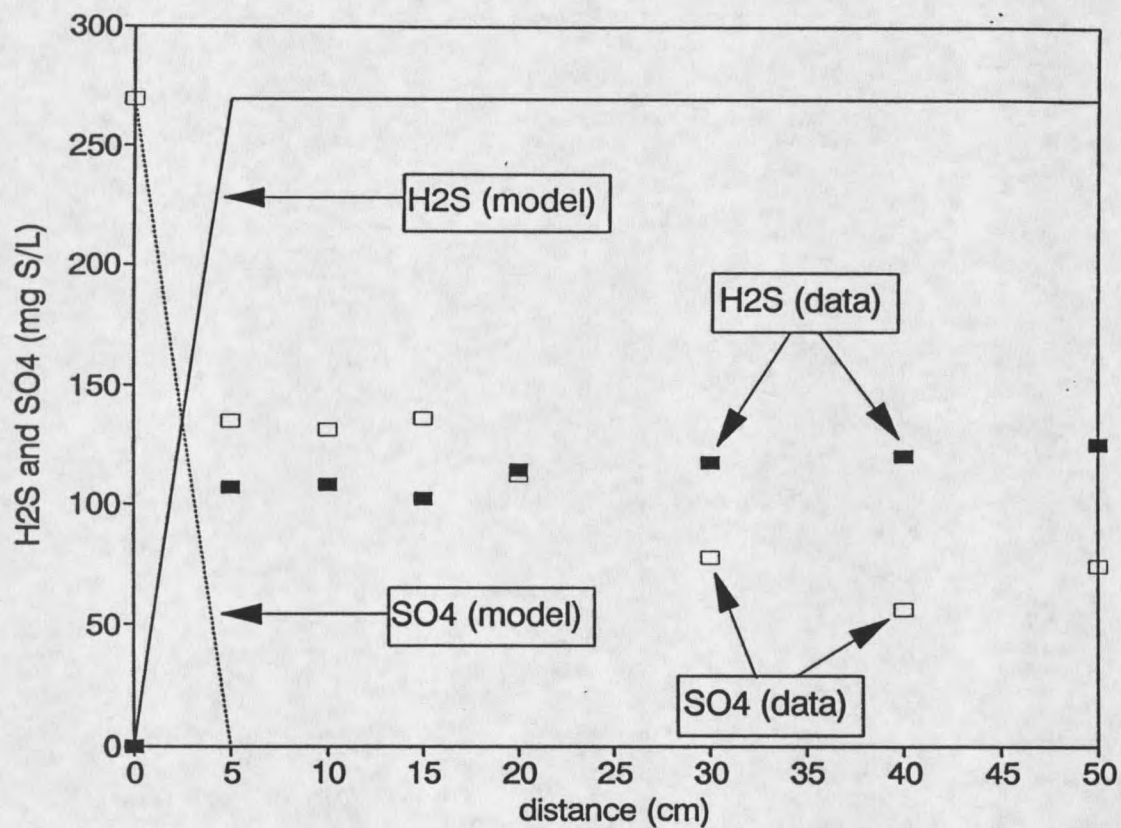


Figure 5.2a. H_2S and SO_4^{2-} concentration profiles in the North Sea column (predicted vs. data) during souring, with the initial set of assumptions.

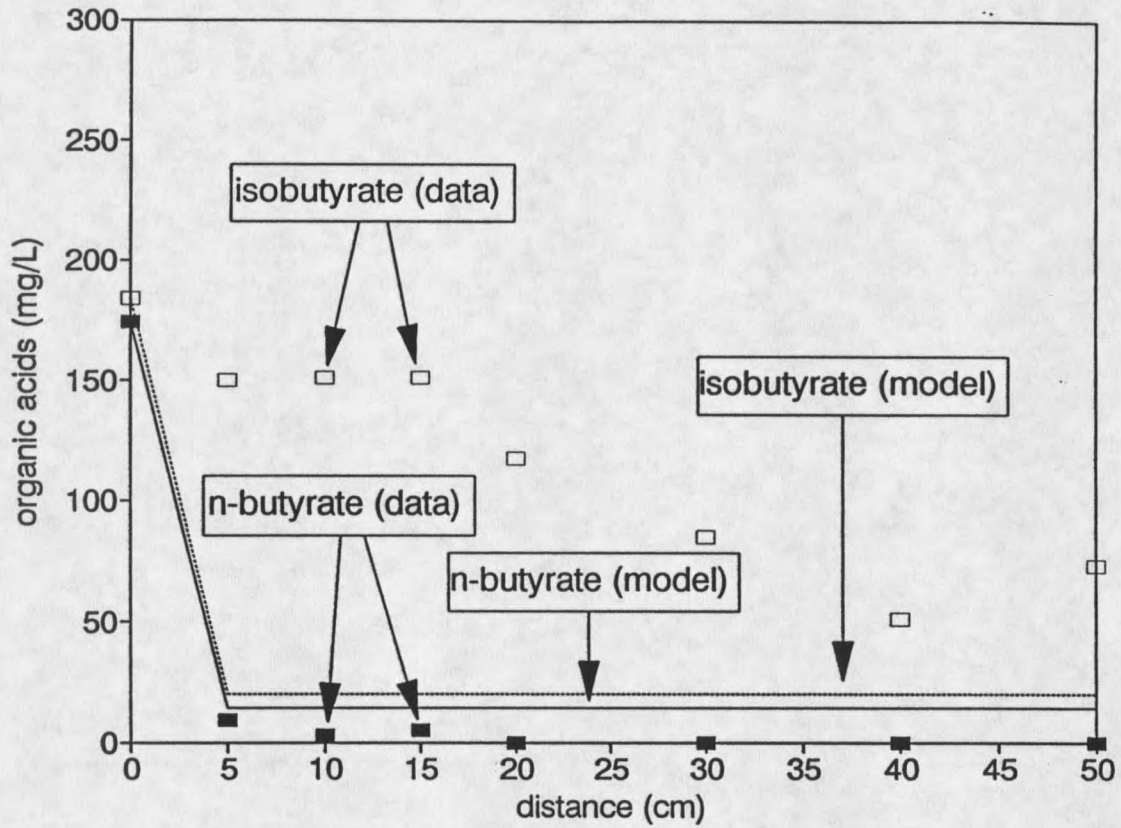


Figure 5.3a. *n*-Butyrate and isobutyrate concentration profiles in the North Sea column (predicted vs. data) during souring, with the initial set of assumptions.

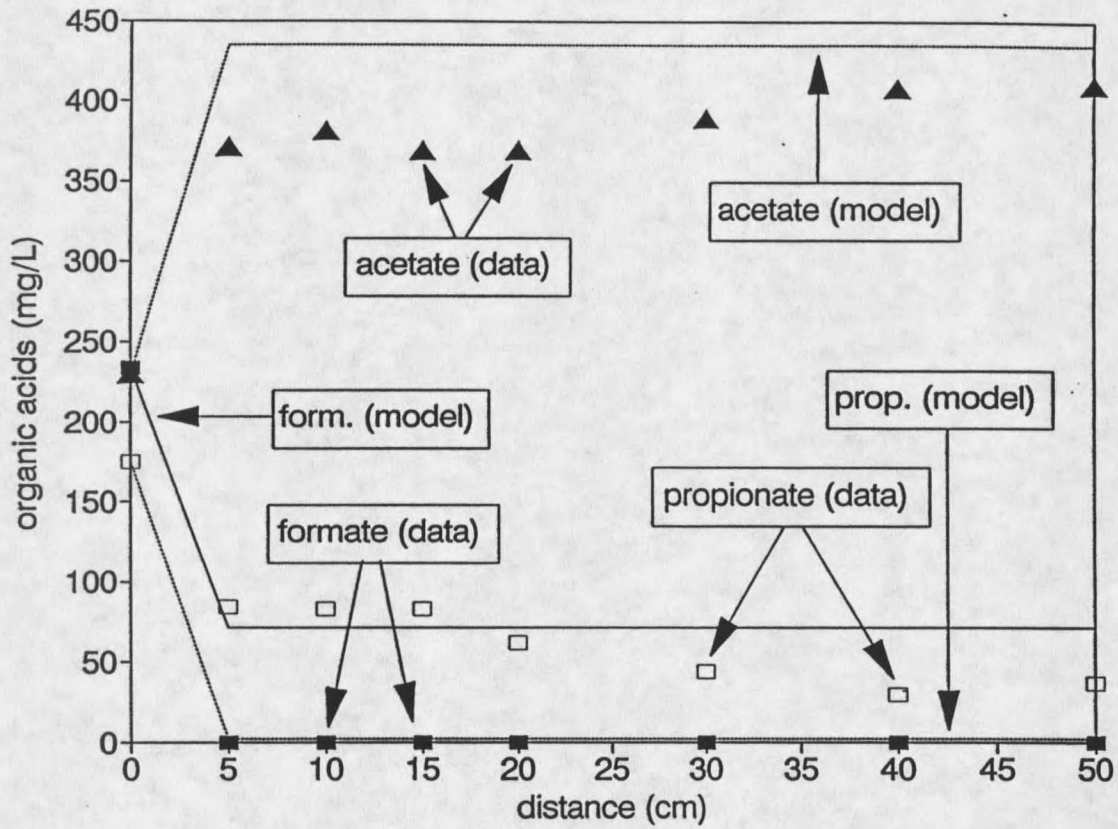


Figure 5.4a. Formate, propionate and acetate concentration profiles in the North Sea column (predicted vs. data) during souring, with the initial set of assumptions.

model inadequately described the porous media column results due to higher activity than actually seen. Several errors in kinetics and stoichiometry for individual substrates were also evident.

Consequently, several of the assumptions were modified:

1. *The biofilm thickness is 0.4 μm throughout the column, not 4.0 μm .*
2. *The maximum specific growth rate (μ_{max}) for SRB on isobutyrate is 0.010/day, not 0.413/day.*
3. *SRB utilize propionate in the column at two different growth rates, with $\mu_{\text{max}1} = 0.20/\text{day}$ and $\mu_{\text{max}2} = 0.005/\text{day}$, depending on whether formate and *n*-butyrate are present.*
4. *The consumption of volatile fatty acid substrates overpredicts the consumption of sulfate, and thus the production of hydrogen sulfide, by 40%.*

These assumptions, which are included in Table 5.2 (the final input to the SRB model) changed the BAM predictions dramatically. Sulfate was still consumed rapidly in the first five cm of the column, resulting in a steep H_2S gradient (Figure 5.2b) but enough sulfate remained to be consumed at a lesser rate in the remaining 45 cm of the column. For H_2S , the model more closely matched the data at the beginning of the column than the original model but still overpredicted sulfide concentrations further up the column.

n-Butyrate, one of the preferred substrates for SRB, was also consumed rapidly in the column (Figure 5.3b). In the adjusted model as well as in the experiment, essentially all of the *n*-butyrate was consumed in the first five cm. The

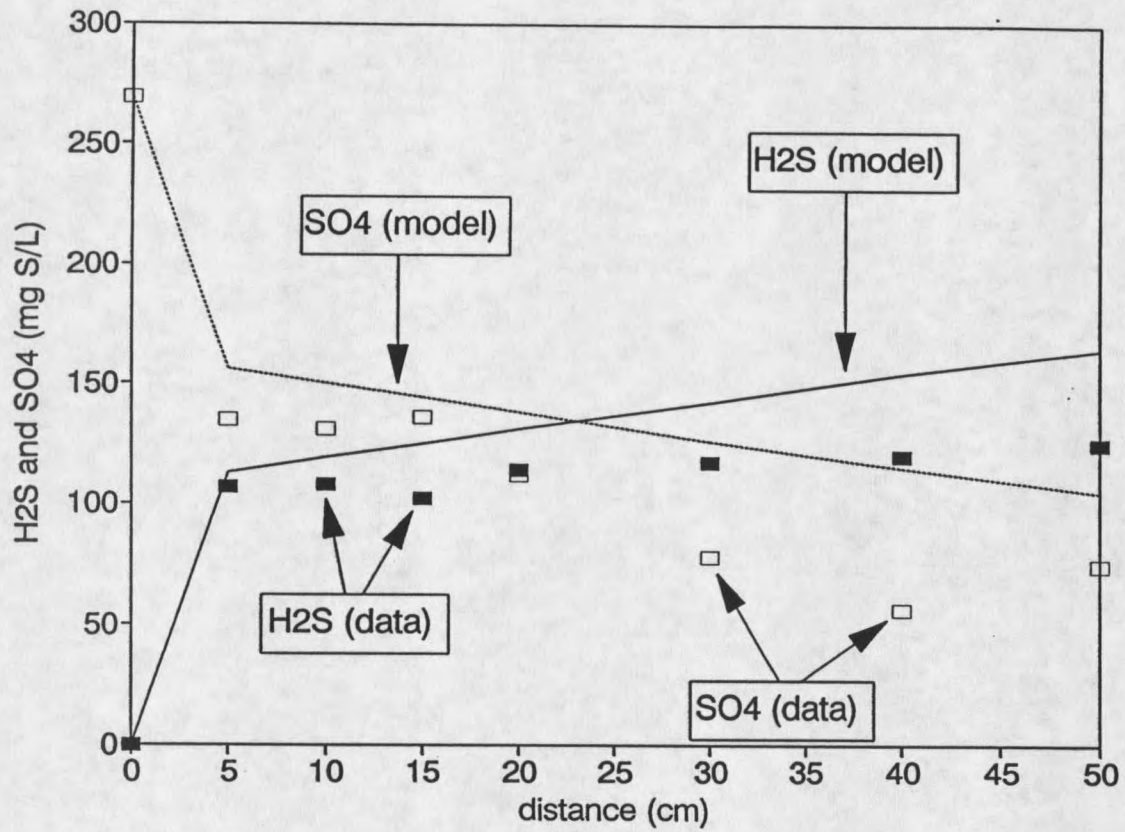


Figure 5.2b. H_2S and SO_4^{2-} concentration profiles in the North Sea column (predicted vs. data) during souring, with the final set of assumptions.

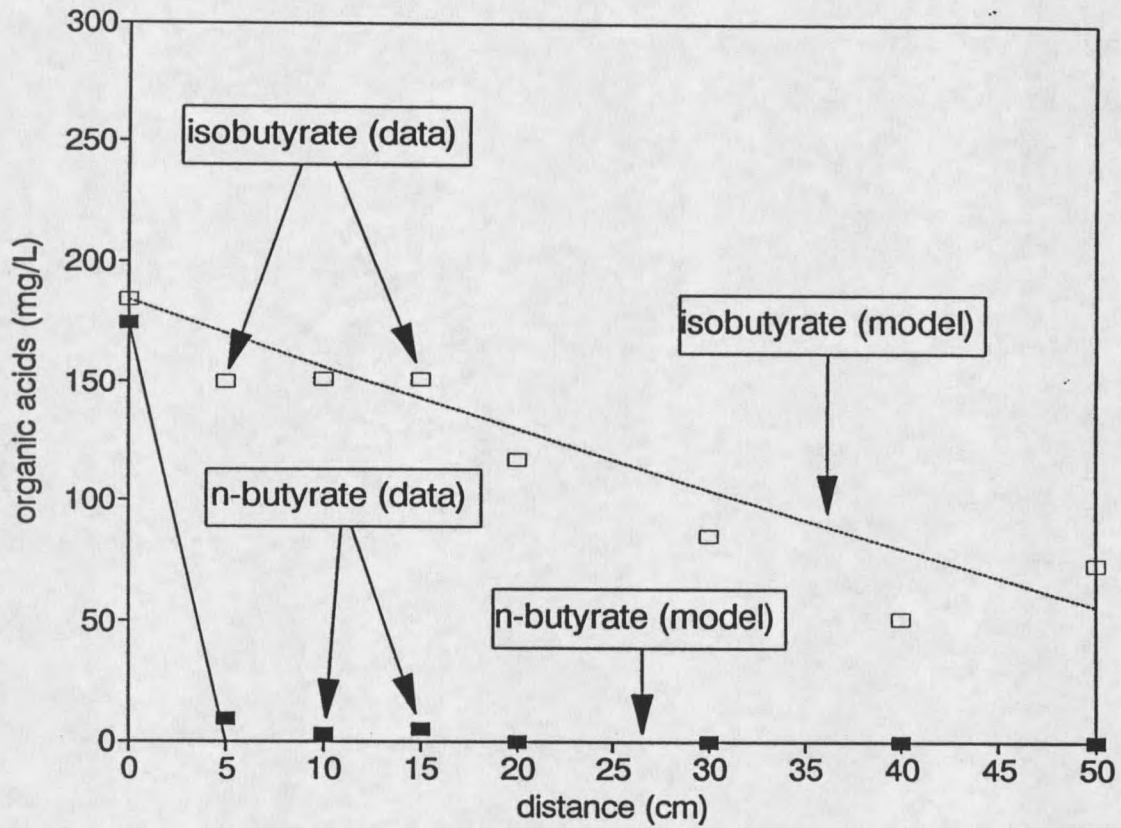


Figure 5.3b. *n*-Butyrate and isobutyrate concentration profiles in the North Sea column (predicted vs. data) during souring, with the final set of assumptions.

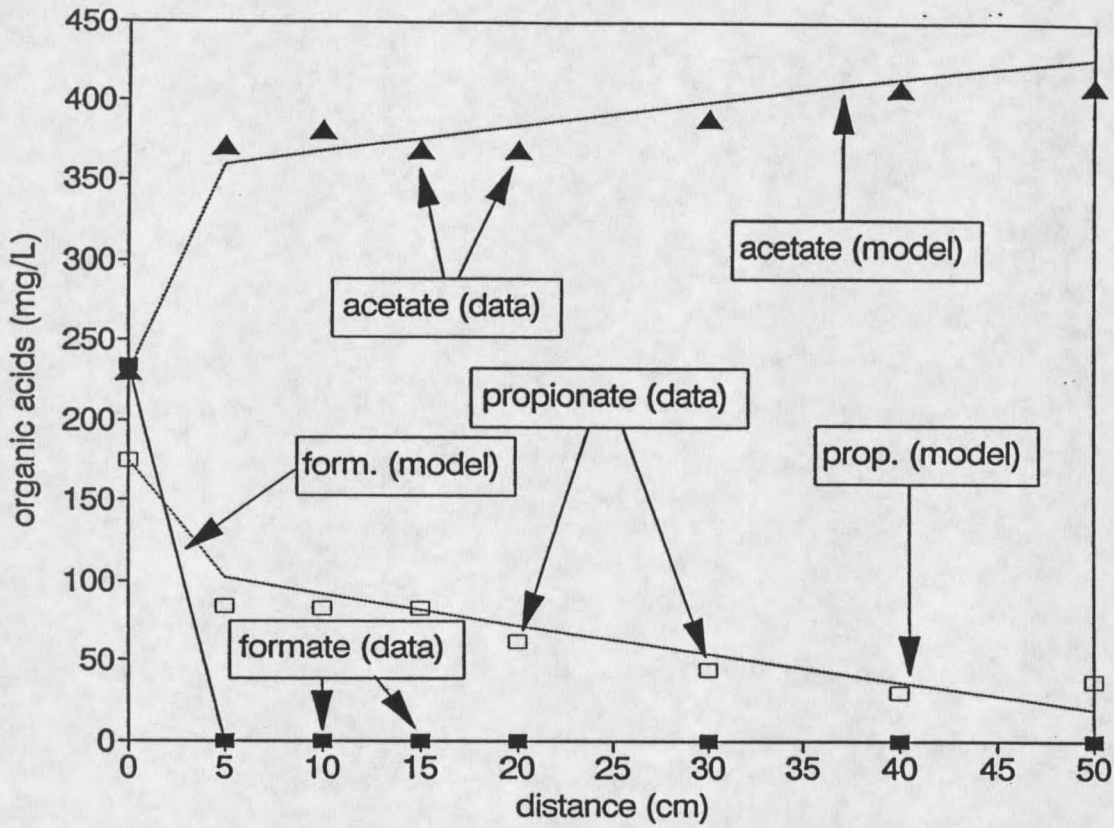


Figure 5.4b. Formate, propionate and acetate concentration profiles in the North Sea column (predicted vs. data) during souring, with the final set of assumptions.

data indicated isobutyrate to be consumed at a slow rate throughout the column; consequently, the original model was modified to use a much lower $\mu_{\max}$ for isobutyrate. A value of $\mu_{\max} = 0.010/\text{day}$, obtained by trial-and-error and inspection, closely fit the data for isobutyrate (Figure 5.3b).

Table 5.2. SRB growth yields and revised kinetics used in the model.

substrate	Y_{xs} (g cells/ g substrate)	Y_{xe} (g cells/ g SO_4^{2-})	$\mu_{\max}$ (1/hr)	K_s (mg/L)	K_e (mg/L)
formate	0.003	0.017	0.312	2.0	4.0
acetate	0	0	0	---	---
propionate (1)	0.002	0.010	0.200	2.0	5.0
propionate (2)	0.002	0.010	0.005	2.0	5.0
isobutyrate	0.003	0.010	0.010	2.0	5.0
<i>n</i> -butyrate	0.003	0.010	0.413	2.0	5.0

Formate, the other preferred substrate, was also completely consumed in the first five cm of the column at steady state (Figure 5.4b), in the model as well as in the column. The much slower sulfate consumption in the last 45 cm of the column (as seen in Figure 5.2b) was a result of the depletion of *n*-butyrate and formate, and a reliance on propionate and isobutyrate for growth. Propionate was rapidly consumed in the first five cm, although more slowly than either *n*-butyrate or formate, and was consumed at a much slower, fairly linear rate in the rest of the column (Figure 5.4b). One way to model this phenomenon was to have SRB

growing on propionate at two different growth rates in the column, such as two rate equations, two different species or one species growing differently at different places in the column. In the model, SRB grew on propionate at the higher growth rate only when formate and *n*-butyrate were present. Acetate was produced as propionate and *n*-butyrate were consumed and incompletely oxidized according to the aforementioned reactions, and acetate was not consumed by SRB in the model. After the modifications to the model, the predicted concentrations for acetate were close to the measured values (Figure 5.4b).

Nitrate Reduction at Steady State

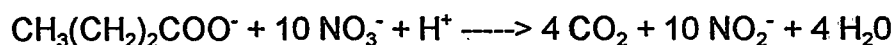
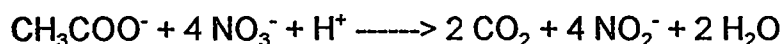
Experiments detailed in Chapter 3 have shown that introducing nitrate to an actively souring column results in production of nitrite (incomplete denitrification), which completely inhibits H₂S production at nitrate concentrations greater than or equal to ten mg N/L. This phenomenon has been shown with both North Sea and North Slope consortia. In this case, it is known that both sulfate-reducing bacteria (SRB) and nitrate-reducing bacteria (NRB) are present in the consortia (obtained from the Ninian North Sea field) and therefore in the column; growth kinetics for both must now be included in the model. The assumptions inherent in the NRB model were as follows:

- 1. The kinetics and yields for SRB remain the same as previously described and modified.*

2. *The intrinsic kinetics and cell yields for NRB are in the range described in the literature.* The kinetic parameters for NRB, such as cellular growth yield and maximum growth rate, were determined by Mueller (1994). The K_i values for the two species were estimated based on experimental data. The values for the half-saturation constants K_S and K_E were extracted from literature data (Grady and Lim, 1980).

3. *n-Butyrate, already shown to be the most favorable substrate along with formate, is the only substrate consumed by SRB.*

4. *Acetate and isobutyrate are the only substrates consumed by NRB.* They react according to the following stoichiometry:



Again, cell yields for NRB growing anaerobically are so low that the cell synthesis reactions may be neglected for each substrate.

5. *The 0.4 μm biofilm is still present in the column but is now composed of both SRB and NRB.*

6. *The inhibition constant K_i for NO_2^- acting on SRB is one mg N/L.* This is an estimate based on experimental data from batch experiments (Mueller, 1994).

7. *The inhibition constant K_i for NO_2^- acting on NRB is 30 mg N/L.* This is also an estimate based on experimental data from batch experiments (Mueller, 1994).

8. *All kinetic equations, diffusivities and parameters for column flow, area, volume, etc. are the same as those stated in the SRB scenario.*

Results of the BAM simulation using these parameters are shown in Figures 5.5a and 5.6a. In each case, the model output is compared to the data measured at steady state after nitrate addition in November 1993. For SO_4^{2-} and H_2S , the model matched the experimental data fairly well, as it predicted a dramatic decrease compared to the souring case shown in Figure 5.2b but underpredicted the souring inhibition actually seen (Figure 5.5a). Nitrate was almost completely consumed in the first five cm, agreeing with the column data (Figure 5.6a) but the production of nitrite was overpredicted. Also, the model failed to account for the decrease in nitrite seen at distances up the column.

Consequently, three more assumptions were modified:

1. *The inhibition constant for NO_2^- acting on SRB activity is 0.2 mg N/L, not 1.0 mg N/L.*
2. *Only 84% of NO_3^- consumed appears as NO_2^- .*
3. *The maximum specific growth rates for nitrate-reducing bacteria are lower: $\mu_{\max} = 1.0/\text{day}$ for acetate (the preferred substrate) and $\mu_{\max} = 0.5/\text{day}$ for isobutyrate.*

These changes also dramatically affected the BAM predictions and provided a much better fit to the experimental data showing inhibition of souring. The final parameters in the NRB model are included in Table 5.3. Almost no sulfate reduction occurred in the column with the modified NRB model (Figure 5.5b), as seen by the small drop in SO_4^{2-} concentration, and the small increase (five mg S/L) in H_2S .

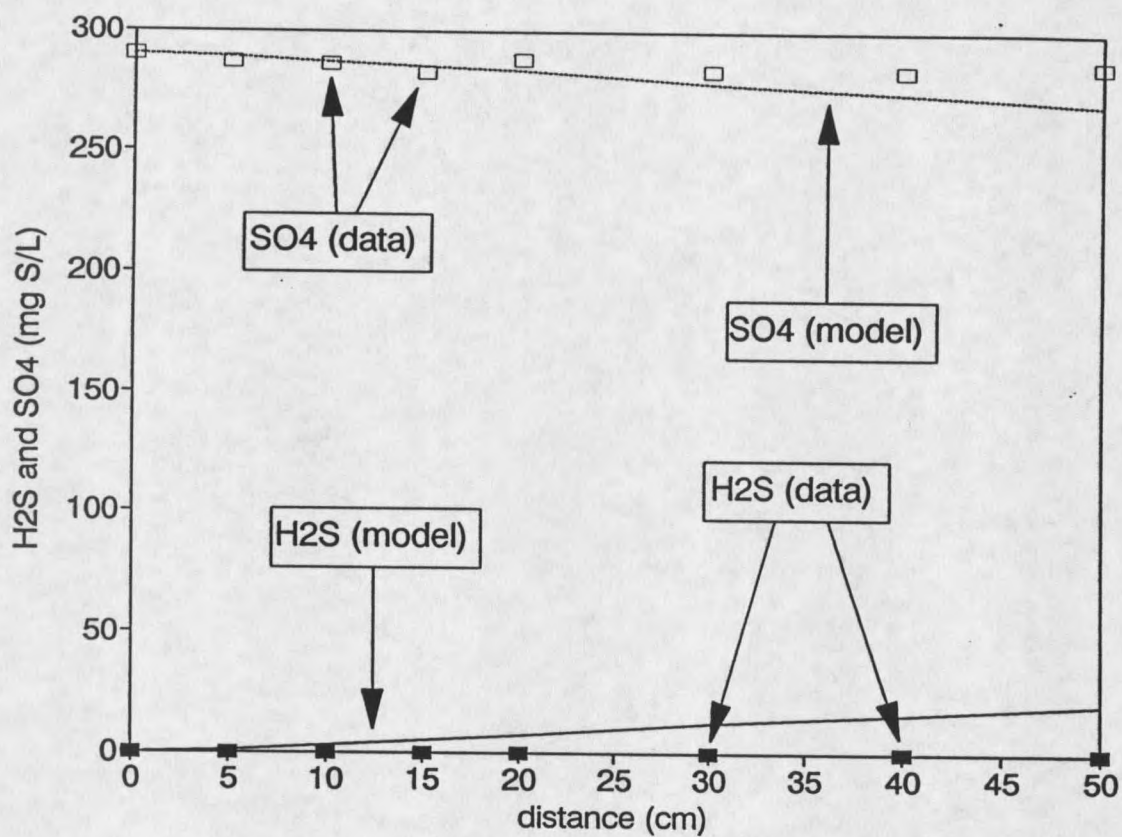


Figure 5.5a. H_2S and SO_4^{2-} concentration profiles in the North Sea column (predicted vs. data) during nitrate reduction, with the initial set of assumptions.

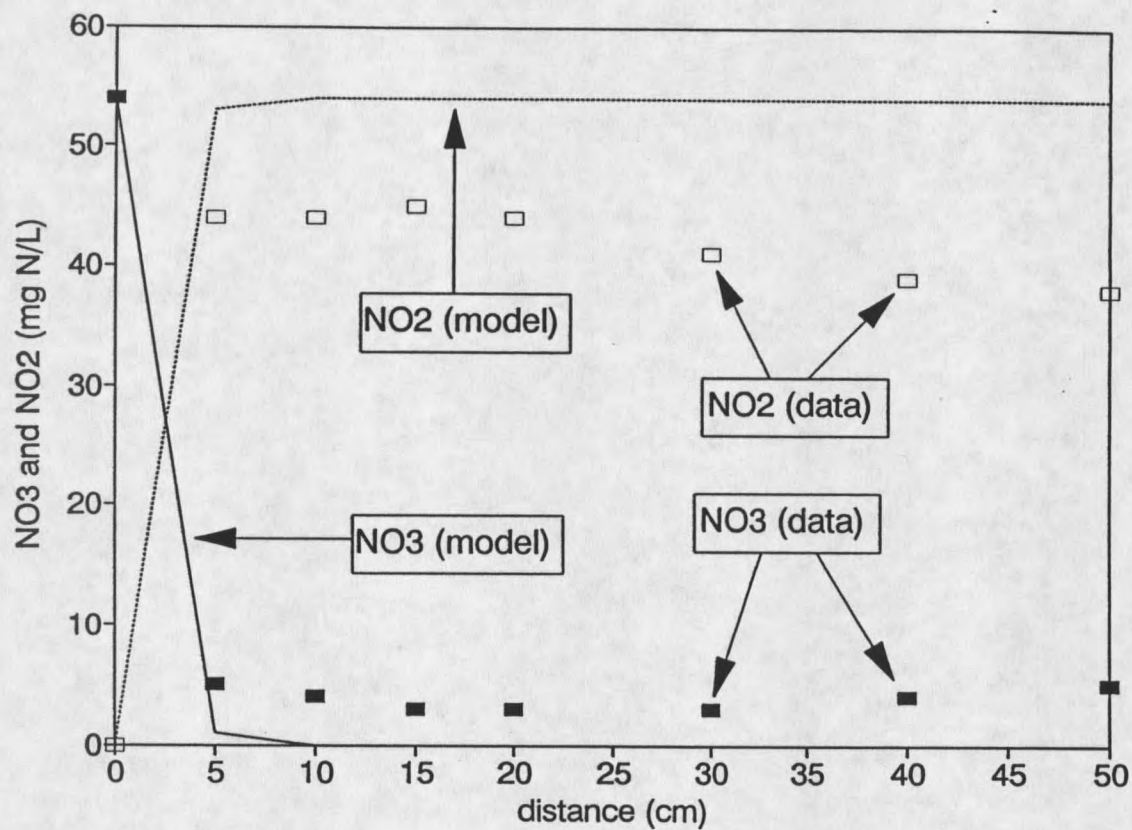


Figure 5.6a. NO_3^- and NO_2^- concentration profiles in the North Sea column (predicted vs. data) during nitrate reduction, with the initial set of assumptions.

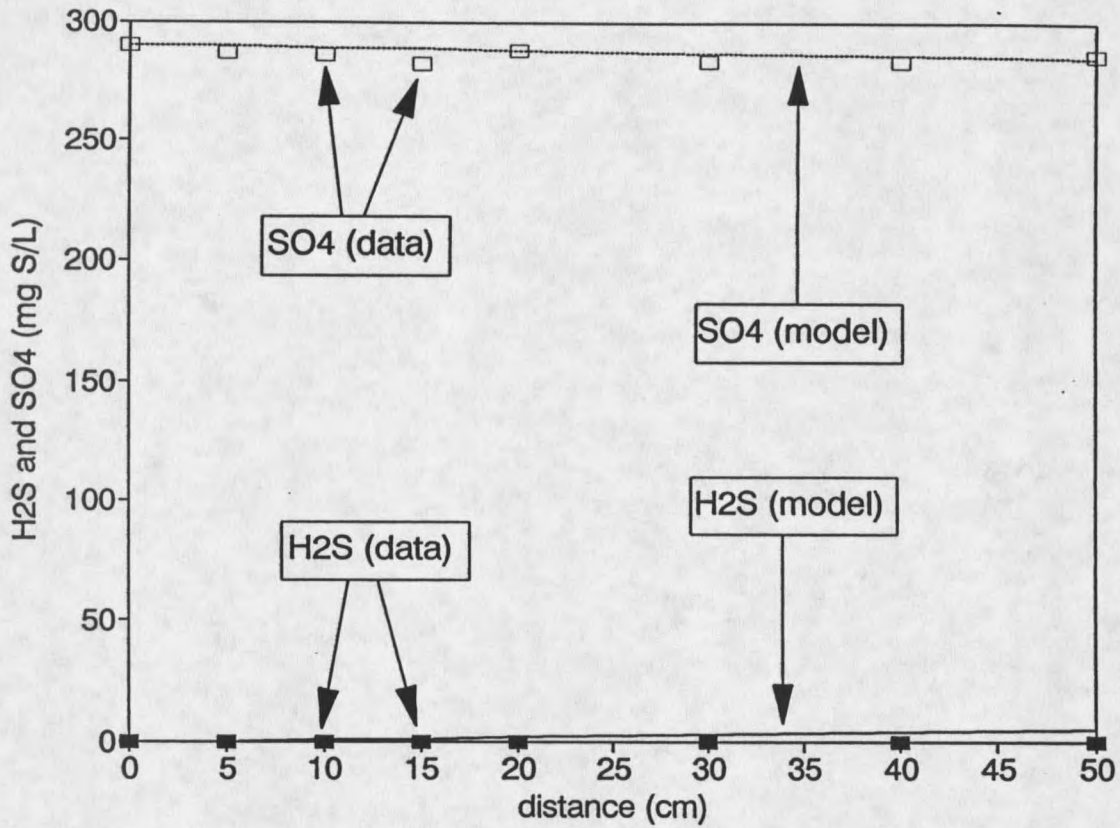


Figure 5.5b. H_2S and SO_4^{2-} concentration profiles in the North Sea column (predicted vs. data) during nitrate reduction, with the final set of assumptions.

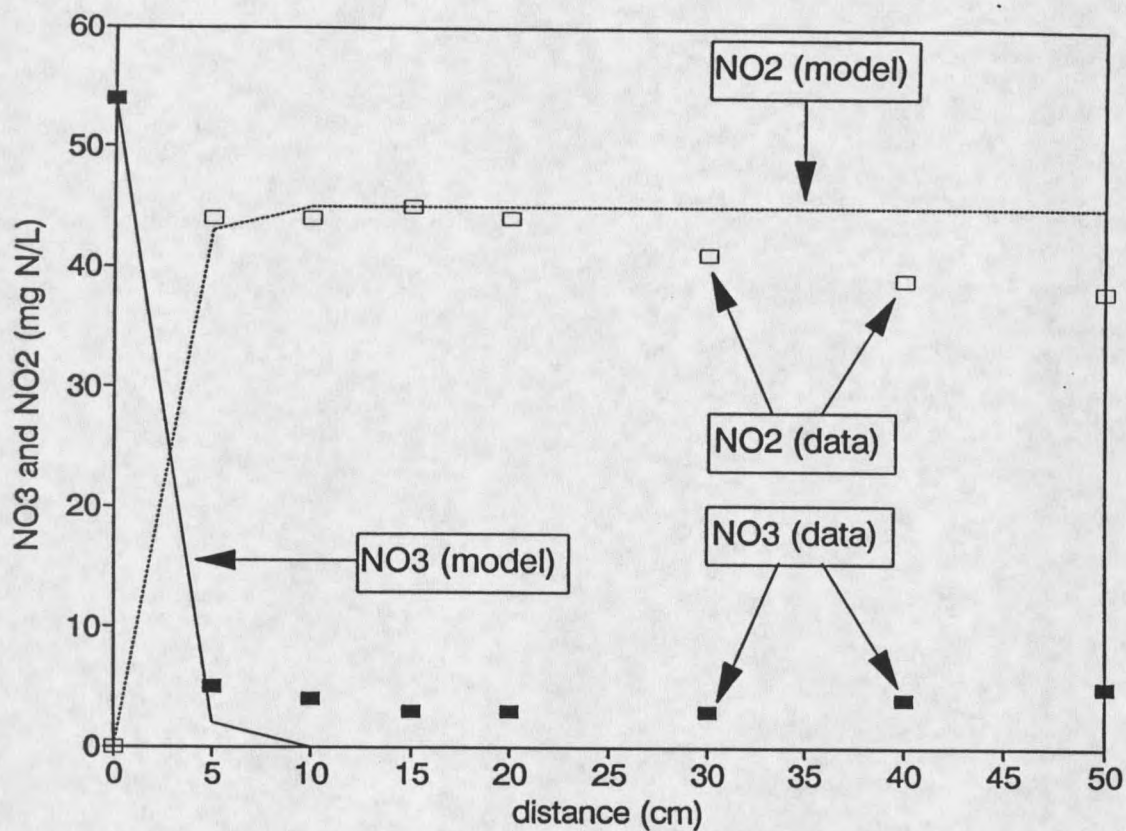


Figure 5.6b. NO_3^- and NO_2^- concentration profiles in the North Sea column (predicted vs. data) during nitrate reduction, with the final set of assumptions.

Table 5.3. NRB growth yields and revised kinetics used in the model.

substrate	Y_{xs} (g cells/ g substrate)	Y_{xe} (g cells/ g NO_3^-)	$\mu_{\max}$ (1/hr)	K_s (mg/L)	K_e (mg/L)
acetate	0.050	0.040	1.0	5.0	5.0
isobutyrate	0.050	0.040	0.5	5.0	5.0

The model also predicted, much better than the original model, that nearly all of the nitrate was consumed before the first sampling port (five cm), as shown in the NO_3^- concentration profile at steady state (Figure 5.6b). The concentration profile for NO_2^- , the product of incomplete denitrification of NO_3^- , is also shown in Figure 5.6b. The lower level of nitrite with these new assumptions more closely resembles the actual data. Concentration profiles for the carbon sources for NRB (acetate and isobutyrate) and the carbon source for SRB (*n*-butyrate) also agreed well with the experimental data (Figures 5.7 and 5.8, respectively), and were much closer than the original predictions.

Transition from Souring to Nitrate Reduction

Transition data from souring mode (where SRB are active) to nitrate-reduction mode (where SRB are inhibited because nitrite-producing NRB are active) may be obtained by using the souring steady state as a starting point, then adding NO_3^- so that NRB in the column become active. This scenario was performed in the column and was simulated in BAM. Steady state results from this scenario were exactly the same as those in the previous section but it is the non-

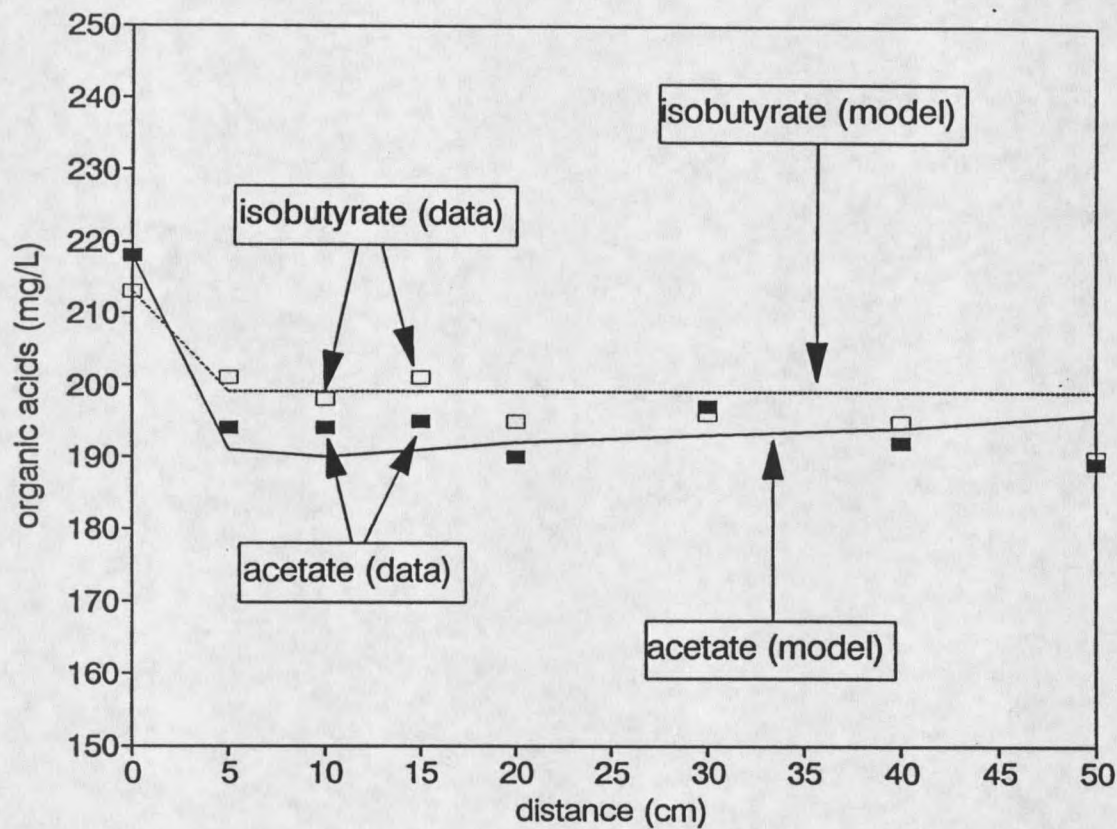


Figure 5.7. Acetate and isobutyrate concentration profiles in the North Sea column (predicted vs. data) during nitrate reduction, with the final set of assumptions.

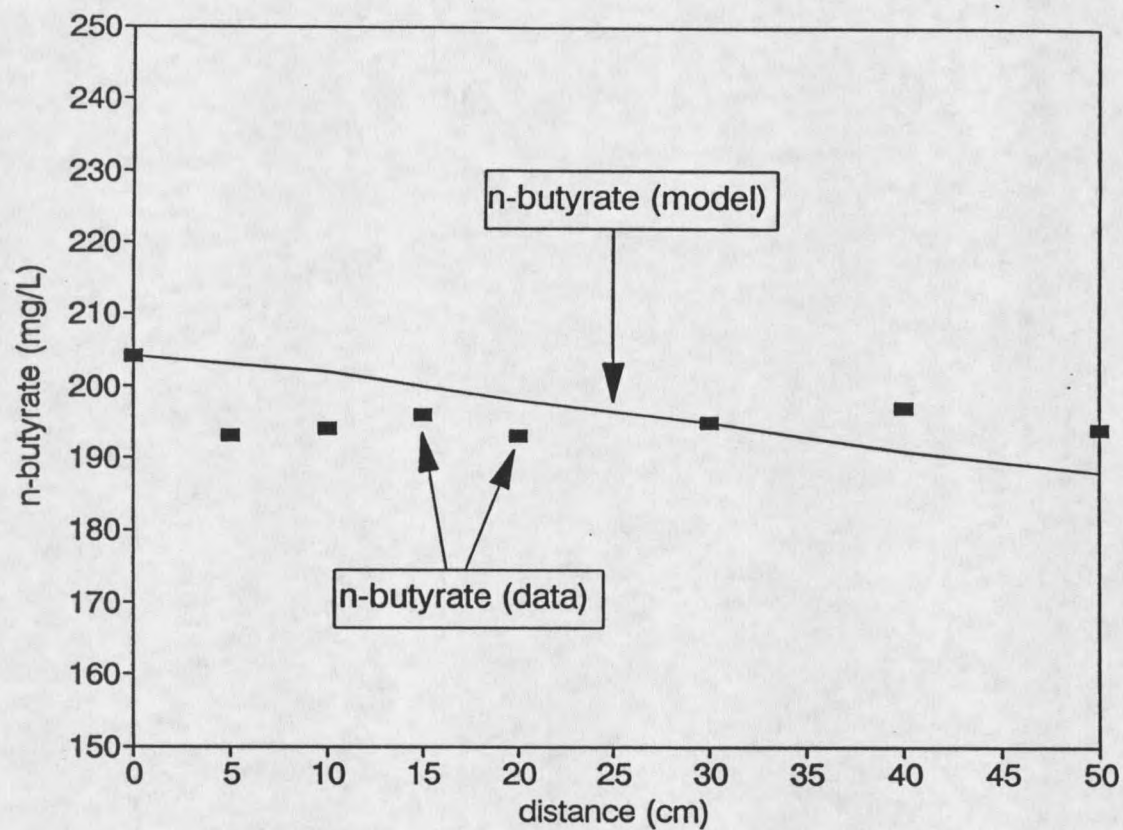


Figure 5.8. *n*-Butyrates concentration profile in the North Sea column (predicted vs. data) during nitrate reduction, with the final set of assumptions.

steady state results that are of interest here. Assumptions made in this scenario were:

1. *Both SRB and NRB are present and ready to grow in log phase.*
2. *Concentrations of all species at time zero are what they are during the souring steady state.*

Plotting the predicted nitrite concentration in the effluent saw it rapidly increase from zero to its steady state value of approximately 45 mg N/L in two days (Figure 5.9). This concentration of 45 mg N/L is the same as that shown at the effluent steady state in Figure 5.6b. The high nitrite concentration quickly inhibited all SRB activity in the model, seen when plotting effluent H_2S and SO_4^{2-} concentrations vs. time (Figure 5.10). After two days, the sulfide concentration dropped from nearly 200 mg S/L to almost zero, and the sulfate concentration rose to almost 300 mg S/L. Another modeling result was that NO_3^- was never seen in the effluent. The model NRB biofilm growing at the described growth rate consumed the NO_3^- so rapidly that it was all converted to NO_2^- before it reached the effluent (the residence time in the column was approximately 20 hours).

Nitrite Addition at Steady State

Complete inhibition of SRB activity has been shown after microbiological conversion of NO_3^- to NO_2^- , the inhibitory substance. The same effect has been seen in these column experiments in which the chemical agent NO_2^- is added directly, instead of requiring its conversion from NO_3^- . A comparison of

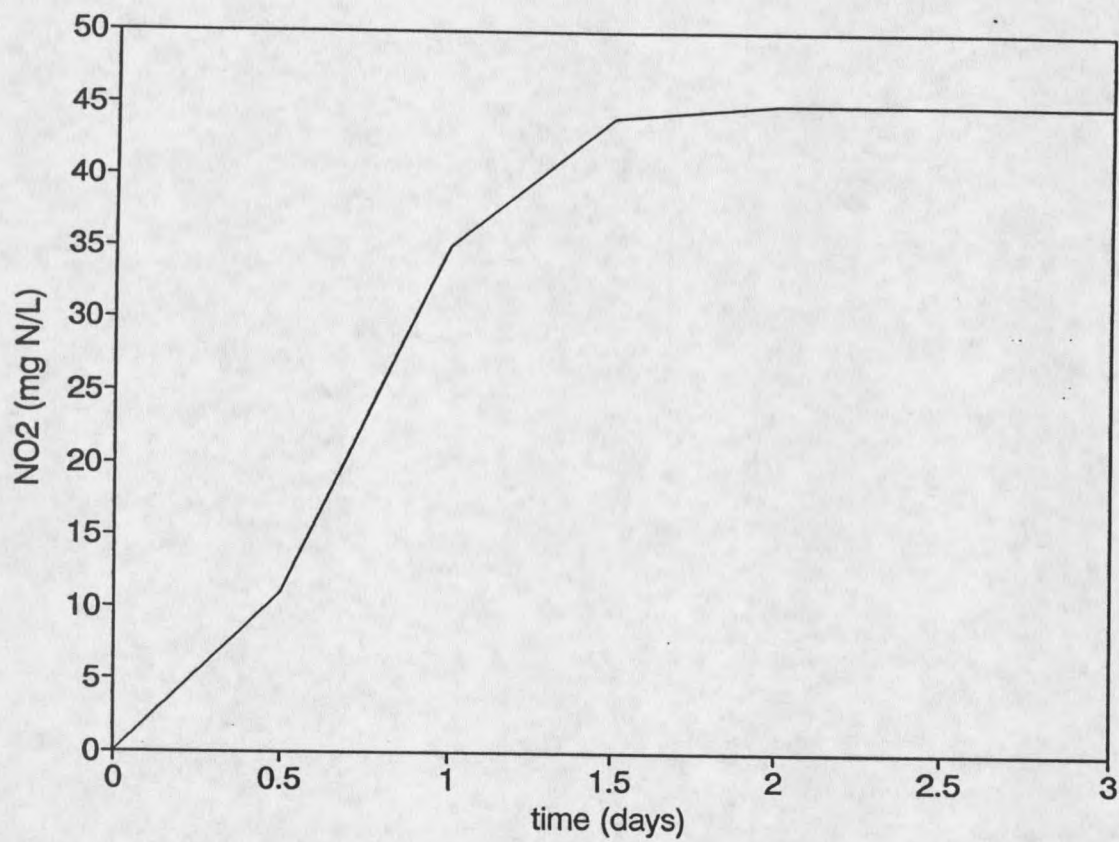


Figure 5.9. Predicted NO_2^- concentration in the effluent after addition of 54 ppm nitrate.

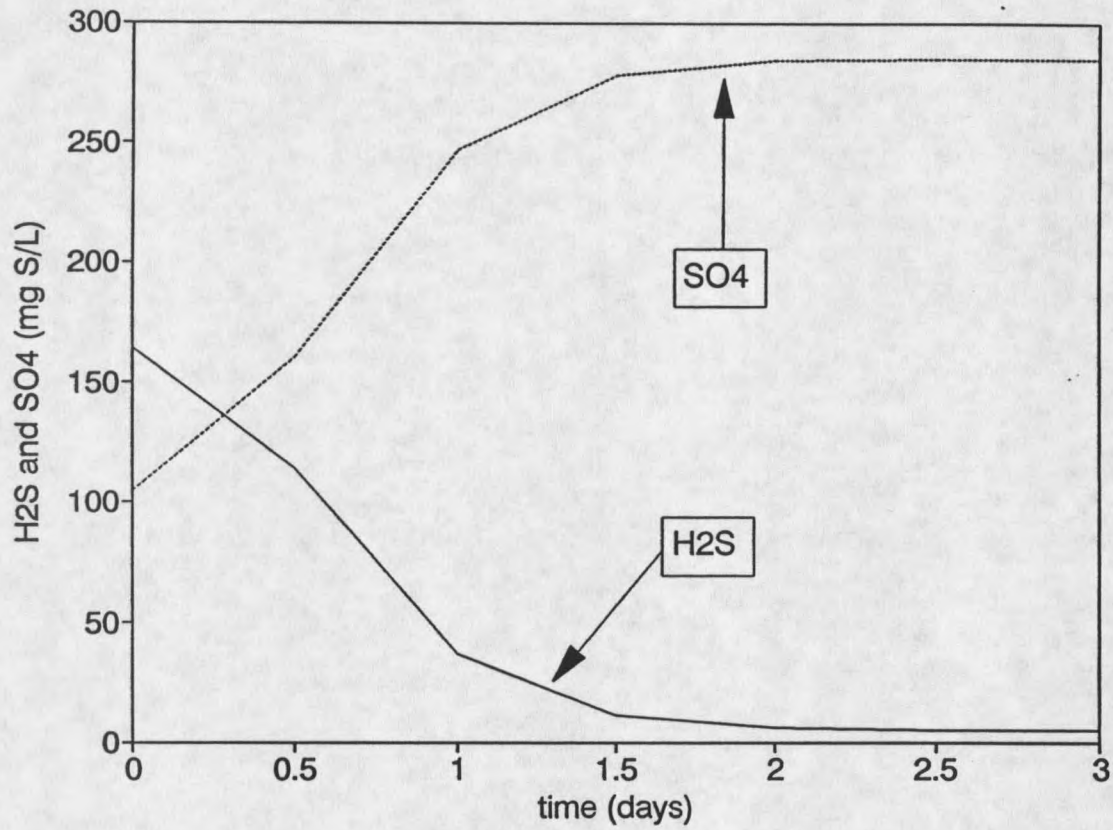


Figure 5.10. Predicted H_2S and SO_4^{2-} concentrations in the effluent after addition of 54 ppm nitrate.

experimental data and BAM predictions follows. Similar to the NRB transition scenario, the model assumed that the column was at a souring steady state at time zero. The only change made at time zero was that NO_2^- at a known concentration, and with the previously known inhibition constant K_i , was added as a step change.

It is known from the experiments detailed in Chapter 4 that 12 mg N/L of NO_2^- will completely inhibit SRB activity and H_2S production (by at least 99%) throughout a 50-cm column using the North Sea consortium. BAM with the previously determined parameters, however, showed that 12 mg N/L of NO_2^- inhibited souring by only 59% (compared to the normal effluent H_2S concentration of 164 mg S/L). Influent NO_2^- concentrations of 20, 30 and 40 mg N/L inhibited souring by 74%, 82% and 86%, respectively. This was much less inhibition than actually seen in the column when approximately 40 mg N/L of NO_2^- was present from reduction of NO_3^- , as zero or close to zero H_2S was measured. Thus, it appears that the model estimate of K_i , the inhibition constant for NO_2^- acting on SRB activity (which was already lowered from the initial estimate of one mg/L), was too high.

Lowering K_i from 0.2 mg N/L to 0.02 mg N/L approximated the column data for H_2S much closer, as shown in Figure 5.11. A K_i value of 0.01 mg N/L in the model provided the best data fit. The sulfate consumption rate and substrate consumption rates (data not shown) were also much lower with the lower K_i value, and approximated the data much closer than with the K_i value of 0.2 mg N/L.

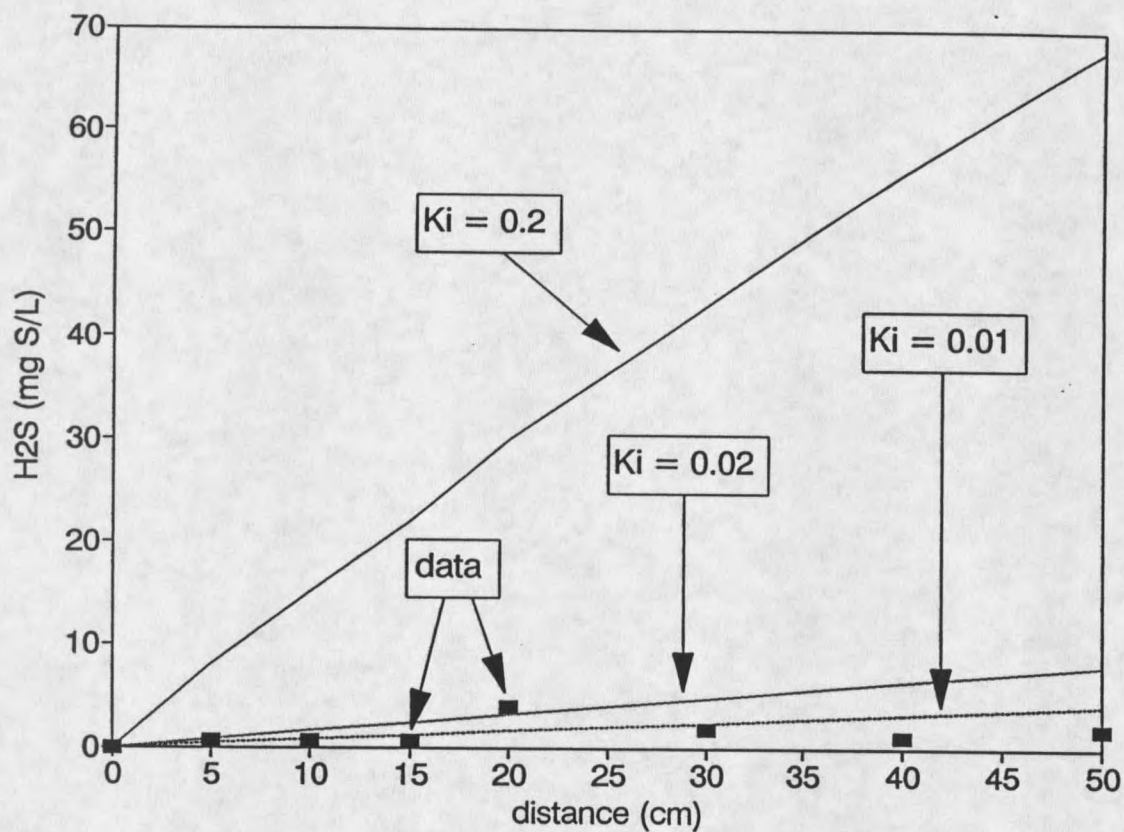


Figure 5.11. H_2S concentration gradients in the North Sea column (predicted vs. data) after addition of 12 ppm nitrite, with different K_i values.

Discussion

Souring at Steady State

The purpose of this study was to use a computer model to attempt to describe the activity of SRB and NRB in a column, using parameters available from our batch studies and previous literature. Model predictions using these initial assumptions were inadequate to accurately simulate the experimental system. Consequently several modifications, which are discussed and justified below, were made to BAM.

The first change was that a thinner biofilm thickness ($0.4\ \mu\text{m}$ vs. $4.0\ \mu\text{m}$) was used in the model. The $4.0\ \mu\text{m}$ thickness was based on ashing the total biomass measured in the column at the conclusion of a similar experiment (detailed in Chapter 2), in which the biomass was found to be fairly uniform throughout the column. This biomass, coupled with the surface area available in the column, equals an average biofilm thickness of $4.0\ \mu\text{m}$. Three factors, however, justify using a thinner SRB biofilm thickness in the model: 1) the biofilm is an unknown mixed consortia of anaerobic bacteria, only a portion of which are sulfate reducers; 2) the small media particle size makes patchiness and less effective substrate utilization much more likely; and 3) acridine orange direct counts of cells in the effluent during active souring, combined with the known surface area of the porous media in the column, produced a calculated biofilm thickness of $\ll 1\ \mu\text{m}$, assuming that the biomass concentration was at steady state. A thickness of $0.4\ \mu\text{m}$, or even lower

(such as a patchy biofilm one or two bacteria thick), is probably realistic. A biofilm thickness greater than 0.4 μm in the model resulted in much steeper concentration gradients than seen in any of the column experiments.

Secondly, several changes were made to the maximum growth rates (μ_{max}) used in the model. Assuming a growth rate for SRB on isobutyrate equal to the growth rate on *n*-butyrate produced a much steeper concentration gradient than seen in the column data. Since no data were available for growth of either bacterial consortium (North Sea or North Slope) on isobutyrate in batch culture, the best fit to the data was used. This predicted a very low growth rate of 0.010/day on isobutyrate.

For SRB growth on propionate, it appeared that two types of growth occurred—fast growth at the beginning of the column (first five cm) and much slower growth in the remaining 45 cm. The best way to model this was to include two different growth rates on propionate. The first growth rate was that found with the North Slope consortium in batch studies and was modeled as also being dependent on the presence of formate and *n*-butyrate—after these two substrates disappeared (in the first five cm), fast growth ceased. This growth rate on propionate may actually be dependent on formate and/or *n*-butyrate, or a fast-growing species of propionate-utilizing SRB may be present only at the beginning of the column.

The second, much lower growth rate on propionate was a best fit to the data in the last 45 cm of the column. This could occur if a second, slower-growing species of propionate-utilizers were present in the column. This scenario cannot

be proved but is possible since the North Sea consortium was an unknown mixed population. Using two vastly different maximum growth rates was the only way found to closely approximate the growth seen on propionate, as trying different inhibition kinetics failed to provide a satisfactory fit. Heyes and Hall (1983) reported at least two subgroups of propionate-using organisms in an anaerobic digester. Their values for $\mu_{\max}$ (0.050/hr and 0.0054/hr) are quite close to the values in this model (0.200/hr and 0.0050/hr).

Another model assumption which must be discussed is the partial oxidation of *n*-butyrate. One mole of *n*-butyrate can either be completely oxidized to four moles of CO₂ or incompletely oxidized to two moles of acetate. Based on stoichiometry from batch studies with the North Sea consortium, it was assumed in the model that "partial oxidation" occurs—half of the *n*-butyrate goes to CO₂ and the other half goes to acetate. This could occur if two species of *n*-butyrate-utilizing SRB were active at approximately equal growth rates, with one species of complete oxidizers and another species of incomplete oxidizers. Model predictions incorporating this theory of acetate production, along with no bacterial consumption of acetate (Figure 5.4b), match the data much closer than does the original model (Figure 5.4a).

The last modification made to the souring model was a change in overall stoichiometry for the column. An electron balance between the sulfate reduced and the substrates oxidized (shown in Chapter 2) indicated only about 60% of the electrons donated by the substrates could be accounted for in sulfate reduction,

based on actual data from the North Sea column. Therefore, the sulfate consumption rate based on the aforementioned stoichiometry with each substrate was adjusted downward by 40%. If this 40% factor were not used, sulfate rapidly became limiting in the model, which was never seen in the column. One explanation for this phenomenon is that fermenters or some other type of organism, consuming carbon sources without consuming sulfate, were present in the column. Products of this metabolism, such as H_2 , CH_4 and CO_2 , were not measured so this theory cannot be proved nor disproved from the experimental data. However, this same "60% electron balance" was also seen with data from the North Slope consortium at a souring steady state, as shown in Chapter 2.

A fairly large discrepancy still exists between the actual and revised model values for H_2S in the column (Figure 5.2b). A small amount of iron in the liquid medium was supplied to the column and a significant amount was attached to the Berea sandstone media, and it was seen that the black precipitate FeS readily formed and moved up the column as souring activity was initiated. It has been reported (Ligthelm et al., 1991) that H_2S scavenging by minerals in a formation will lower the H_2S concentration. Adding a simple zero-order reaction between H_2S and iron to the model with a rate constant of about 10/day allows one to very closely match the experimental data for H_2S (data not shown). Reversing the reaction during inhibition of souring is also plausible since the black precipitate was seen to slowly disappear over time. The dissolution reaction with this rate constant, however, puts far more H_2S into the aqueous phase than was seen in any of the

souring inhibition experiments. The discrepancy between actual and model H_2S values, therefore, was probably due to the system not yet being at steady state, as much of the H_2S being generated was still precipitating with available iron farther up the column and was not measured in the aqueous phase samples. It is also possible that the dissolution reaction is slower than precipitation. For simplicity of the model, however, it was decided to exclude any Fe- H_2S reaction.

Inhibition of SRB by acetate, with a K_i as low as 54 mg/L, has been noted by several researchers (Reis et al., 1990). Incorporating this inhibition constant into the model predicted slower growth than observed, especially at the beginning of the column. This fact, along with measured acetate concentrations of 500-1000 mg/L in the two oilfields where active SRB have been obtained for this research, also led to the exclusion of this phenomenon from the model.

This modeling scenario of souring at steady state essentially predicts the outcome of initiating a column which contains the requirements for souring (SRB, SO_4^{2-} and SRB-preferred carbon sources) and which does not contain at least one of the requirements for nitrate reduction (NRB, NO_3^- and NRB-preferred carbon sources). Until field test data are available, it is the best prediction of what will occur when waterflooding (SO_4^{2-} injection) is initiated in a reservoir. Since it appears that SRB, NRB and many carbon sources are already present in a reservoir, addition or withholding of NO_3^- in the injection water is the key parameter in this scenario.

Nitrate Reduction at Steady State

Two changes to kinetic parameters for nitrate-reducing bacteria were made in BAM to account for the actual behavior in the column. Estimated maximum growth rates were lowered from 1.44/hr to 1.0/hr (growth of NRB on acetate) and to 0.50/hr (NRB on isobutyrate). These growth rates for NRB, which are still significantly higher than those for SRB, matched the actual results much better than did the original model.

These changes in kinetics do not explain the five mg N/L of NO_3^- which actually remained in the column after five cm (Figure 5.6b). This level is close to the detection limit for nitrate using the ion chromatograph at the given dilution but is still measurable. The unreacted nitrate could not be explained by varying the kinetics and still match the rapid consumption rate at the beginning of the column. Phenomena which could possibly cause this small amount of nitrate to remain unreacted include non-Monod kinetics or nitrate complexing into a non-bioavailable form. The 40% factor for adjusting the stoichiometry of sulfate reduction was also used in this model, although this was probably not important, since very little sulfate reduction occurred. A small amount of H_2S (< 5 mg S/L) could have been produced and gone undetected in the column, since this was the approximate detection limit.

Another discrepancy exists in the NO_2^- concentration, as the model did not predict the decrease in NO_2^- concentration at greater distances through the column (Figure 5.6b). One hypothesis is that nitrite, a very reactive ion, reacts with H_2S to produce N_2 and elemental sulfur. Batch experiments, however, failed to produce

this reaction. Another possibility is that the intermediate NO_2^- was slowly proceeding to N_2 through complete microbial denitrification. However, this metabolism, when it occurs, is ordinarily so rapid that NO_2^- cannot be detected. A third possibility, and the most likely, is that nitrite was chemically reacting with a species other than H_2S in the column.

One change in stoichiometry was made from the original NRB model—only 84% of the nitrate reduced was measured as nitrite in the column. Thus, although 54 mg N/L of NO_3^- was consumed in the first five cm of the column, no more than 45 mg N/L of NO_2^- was measured at any point in the model. It was seen in batch studies by Mueller (1994) at 60°C and high salinity (the same conditions at which the column was operated) that 80-100% of the nitrate reduced went to nitrite. The remainder probably went to N_2 gas or another nitrogen intermediate. No analysis of these species was performed.

The last change to BAM for nitrate reduction involves K_i , the inhibition constant for NO_2^- acting on SRB. From behavior in the column when NO_3^- was introduced, it was known that K_i must be small—therefore, an initial estimate of one mg N/L was used. It was found that K_i could be no higher than 0.2 mg/L in the model, however, to predict the degree of souring inhibition seen. This K_i value is the maximum value to explain the column behavior during nitrate reduction—it may be even lower.

This scenario simulates the suppression or prevention of souring in a column where SRB and NRB are both present along with their respective growth substrates

(SO_4^{2-} and SRB-preferred carbon sources, and NO_3^- and NRB-preferred carbon sources). Whether the NO_3^- was introduced initially or after souring had begun, the same steady state (in which little or no soluble H_2S was measured in the column) was quickly reached.

Transition from Souring to Nitrate Reduction

Model results showing the unsteady state behavior of the column after NO_3^- was added to the medium approximated the column data. The main difference is that a lag phase was observed in the column, in which nitrate-reducing bacteria required approximately two weeks to become active after the addition of nitrate. During this lag phase, souring activity carried on as normal. But within three days of the first observed consumption of nitrate (data not shown), the column had very nearly reached the steady state modeled in the previous scenario. Nearly all of the NO_3^- was consumed in the first five cm, and no H_2S was measured anywhere in the column within three days after the first measured disappearance of NO_3^- . This is consistent with the modeling of this transition, which predicts that the sulfide concentration will drop to nearly zero within two days (Figure 5.10), and the nitrite concentration will reach steady state after two or three days (Figure 5.9). More support for the model comes from the nitrite addition experiment detailed in Chapter 4, in which addition of 50 mg N/L nitrite completely inhibited souring at the beginning of the column in less than a day.

Nitrite Addition at Steady State

It was found in the second scenario, nitrate reduction at steady state, that a K_i value of 0.2 mg N/L for the inhibition of NO_2^- on SRB more accurately modeled the data profiles seen for H_2S production and substrate consumption than did the original model. In that case, approximately 56 mg N/L of NO_3^- was added and about 40 mg N/L of NO_2^- was observed in the column. In subsequent column experiments (Figure 5.1), it was seen that the NO_3^- level could be reduced to as low as five mg N/L while maintaining inhibition, and that an actively souring column could be effectively "unsoured" with as little as 10 mg N/L of NO_3^- . Experiments involving the direct addition of nitrite, instead of requiring its biological production from nitrate, were run later and thus employed the lower levels of nitrite known to be effective.

Using this low level of nitrite in the model, such as 12 mg N/L, combined with the previously determined K_i value of 0.2 mg N/L, was found to be insufficient to completely inhibit souring via nitrite addition. A lower K_i value, 0.01 mg N/L, was found to be required in the model. This is a revised maximum value for the inhibition constant—again, it may actually be lower. Including the lower K_i in the nitrate reduction model also predicted H_2S and SO_4^{2-} concentrations another increment closer to their measured values (data not shown).

The model was found to be quite insensitive to many of the other parameters which could be varied in BAM, such as the boundary layer between the biofilm and the bulk liquid (10^{-5} m was used), the diffusivities of the dissolved species, and the diffusivity in the biofilm divided by the diffusivity in the bulk liquid (a ratio of 0.8 was

used). It was, however, found to be quite sensitive to the biofilm thickness and the growth parameters, which is why some of these were ultimately adjusted in the model.

This study illustrates the utility of computer modeling as a tool to be used in the formulation of conceptual models and in guiding continued experimental work. The modeling results reported here are exploratory; they do not prove or disprove hypotheses. The results are quite useful, nevertheless, as they help diagnose weak points in the formulation of a conceptual model of microbial dynamics in porous media. In this case, the computer modeling results indicated that there were phenomena missing from the original formulation. Further simulations suggested candidate phenomena that, when added, could better account for the experimentally observed behavior. The computer modeling studies also helped identify parameter values that were important but not adequately determined by the available experimental data. In summary, the value of computer modeling in this study was to provide focus. From a complex melange of phenomena and parameters, the modeling results have highlighted a few processes or key parameters that appear to govern the remaining discrepancies between experimental and theoretical observations.

Conclusions

A computer model was developed to describe the activity of a mixed microbial population growing as a biofilm in a porous media column, and model

predictions were compared to experimental data. The experimental system simulated the environment in an oil field reservoir subjected to secondary oil recovery by injection of seawater. The activity of the microbial consortium, which contained sulfate-reducing bacteria (SRB) and nitrate-reducing bacteria (NRB), was modeled in four scenarios: souring (production of hydrogen sulfide by SRB); nitrate reduction (production of nitrite by NRB), with subsequent inhibition of souring; transition from souring to nitrate reduction; and souring inhibition by the addition of nitrite. Initial model runs made using independent estimates of physical and biological parameters (from batch culture kinetic studies and from literature, for example), were not able to adequately describe all features of the experimental results. Additional computer model simulations were conducted to identify potential phenomena missing from the original model and to focus on key parameter values that were particularly important in governing the remaining discrepancies between experiment and theory. Additional phenomena that were not thought to be important after performing additional simulations were the reaction of hydrogen sulfide with nitrite and the inhibition of SRB by acetate. Candidate phenomena for inclusion in a revised model were partial denitrification of nitrite to nitrogen gas, and utilization of propionate by two distinct pathways or microorganisms. Key parameters identified by the simulations were the biofilm thickness and the inhibition constant for nitrite. A modified version of the original model captured the qualitative behavior of the experimental system. This study illustrates the utility of

computer modeling in the formulation of new hypotheses and in suggesting appropriate areas to focus further experimental effort.

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CHAPTER 6

SUMMARY AND IMPLICATIONS

General Conclusions

Similar souring activity was found in both consortia tested in porous media columns: most of the H_2S production was at the beginning of the columns; formate and *n*-butyrate were preferred carbon and energy sources, with no utilization of acetate; and cell yields were very low. While essentially all of the column activity was performed by biofilm cells, these results are consistent with those from batch experiments using suspended cells (Mueller, 1994). Biomass and iron sulfide were distributed fairly evenly within the columns and some plugging was evident, especially in systems with a lower initial permeability.

When nitrate was added to the columns, nitrate-reducing bacteria (NRB) in the original mixed consortia produced nitrite and inhibited sulfate-reducing bacteria (SRB). Ten mg N/L nitrate or greater completely inhibited souring and five mg N/L was required to maintain the inhibition. Changes in E_h were noted during this time but the effect of nitrate/nitrite on E_h is not completely understood. It appears that nitrite is the active inhibitory agent. It also appears that nitrite is slowly reduced to nitrogen gas or can be biologically converted to ammonia, both of which lower the nitrite concentration and thus its inhibitory effects. Low cell yields were again noted with both consortia during nitrate reduction, and cells present in the column had a different morphology than during sulfate reduction. Removal of nitrate allowed

souring to resume in the columns, even after SRB had been completely inhibited for as long as three months.

Direct addition of nitrite to the columns achieved the same results as nitrate addition. About 12 mg N/L nitrite completely inhibited souring, and pulses of nitrite, in which the same total amount of nitrite was added as during continuous addition, were even more effective. Nitrite addition decreased plugging, apparently due to the dissolution of precipitated iron sulfide. Nitrite was a more effective and longer-lasting inhibitor than was glutaraldehyde. It appears that glutaraldehyde kills SRB while nitrite provides some type of long-term inhibition to enzyme activity.

An initial computer model, developed to simulate and predict activity of SRB and NRB in the porous media columns, differed greatly from the experimental data. Biofilm thickness and K_i , the Monod inhibition constant for nitrite acting against SRB, were found to be key parameters in the model. Other phenomena examined in revised models included the possible reaction between H_2S and NO_2^- , the K_i for acetate acting against SRB, reduction of nitrite to nitrogen gas, utilization of propionate as a carbon source, and kinetic coefficients for other substrates consumed by SRB and NRB. A final model captured the qualitative behavior of the system.

Implications for a Sour/Potentially Sour Reservoir or Facility

Two scenarios are commonly discussed when describing oilfield souring. In the first scenario, all of the nutrients required for SRB growth (carbon source as

electron donor and sulfate as electron acceptor) are contained in the injection water; consequently, most of the souring occurs close to the injection well (within several meters). In the second scenario, sulfate is contained in the injection water but the carbon source is contained in the reservoir; consequently, souring occurs in a "mixing zone" deeper within the reservoir.

Injection Well Souring

Control of souring by way of any strategy would be simplified if the first scenario were true. Pulse addition of nitrite would probably be very effective if souring were occurring close to the injection well. This was the scenario simulated by the column experiments and in the computer model, in which the carbon source and sulfate were added simultaneously to the beginning of the column (where SRB were located after inoculation) and most of the souring occurred within a short distance. It also appears that continuous addition of nitrite would also be effective, as long as the concentration reaching the SRB's were 12 mg N/L or greater. Higher concentrations may have to be added for souring occurring slightly away from the injection well to compensate for dispersion (in the case of a pulse) and for nitrite reaction with other constituents in the reservoir. In the 20-hour residence time in the column, the nitrite concentration decreased by 10-20%. With high flow rates near the injection well, it is expected that nitrite, whether chemically added or biologically produced, would reach the active souring zone within a day and effectively inhibit souring there. If nitrite is continually depleted through reaction,

however, the possibility exists that souring could then occur deeper in the formation. If this scenario were to occur, reservoir souring has essentially been substituted for injection well souring, which is probably not an advantage.

Batch experiments showed that high salinity and low temperature both limit the effectiveness of nitrate addition, as much of the produced nitrite is further reduced to nitrogen gas (Mueller, 1994). The highest salinities and lowest temperatures in the formation would occur at the injection well, especially in pure seawater injection, so these factors would have to be taken into account when considering nitrate addition to control injection well souring. Salinity and temperature would probably affect nitrite addition in the same manner, as added nitrite could also be further reduced.

Deep Souring

In the second scenario, deep reservoir souring, pulse addition of nitrate or nitrite would not be as effective due to diffusion, mechanical dispersion and possibly nitrite reaction. Factors such as increased distance from the injection well, shortened time of pulse and increased permeability of the formation would all serve to dilute the concentration of the nitrite eventually reaching the SRB, thus lowering its effectiveness. If the reservoir flow pattern is well characterized, the concentration at different depths within the reservoir can be modeled.

If this second scenario accurately describes the souring, continuous addition of nitrite may be a better option. With continuous addition, there would be some lag

time between the initial introduction of nitrite and an effective concentration reaching the souring zone (due to dilution), but this concentration in the reservoir would increase over time and should reach a steady-state value approaching the nitrite concentration in the injection water. Continuous addition would rely less on the characteristics of the reservoir than would pulse addition. For deep souring, inhibitory concentrations of nitrite would probably reach the active SRB within several weeks or months, and the results would probably be seen several months later at a producing well. For injection well souring, the results could be seen almost immediately by "back-flowing" the injection well to check for H_2S and nitrite.

An intriguing idea which has been discussed (Mueller, 1994) involves pulse addition of nitrate, which was not done experimentally. The theory behind this method is to add large amounts of nitrate, with a limited carbon source, so that substrate is available only for the first step in nitrate reduction (nitrite production), and the reaction cannot proceed to nitrogen gas or ammonia. If nitrite reaction is determined to be significant, then addition of nitrate (especially pulse addition) would be the best solution for reservoir souring.

Deep in the formation, the higher temperature and lower salinity would reduce the reaction rate of nitrite going to nitrogen gas or ammonia, increasing its inhibitory effects. So although deep reservoir souring is less understood and more difficult to monitor, it may actually be easier to control because of the environmental conditions normally existing there.

Souring in Surface Facilities

Control of souring in surface facilities, which is very common, would be easier than at either the injection well or in the deep reservoir. The surface system is better defined and easier to manipulate, and it is simpler to monitor results. Nitrite addition would be the easiest method of control here. With nitrite, one would not have to be concerned about the presence of NRB or an anaerobic environment for their growth. Although it has not been proven experimentally, a reaction between H_2S and NO_2^- may occur which would "remediate" souring even without microbial inhibition.

No matter where the souring is occurring or how the nitrate/nitrite is added, control should be very cost-effective compared to glutaraldehyde addition. Glutaraldehyde and nitrite have about the same raw material cost on an active-weight basis, and it has been shown that low concentrations of nitrite are more inhibitory and maintain longer-lasting control than glutaraldehyde.

Recommendations for Future Research

Experiments should be done to more precisely define an effective scheme for pulse addition of nitrite to a souring environment. The nitrite concentration, pulse duration and time between pulses could be optimized to determine the least amount of nitrite required to achieve a certain degree of souring inhibition. The only pulse data now available shows that a five-minute pulse of 480 mg N/L of NO_2^- every four hours more completely inhibits souring occurring close to the point of

injection in a laboratory column than the same amount of nitrite in continuous addition. The slow recovery of H_2S production after nitrite removal suggests that pulses of nitrite at infrequent intervals may still be very effective. Nitrate pulsing should also be done to determine whether this would indeed decrease the amount of nitrite reduction by having a limiting carbon source.

A larger-scale demonstration should be done to more precisely define an effective scheme for pulse addition of nitrate/nitrite to a souring environment. Porous media permeability should be varied and the point of carbon source introduction could also be varied to simulate deep reservoir souring. With a large system and a low flow rate, the phenomenon of nitrite disappearance could be studied in-depth and the fate of the reacted nitrite (e.g., as N_2 , N_2O , NH_3) should be determined.

Another experiment which should be performed is to initially add nitrate or nitrite to a potentially souring system (i.e., sulfate, carbon sources and SRB are also introduced). This would simulate the initial stages of a water-injected oil field. All indications are that any souring activity in a laboratory system would be prevented but this has not been tried experimentally.

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APPENDICES

APPENDIX 1**TRACER EXPERIMENTS IN A POROUS MEDIA COLUMN**Introduction

Flow in a porous media reactor may range from nearly plug-flow, in which there is minimal mixing in the axial direction, to a CSTR (complete stirred tank reactor), in which there is complete mixing in the axial direction and the concentration anywhere within the reactor equals the concentration of the effluent. In a fairly long, narrow reactor such as the columns used in these experiments, it was envisioned that the flow pattern would be better approximated by a PFR (plug-flow reactor) than by a CSTR. Phenomena such as dispersion, retardation and irreversible adsorption, which can be estimated from tracer experiment results, may become important in analysis of the column experimental results. It was decided to characterize the flow and dispersion within the column to aid in the interpretation of the chemical and microbial data gathered in previous experiments.

Biofilm development can significantly change the porosity and flow patterns within porous media. Diffusion of tracer in and out of a thick biofilm could create significant distortion of residence time-distribution (RTD) curves (Stevens et al., 1986). Retardation due to sorption may also be significant for certain solutes (Roberts et al., 1986). Therefore, it was desired to obtain concentration vs. time curves before and after biofilm development to explore the effects of the biofilm. It was also important to explore the difference between flow of an inert tracer and

flow of a microbial cell (or cell-sized particle). The goal of these experiments was to explore the effect of a biofilm on the flow properties (dispersion and retardation) of both an inert tracer and microbial cell-sized beads.

A computer model was developed to explore the behavior of the mixed microbial consortia in the column (Chapter 5). Another objective of these tracer experiments was to compare the flow characteristics of the actual column to those of the simulated column, in order to gain information about the model's usefulness and limitations.

Materials and Methods

Reactor System

The North Slope produced water column described in Chapter 2 was the anaerobic porous media reactor system used for these experiments. The reactor was a packed bed (50 x 5.5 cm) column made of polycarbonate and equipped with six sampling ports. Only the four sampling ports located at regular intervals on one side of the column (9, 19, 29 and 39 cm from the inlet) were used. The porosity of the column after packing with crushed Berea sandstone was 0.49. The column was maintained at 60°C in an incubator. The flow rate of the medium was set at 650 ml/day, which equals an average pore velocity of 55 cm/day. Other details of the column set-up and operation were reviewed in Chapter 2.

Pulse inputs were used in these experiments. A tracer input, introduced in a time shorter than five percent of the mean residence time of liquid in the reactor,

is considered a pulse input (Riemer et al., 1980). The volume of tracer should be less than one percent of the reactor volume.

Bacterial Consortia and Cultivation

A sample of produced water from the Kuparuk North Slope oil field was used for the microbial consortia in this experiment. The column was inoculated by flowing one pore volume (approximately 600 ml) of freshly obtained produced water into the column. The produced water was purged with nitrogen before and during the introduction to the column. Details of the consortium and growth medium are included in Chapter 2.

Experimental Procedure

Four different types of tracer experiments were run in the North Slope PW column, all using a pulse injection method. Inert tracer experiments were performed before and after biofilm development, and fluorescent bead experiments (to simulate cell migration) were also performed before and after biofilm development. Experiments "before biofilm development" were run before inoculation of the column with produced water, so were all done within the first month of column operation. A summary of tracer experiments in the PW column is shown in Table A1.1.

In all experiments, a small volume (≤ 1 ml) of solution (compared to a total column liquid volume of approximately 600 ml) was quickly injected into the tubing immediately preceding the column inlet. Subsequent concentrations at each of the four sampling ports were determined by sampling small volumes (≤ 0.5 ml) at

regular time intervals. It was determined that the sample volumes removed were less than five percent of the total flow.

Table A1.1. A summary of pulse-injected tracer experiments in the North Slope PW column.

<u>experiment</u>	<u>date</u>	<u>pulse</u>
1	3/3/94	0.1 ml of 25,000 ppm SO_4^{2-} (before biofilm)
2	3/10/94	0.1 ml of 25,000 ppm SO_4^{2-} (before biofilm)
3	3/23/94	0.1 ml of 2.0×10^{10} beads/ml (before biofilm)
4	3/29/94	1.0 ml of 2.0×10^{10} beads/ml (before biofilm)
5	4/27/94	1.0 ml of 25,000 ppm Br^- (after biofilm)
6	5/2/94	1.0 ml of 2.0×10^{10} beads/ml (after biofilm)

In the first experiment, 0.1 ml of a sulfate solution containing 25,000 mg S/L was added in a pulse. This experiment was repeated one week later (the second experiment). In the third experiment, also before biofilm development, 0.1 ml of a fluorescent bead solution was injected. This solution, originally containing 4.0×10^{10} beads/ml, was diluted 1:2 with bovine serum albumin (BSA) to minimize sticking to the porous media, based on previous experience with these beads (Stoodley, 1994). The beads (L-5280, Molecular Probes, Inc., Eugene, OR) were 1.0- μm blue fluorescent spheres, latex and carboxylate-modified, in a 2% solids solution. A 2% BSA solution was made by mixing 0.05 g BSA in 2.5 ml nanopure H_2O . At the same time, two ml of the bead solution was autoclaved in a test tube. Two ml of 2% BSA solution was then added to the test tube through a 0.22- μm filter

(Gelman). After vortexing, 0.1 ml of this 1% BSA/bead solution was injected into the column.

A fourth experiment, again without biofilm in the column, was run one week later by injecting 1.0 ml of the same BSA/bead solution. A larger volume was used in an attempt to gain greater precision by sampling larger numbers of beads.

The last two experiments were run with a mixed-consortium biofilm actively souring in the column, about four weeks after inoculation with produced water. Bromide (Br^-) was used as the inert tracer in this case, as sulfate would have been consumed by sulfate-reducing bacteria. Bromide was not used in the "before biofilm" experiments because a satisfactory analytical technique for bromide had not yet been developed in our laboratory. One ml of a solution containing 25,000 ppm Br^- was added in a pulse. The final experiment, flow of beads through a column with biofilm, was run by again adding 1.0 ml of the BSA/bead solution.

Analytical Methods

Sulfate was measured using a Dionex ion chromatograph (model AI-450; Dionex Co., San Francisco, CA) with a pulse electrochemical detector (model DX-300) using a Dionex Ionpac AS4A-SC column (2 mm) and a sodium carbonate eluant. Sulfate was expressed in terms of sulfur content (S). Bromide was measured using the same Dionex system and column, with a sodium carbonate/sodium bicarbonate eluant.

Fluorescent bead counts were performed in a manner similar to the microbial cell count method referred to in Chapter 2. Aqueous 0.5-ml samples were removed from the column by inserting a needle through the thick rubber stopper located at each sampling port, then diluting 1:2 with nanopure water. Each one-ml sample was filtered through a 0.22- μm black filter (Fisher Scientific) and placed on a microscope slide. Slides were viewed under an ultraviolet-light microscope at 1000x and the number of beads per field-of-view (FOV) was counted. For low bead counts (<10 beads/FOV), 20 FOV's per sample were measured; for high bead counts, 10 FOV's per sample were counted.

Data Analysis Methods

Concentration vs. time data were analyzed in several different ways to determine D_I , the dispersion coefficient. D_I in a flowing system is the hydrodynamic dispersion coefficient, defined by Freeze and Cherry (1979) as:

$$D_I = \alpha_l v + D^* \quad (1)$$

where:

α_l = dispersivity (property of the media)

v = average pore velocity

D^* = molecular diffusion coefficient in porous media

and:

$$D^* = \omega D \quad (2)$$

where:

D = molecular diffusion coefficient in water

ω = property of porous media ($0.01 \leq \omega \leq 0.5$)

Therefore, if values for D , ω , v and α_l are known, then D_l can be calculated. Or, if D_l is calculated from another method and all but one of the other variables are known, ω or α_l can be determined. From equation (1), it is seen that D_l is dependent on both advection and molecular diffusion. At higher flow rates, the $\alpha_l v$ term becomes larger and the effect of molecular diffusion may become insignificant.

Four different methods were used to estimate D_l , the hydrodynamic dispersion coefficient for the column: 1) moments analysis, 2) a best-fit to the analytical solution of the advection-dispersion equation given by Robbins (1989), 3) a best-fit of concentration curves to curves at known Peclet numbers, and 4) a prediction based on d_p , the average particle diameter.

The first method, moments analysis, can be used to calculate the mean residence time, dispersion coefficient and skewness for the data. The dispersion coefficient was calculated by utilizing the concentration vs. time data at each sampling port in the equations listed in Levenspiel (1972), but this method provided unsatisfactory values, as two of the coefficients were below zero. I believe that the data obtained was not precise enough for the moments analysis method, so other methods were then researched. As noted by Wen and Fan (1975), "A serious fault of this method is that too much weight is placed on the tail.....As has been stated, the tail ... may not be reliable due to the difficulties of measuring tracer

concentration accurately at low concentrations." The other three methods were successful in obtaining estimates for the dispersion coefficient in the column, and these will be detailed in the next section.

Results

Before Biofilm Development

Inert tracer experiments. In the first inert tracer experiment, 0.1 ml of 25,000 ppm SO_4^{2-} was injected into the column (it was determined in an earlier experiment that sulfate did not react with or adsorb onto the crushed Berea sandstone packing). Samples were taken and concentration vs. time curves were plotted but by saving effluent from the column, it was found that SO_4^{2-} recovery was approximately 1000% (i.e., ten times as much sulfate exited the column as was pulse-injected). This was due to residual sulfate from the sandstone washing out with the medium, as flow had been started only a day or two earlier. Medium was run through the column for another week, after which time no sulfate was detected, so the experiment was then repeated.

Results of the second sulfate experiment were much better. This time, a mass balance on sulfate collected at the effluent was within eight percent of the amount injected. The error decreased to four percent after accounting for sampling losses. This could easily be explained by inaccuracies in the amount injected or in the ion chromatograph analysis.

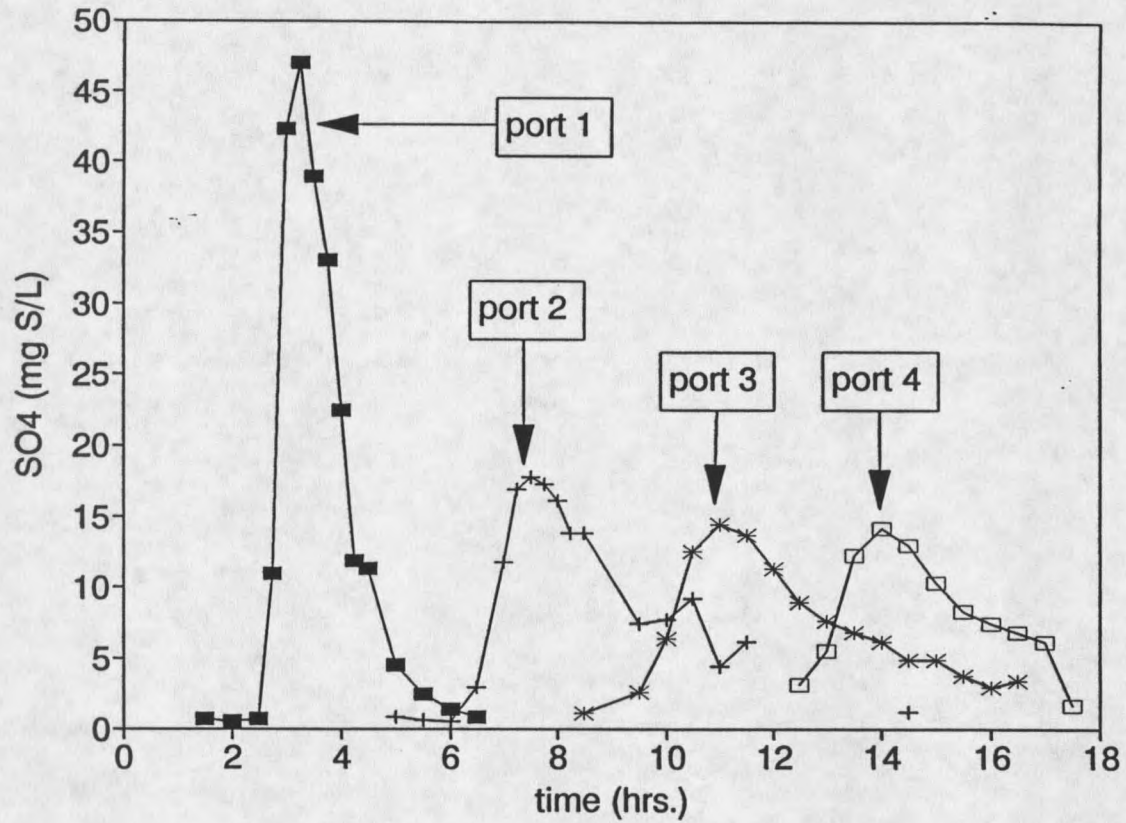


Figure A1.1. Tracer output curves at different distances after injection of a 0.1 ml pulse of 25,000 mg S/L at the North Slope PW column inlet, before biofilm development. Distance at port 1 is 9 cm; port 2, 19 cm; port 3, 29 cm; and port 4, 39 cm.

A plot of concentration vs. time at each sampling port (Figure A1.1) shows the pulse decreasing in concentration and increasing in width as it progressed up the column. The dispersion coefficient at each port was calculated by modifying the method detailed by Robbins (1989), as the concentration at any time and place in the column can be calculated from:

$$C = \frac{C_0}{2} \left\{ \operatorname{erf} \left[\frac{(x - vt + \frac{X}{2})}{2\sqrt{D_t t}} \right] - \operatorname{erf} \left[\frac{(x - vt - \frac{X}{2})}{2\sqrt{D_t t}} \right] \right\} \quad (3)$$

where:

C_0 = initial concentration of pulse (i.e., 25,000 ppm)

x = distance (i.e., 9 cm at port 1)

v = velocity = 7.16×10^{-4} cm/sec

t = time

X = initial length of pulse = volume/(cross-sectional area x porosity)
= 0.0084 cm for a 0.1 ml pulse

D_t = dispersion coefficient

Using the Excel software package, D_t was determined by a best-fit solver using the concentration vs. time data. A dispersion coefficient was determined from the data obtained at each sampling port (i.e., each value of x). An example of the actual vs. predicted concentrations using this method to determine D_t is shown in Figure A1.2. The predicted concentration curve for each sampling port was

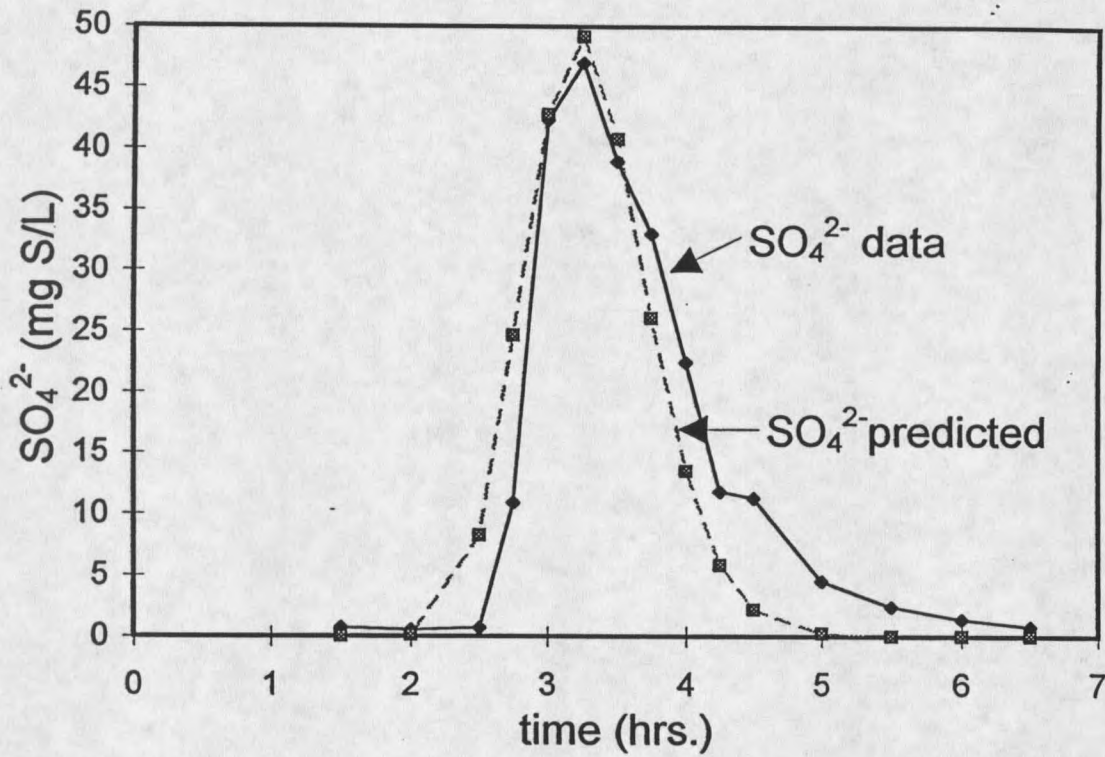


Figure A1.2. A comparison of pulse data (before biofilm development) at port 1 vs. the predicted pulse using the best-fit dispersion coefficient according to the method detailed by Robbins.

adjusted in two ways to obtain the most accurate fit: 1) a retardation factor was included so that the time of the predicted peak of the pulse matched the actual time of the peak (i.e., v was adjusted upward or downward, depending on whether apparent channeling or sorption was seen); and 2) a tracer recovery factor was used to decrease the predicted area under the curve, based on the actual area under the curve (i.e., C_0 was adjusted downward). The dispersion coefficient which provided a predicted curve with the best fit to the actual curve (minimizing the least-squares error) was then calculated automatically in Excel. The tail of the curve was neglected, according to the reasoning already given by Wen and Fan (1975). The dispersion coefficients, retardation factors, and tracer recoveries for each port are listed in Table A1.2. The tracer recoveries are based on the area under the concentration curves during each sampling interval (Figure A1.1), and are all less than the total recovery in the effluent already mentioned.

Table A1.2. A summary of D_i (dispersion coefficient) for each port before biofilm development, calculated using the method detailed by Robbins.

port	distance (cm)	retardation factor	tracer recovery	D_i (cm ² /sec)
1	9	0.93	71%	6.26×10^{-5}
2	19	1.02	64%	1.80×10^{-4}
3	29	0.98	68%	2.24×10^{-4}
4	39	0.93	53%	9.97×10^{-5}

The dispersivity α_l may then be calculated using Equation (1) and a D value from the literature (Perry and Chilton, 1973). The calculated dispersion coefficients are large enough that they are dominated by the flow term and less affected by molecular diffusion (D for $\text{SO}_4^{2-} = 4.2 \times 10^{-5} \text{ cm}^2/\text{sec}$). Consequently, ω (which is unknown) does not significantly affect D_h , so a value of $\omega = 0.01$ was assumed (i.e., diffusion is negligible). This results in an α_l value of 2.0 mm, which agrees well with values in the literature. According to Freeze and Cherry (1979), "Values of longitudinal dispersivity (for relatively homogeneous sandy materials under controlled conditions in the laboratory) are typically in the range of 0.1 to 10 mm."

Fluorescent bead experiments. In the first bead tracer experiment, 0.1 ml of the bead solution was injected into the column, and provided the pulse data shown in Figure A1.3. It can be seen that a significant quantity of beads were permanently sorbed or their flow was highly retarded by the porous media, as the area under the curve was significantly reduced at greater distances up the column.

This experiment was repeated with 1.0 ml of bead solution in an attempt to see significant quantities of beads throughout the column. Results, shown in Figure A1.4, show higher concentrations of beads at the beginning of the column (note the different y-axis) but minimal concentrations at the end. Because of the sketchy data and the lack of beads after the second port, dispersion coefficients for the beads using Robbins' method were not calculated.

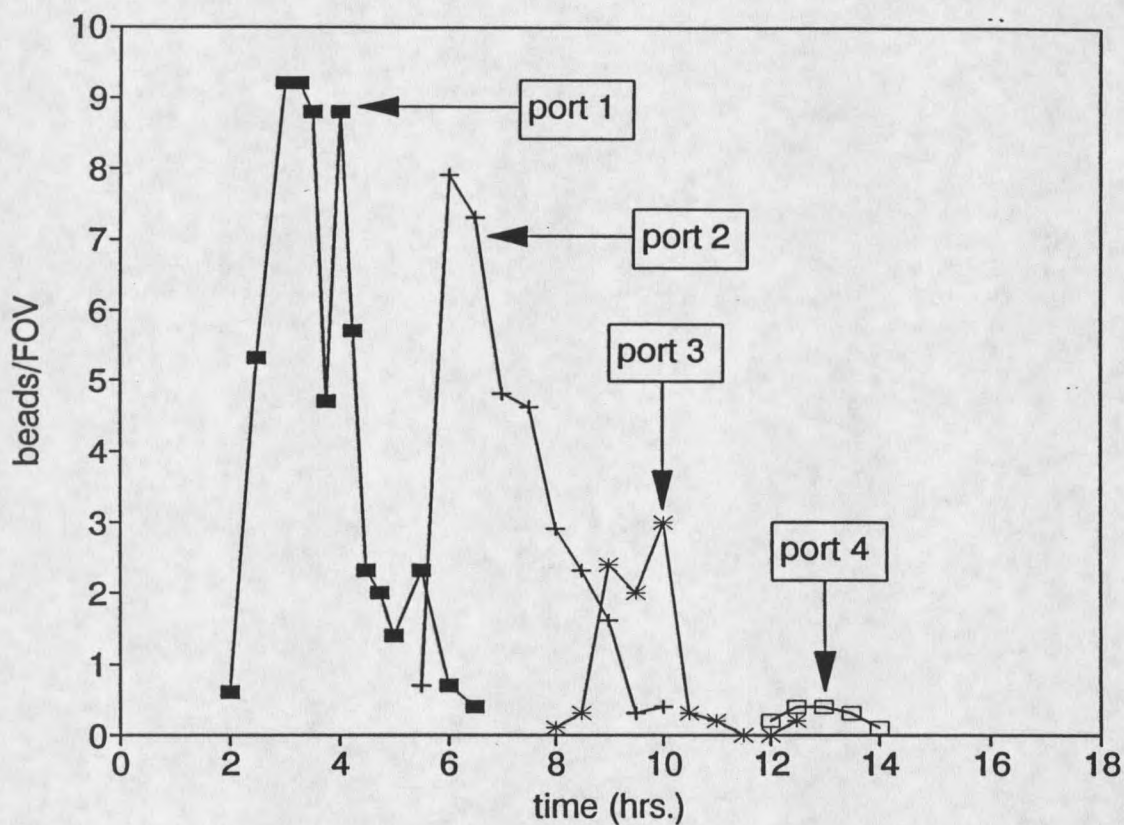


Figure A1.3. Tracer output curves at different distances after injection of a 0.1 ml pulse of 2.0×10^{10} fluorescent beads (diameter = $1.0\mu\text{m}$) at the North Slope PW column inlet, before biofilm development.

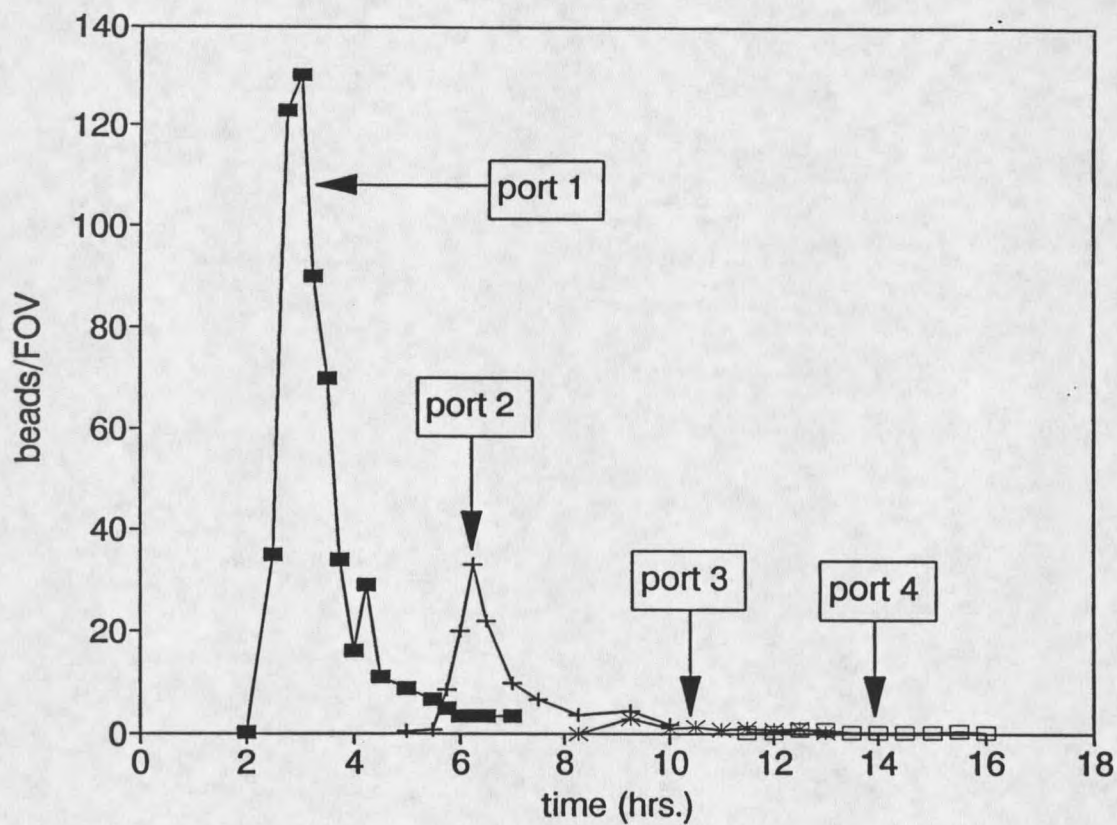


Figure A1.4. Tracer output curves at different distances after injection of a 1.0 ml pulse of 2.0×10^{10} fluorescent beads (diameter = $1.0 \mu\text{m}$) at the North Slope PW column inlet, before biofilm development.

After Biofilm Development

Inert tracer experiments. Approximately one month after microbial inoculation, 1.0 ml of a 25,000 ppm Br⁻ solution was pulse-injected into the column. A larger volume was used because the ion chromatographic method of analysis is less sensitive to bromide compared to sulfate. Concentration curves for the first two ports are shown in Figure A1.5 (data was not obtained at the last two sampling ports). It may be seen that after biofilm development, concentration peaks occurred much later than those predicted by plug flow (Table A1.3), in contrast to the sulfate data without a biofilm. The same best-fit procedure using Excel and Robbins' method to determine the dispersion coefficients was employed (Figure A1.6). A summary of the inert tracer results after biofilm development is provided in Table A1.4. It was not possible to do a mass balance on Br⁻ because of the large chloride peak (from the simulated salt-water medium) on the ion chromatograph, which masked the low-concentration Br⁻ peak in the effluent.

Table A1.3. Times of pulse peaks obtained in the column (hrs.), at a flow rate of 30 ml/hr and porosity of 0.49.

	port 1	port 2	port 3	port 4
plug flow	3.49	7.37	11.25	15.13
SO ₄ ²⁻ before biofilm	3.25	7.5	11.0	14.0
Br ⁻ after biofilm	4.0	9.0	---	---
beads before biofilm	3.0	6.25	9.25	12.5
beads after biofilm	5.0	---	---	---

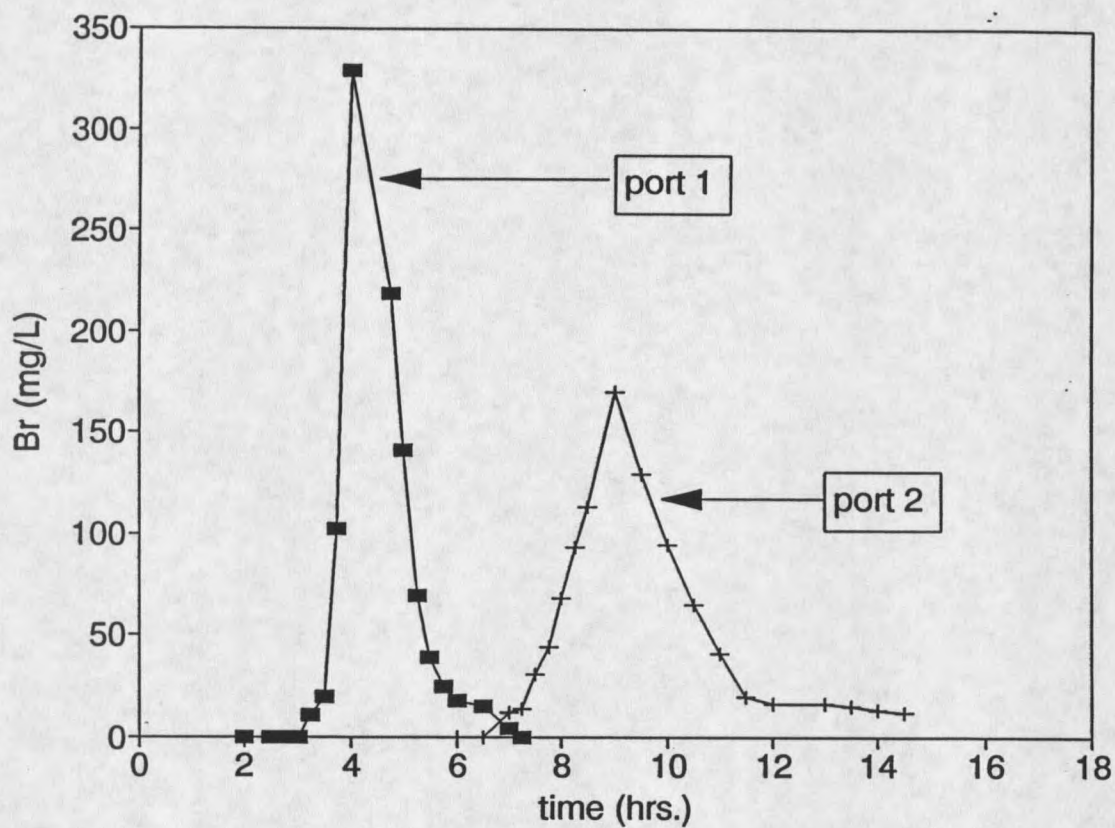


Figure A1.5. Tracer output curves at different distances after injection of a 1.0 ml pulse of 25,000 ppm bromide, after biofilm development.

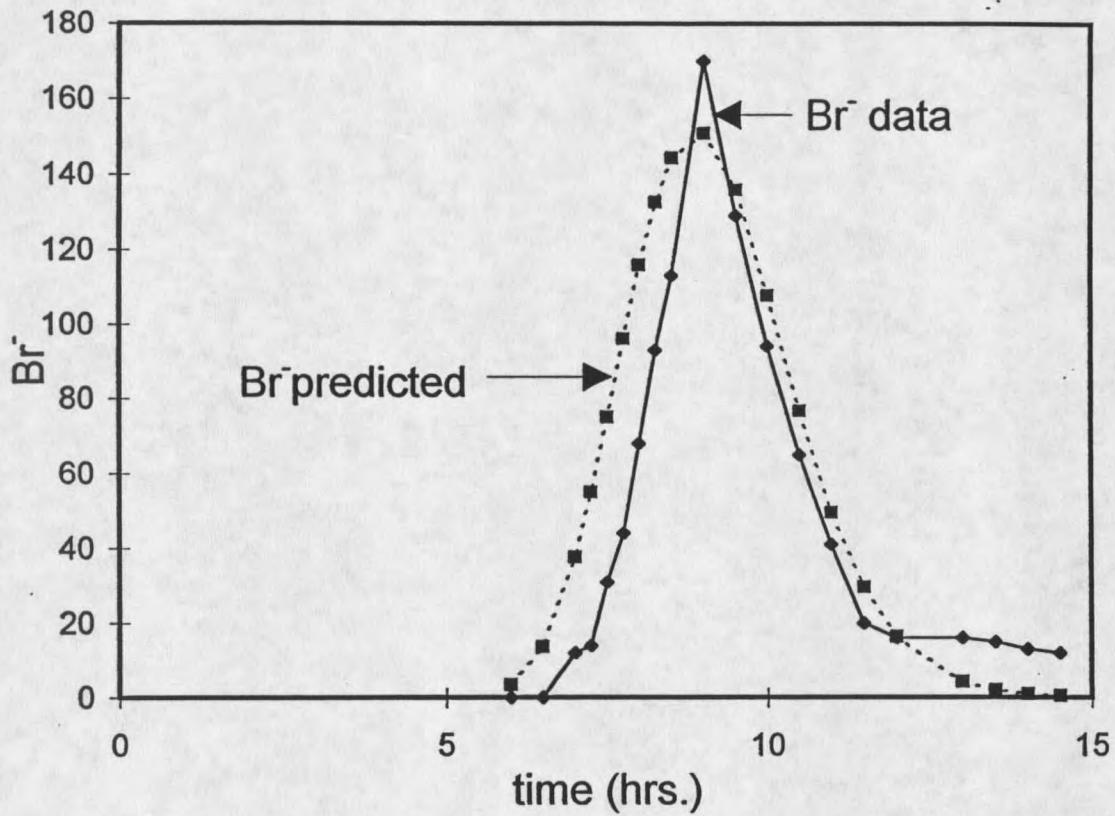


Figure A1.6. A comparison of pulse data (after biofilm development) at port 2 vs. the predicted pulse using the best-fit dispersion coefficient according to the method detailed by Robbins.

Table A1.4. A summary of D_i (dispersion coefficient) for the first two ports after biofilm development, calculated using the method detailed by Robbins.

port	distance (cm)	retardation factor	tracer recovery	D_i (cm ² /sec)
1	9	1.22	43%	2.25×10^{-5}
2	19	1.22	48%	1.14×10^{-4}

Fluorescent bead experiments. One week after the inert tracer experiment, 1.0 ml of bead solution was injected into the column. The results (Figure A1.7) show several significant differences compared to before biofilm development: about two orders-of-magnitude fewer beads were detected, essentially zero beads were detected after the first port, and the few beads which were counted were highly retarded compared to plug flow (Table A1.3). Again, dispersion coefficients for the beads were not calculated.

Peclet Number Method

Dispersion coefficients for the inert tracer before biofilm development (sulfate) were calculated by another method. Figure A1.8 (Froment and Bischoff, 1979) shows the effect of Peclet number (Pe) on the dispersion of a pulse. A high Pe means a small dispersion coefficient (closer to plug flow), as the Peclet number is the ratio of the convective effects compared to the dispersive effects. The plots of sulfate concentration vs. time at each port (Figure A1.1) were compared to the plots in Figure A1.8 for different Peclet numbers. A linear interpolation between Peclet numbers 40 and 500 was assumed. Since my curves were less

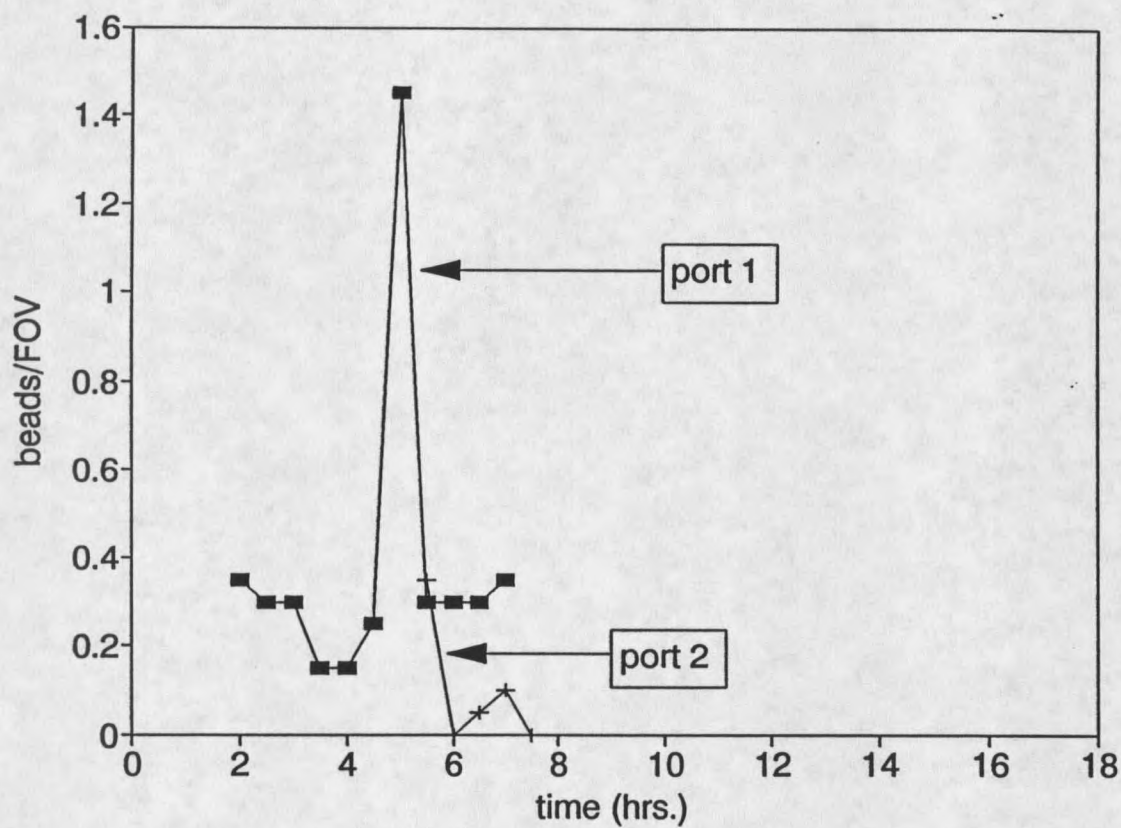


Figure A1.7. Tracer output curves at different distances after injection of a 1.0 ml pulse of 2.0×10^{10} fluorescent beads (diameter = $1.0 \mu\text{m}$) at the North Slope PW column inlet, after biofilm development.

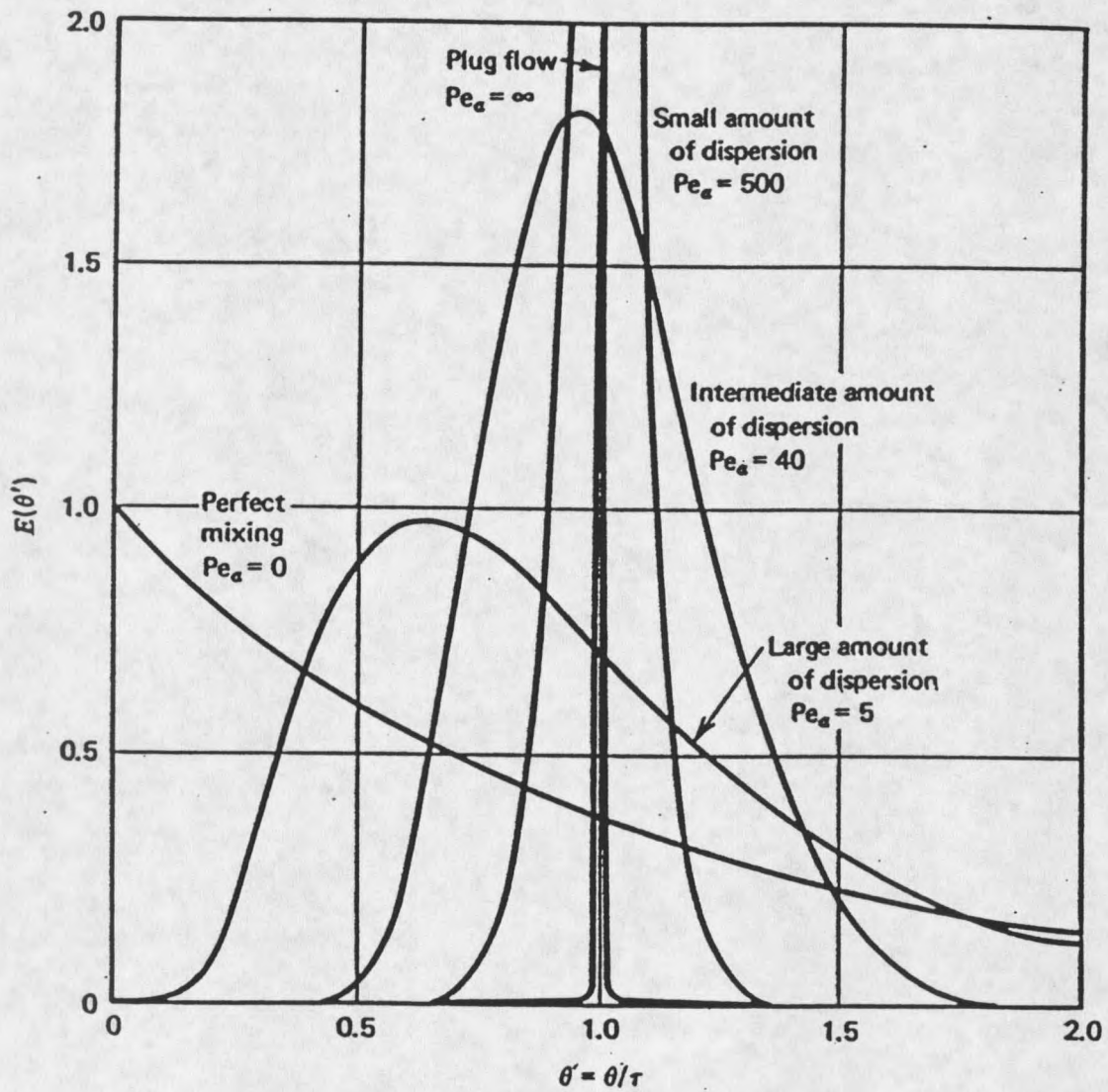


Figure A1.8. The effect of Peclet number on dispersion of a pulse (Froment and Bischoff, 1979).

symmetrical than those in Figure A1.8, the best-fit Peclet numbers were picked (by inspection) for both the leading half and trailing half of the curve (Pe_1 and Pe_2 , respectively). The equation relating Pe to D_i (Bailey and Ollis, 1986) is:

$$D_i = v x / Pe = \frac{(7.16 \times 10^{-4} \text{ cm/sec}) (x)}{Pe} \quad (4)$$

A summary of Peclet number and dispersion coefficient for each port is provided in Table A1.5. The dispersion coefficients for the last three ports are in close agreement and are slightly lower than the coefficients calculated from Robbins' method. The average hydrodynamic dispersion coefficient for the last three ports using this method is $1.2 \times 10^{-4} \text{ cm}^2/\text{sec}$, which is three times larger than the molecular diffusion coefficient for sulfate ($4.2 \times 10^{-5} \text{ cm}^2/\text{sec}$). The calculated value at the first port is even smaller than the diffusion coefficient, so this must be in error.

Table A1.5. A summary of D_i (dispersion coefficient) for each port before biofilm development, using the Peclet number (Pe) method.

port	distance (cm)	Pe_1	Pe_2	Pe_{avg}	D_i (cm^2/sec)
1	9	100	400	250	2.6×10^{-5}
2	19	224	30	127	1.1×10^{-4}
3	29	224	40	132	1.6×10^{-4}
4	39	380	190	285	9.8×10^{-5}

Prediction Method

The third successful method to estimate dispersion coefficients used the method explained by Perkins and Johnston (1963). Figure A1.9 is used to predict K_l , the longitudinal dispersion coefficient, which is the same as D_l . The following equation is used:

$$\frac{K_l}{D_o} \propto \frac{U d_p}{D_o} \quad (5)$$

The quantity $U d_p / D_o$ equals 0.37 in my system. To use Figure A1.9, the quantity $U \sigma d_p / D_o$ must be calculated. Since $\sigma = 3.5$ for random packs (Perkins and Johnston, 1963), the quantity $U \sigma d_p / D_o$ equals 1.3. From Figure A1.9, $K_l / D_o = 1.5$, leading to a value of $6.3 \times 10^{-5} \text{ cm}^2/\text{sec}$ for K_l . Since K_l (the hydrodynamic dispersion) is equal to the mechanical dispersion plus D_o (the molecular diffusion), this means that the mechanical dispersion term is only half as large as the diffusion term ($4.2 \times 10^{-5} \text{ cm}^2/\text{sec}$). This is undoubtedly due to the small packing and the low flow rate. The predicted dispersion coefficient is the same at all locations in the column.

The calculated or predicted dispersion coefficients from all three methods are shown in Table A1.6, with close agreement between all three methods. The coefficients determined from Robbins' method are slightly higher, which means that the effect of mechanical dispersion (due to flow) would be greater. The same trend

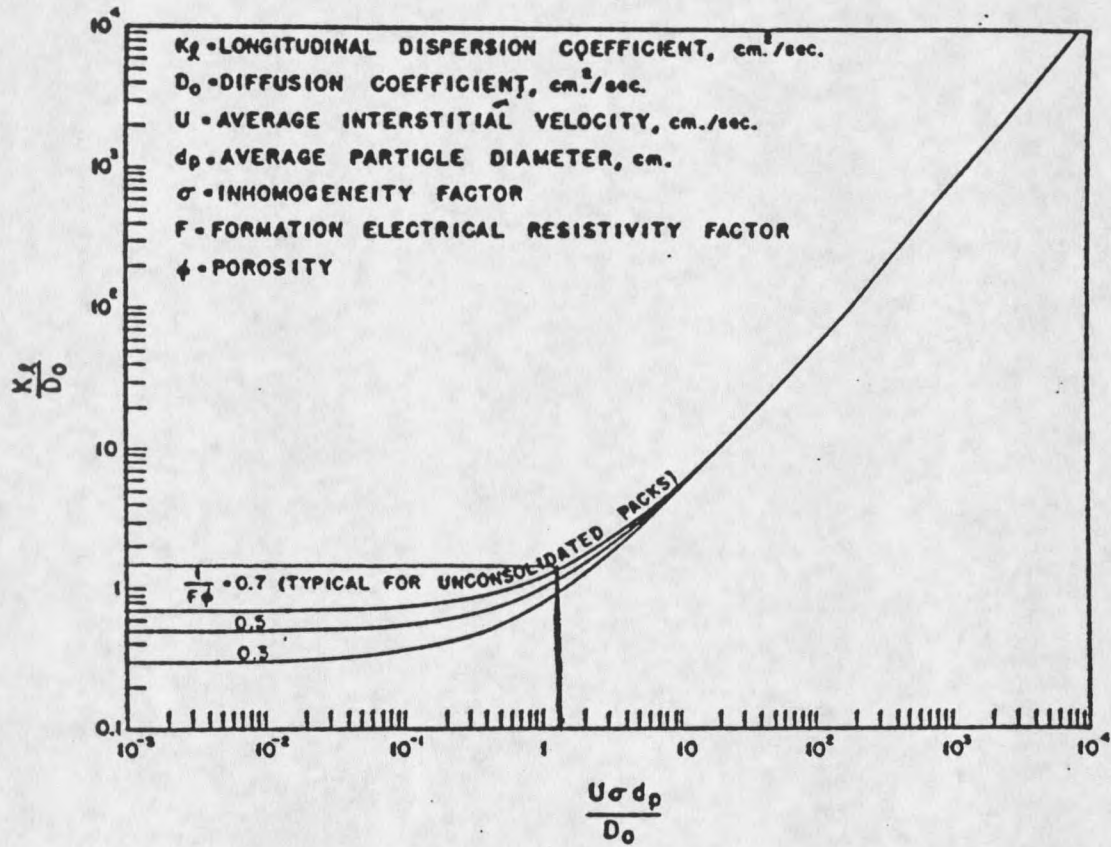


Figure A1.9. Estimation of K_L , the longitudinal dispersion coefficient, with the method from Perkins and Johnston (1963).

(D , highest at port 3 and progressively lower at ports 2, 4 and 1) was seen with both the Robbins and Peclet number methods.

Table A1.6. Comparison of dispersion coefficients from all three methods (ranges shown are calculated values from the four ports of the column).

method	D_i (cm ² /sec)	α (mm)
Robbins (calculated)	$0.6 - 2.2 \times 10^{-4}$	0.9 - 3.1
Peclet number (calculated)	$0.3 - 1.6 \times 10^{-4}$	0.4 - 2.3
Perkins (predicted)	0.6×10^{-4}	0.9

Dispersion in the BAM Model

Pulse injection in the BAM model described in Chapter 5 was simulated by adding 0.1 ml of a 25,000 ppm sulfate solution to the model at time zero, before biofilm development. The sulfate concentration was followed at port 1 (distance = 10 cm), which flowed through two 2.5-cm sections and one 5-cm section in the model. Figure A1.10 shows the BAM concentration data compared to the best-fit data using Robbins' method to calculate the dispersion coefficient. As the graph shows, the pulse at port 1 in this case is much broader than the actual data (Figure A1.2) and the predicted D_i of 2.8×10^{-3} cm²/sec is an order-of-magnitude larger than the value based on column data.

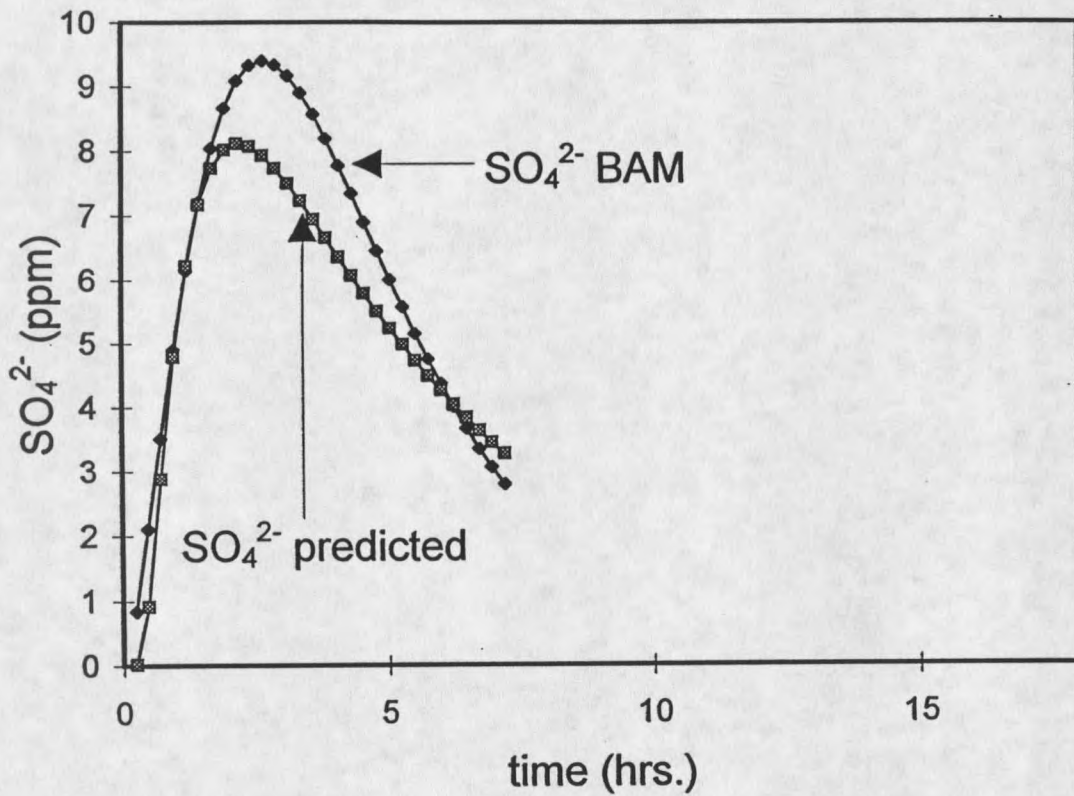


Figure A1.10. The simulated tracer output curve using the BAM column detailed in Chapter 5 vs. the predicted output curve using the best-fit dispersion coefficient according to the method detailed by Robbins. The column distance is 10 cm.

Discussion

Tracer Recovery

In the inert tracer experiment without biofilm, 53-71% of the sulfate was recovered at each port, based on a smooth curve using the data points collected (Table A1.2). Recovery would be higher if the height of the pulse peak were underestimated, which is very possible if the true peak were missed due to the sampling intervals used. This problem could be avoided if a continuous sampling method (such as a probe) were used. Recovery during the sampling time was highest at the beginning of the column and lowest at the end, as expected. Total recovery based on collection of sulfate in the effluent was close to 100%, however, as shown previously.

In the inert tracer experiment with biofilm, significantly less tracer was recovered (43-48%). This could be due to several factors, among them: more reaction or permanent sorption of bromide within the column than with sulfate (unlikely since bromide is a commonly used tracer in biofilm systems); a long tail due to diffusion in and out of the biofilm (also unlikely, since it was shown in Chapter 2 that the biofilm is very thin); or poor calibration of the bromide analysis method at low concentrations. Tracer diffusion in the biofilm was considered to be the dominant mechanism acting to create the long tails on the RTD curves, in experiments by Stevens et al. (1986). Their biofilms, however, were 14 to 68 μm , considerably thicker than in my column.

Recovery of the fluorescent beads was very low without biofilm and was almost non-existent with a biofilm. Percent recovery in both experiments without biofilm was approximately 10^{-8} (i.e., 100 beads recovered out of 10^{10} injected). It appears that the beads were adsorbed to the media permanently or at least long-term--after taking samples for cell counts one to two weeks after the last bead experiment was run, occasional beads were seen.

Retardation Factor

The retardation factors (RF) in the first inert tracer experiment, calculated by comparing the time of the pulse peak to the time the peak should have arrived in plug flow (Table A1.3), ranged from 0.93 to 1.02. Values below one indicate one of two phenomena: significant channeling; or non-homogeneities in the column packing, since the average porosity was used in calculating the plug-flow pulse time. The fact that the retardation factors varied somewhat between sampling ports indicates non-homogeneities, probably both in the porosity and in the amount of channeling. The low RF at the first port (0.93) was most likely caused by a local porosity which was lower than the average porosity for the column, caused by media settling during packing.

RF's for bromide after biofilm development were uniformly higher at 1.22 for both sampling ports. This is probably due to the diffusion effects of the biofilm, which also affected the percent recovery. Taylor and Jaffe (1990) suggest that retardation could also be due to chemical adsorption of the tracer onto compounds

comprising the biofilm. If the porosity had been significantly lowered by biofilm growth (unlikely, since a biofilm in an identical column was calculated to be only about 4 μm , compared to an average particle diameter of 220 μm), this would have actually lowered the retardation factor. Even in thick biofilm systems with significant plugging, no significant effect of biofilm on porosity was seen (Mueller, 1988).

The retardation factor for fluorescent beads without biofilm was actually less than one at the first two ports (Table A1.3). I see two possible explanations for this: too little data for this to be a real effect; or more likely, the only beads counted were those channeled through regions of high flow, where their velocity was increased and their chance of being adsorbed onto media decreased. After biofilm development, not enough beads were counted (due to capture) to calculate a retardation factor. In all experiments, the retardation factor based on the mean residence time instead of the time to the pulse peak, would be slightly larger due to the long tail and some channeling.

In addition to diffusion, retardation may also be due to "dead zones" within the column where flow is stopped or redirected. Although an asymmetric dispersion curve is normal, it appears that the curves for bromide after biofilm development (Figure A1.5) have less tailing than those without biofilm. A biofilm could conceivably cause less tailing (retardation) by plugging some of the dead zones.

Retardation factors as high as 9.0 have been reported by Roberts et al. (1986) in an underground aquifer. A biofilm was present as evidenced by

biotransformation of several compounds. The highest retardation was seen for hydrophobic compounds and was attributed to sorption.

Comparison to Other Work

Hydrodynamic dispersion coefficients have been calculated for pulse addition to similar systems by other researchers. Robbins (1989), working with larger particles and a higher flow rate, obtained dispersion coefficients almost identical to the coefficients for my system according to his method (Table A1.7). When estimating dispersion coefficients for Robbins' system using Perkins' method, however, I obtained higher values than those reported by Robbins. Taylor and Jaffe (1990), working with larger sand particles and an intermediate fluid velocity, reported dispersion coefficients about one order-of-magnitude larger than in my system. Estimating their coefficients gave values nearly the same as that predicted for Robbins, since the larger d_p and smaller v offset each other, and these values were only slightly lower than Taylor and Jaffe reported.

Retardation due to biofilm has also been reported by other investigators. Both Taylor and Jaffe (1990) and Stevens (1986) reported significant retardation of inert tracers after biofilm growth in porous media systems. Taylor and Jaffe (1990) also reported a significant increase in the dispersion coefficient after biofilm growth, with a corresponding increase in the dispersivity α (Table A1.7). This was not seen in my system, probably due to the biofilm thickness. Taylor and Jaffe's biofilm was much thicker than the one in my system due to their aerobic conditions

and larger particle size. The dispersion data from my system with a biofilm is difficult to interpret, as both dispersion and sorption were affected and appear to be related.

Table A1.7. Comparison of inert tracer dispersion coefficients from different systems with different calculation methods.

system	method	d_p (μm)	v (cm/sec)	D_i (cm^2/sec)	α (mm)
my column	Robbins	220	7×10^{-4}	$0.6\text{-}2.2 \times 10^{-4}$	0.9-3.1
my column	<i>Pe</i> fit	220	7×10^{-4}	$0.3\text{-}1.6 \times 10^{-4}$	0.4-2.3
my column	Perkins	220	7×10^{-4}	0.6×10^{-4}	0.9
Robbins	Robbins	480	1×10^{-2}	$0.7\text{-}2.3 \times 10^{-4}$	0.07-0.23
Robbins	Perkins	480	1×10^{-2}	8.4×10^{-4}	0.84
Taylor & Jaffe	---	700	6.7×10^{-3}	$1.1\text{-}1.7 \times 10^{-3}$	1.6-2.5
Taylor & Jaffe	Perkins	700	6.7×10^{-3}	8.2×10^{-4}	1.2
my column (w/biofilm)	Robbins	220	7×10^{-4}	$0.2\text{-}1.1 \times 10^{-4}$	0.3-1.6
Taylor & Jaffe (w/biofilm)	---	700	6.7×10^{-3}	2.8 - 16	4200-24,000

Fdez-Polanco et al. (1994) noted that porosity was the controlling factor for hydrodynamic dispersion. Their system, with porosities of 0.48 to 0.78 and a 4-mm particle diameter, also closely approximated plug flow.

Dispersion Effects in BAM

The dispersion coefficient for the pulse simulated in the BAM model is about one order-of-magnitude higher than the coefficients anywhere in the actual column,

regardless of which calculation method was used. This means that the BAM column is more like a CSTR than is the actual column. This makes sense, since (as explained in Chapter 5) the BAM column is actually a series of eight CSTR's. In order to more accurately model the flow in BAM, one would need to significantly increase the number of sections (CSTR's) comprising the column. It was also difficult to simulate a short pulse in the current BAM configuration, which may also have added to the discrepancy between the model and the real system. The conclusions reached by using the BAM model in Chapter 5 still appear to be valid, however, as those results were insensitive to the diffusion coefficients used. Kinetics appear to dominate over transport phenomena such as dispersion in both the model and actual column.

Conclusions

The hydrodynamic dispersion coefficients for an inert tracer in the porous media column, D_h , closely agreed when calculated by two different methods. D_h was slightly higher than that predicted by the media particle size. Calculated values for D_h showed that the column was much closer to a plug-flow reactor than to a perfectly mixed system. Coefficients calculated at different places within the column showed some variation, probably due to packing heterogeneities, but agreed within an order-of-magnitude, and agreed with those calculated by other researchers working with similar systems. The inert tracer was conserved in the column and no retardation (but some channeling) was seen without a biofilm.

Fluorescent beads, similar in size to microbial cells, were adsorbed by the media but non-adsorbed beads also showed no retardation, which is further evidence of channeling.

After a thin biofilm was grown in the column, a lower recovery rate and some retardation was seen with the bromide tracer, probably due to sorption and diffusion into the biofilm, respectively. Less retardation, and much lower dispersion coefficients and dispersivities were seen compared to other researchers working with biofilms, however, probably because of the thin biofilm in my system. With fluorescent beads, nearly zero recovery and some retardation was noted. Therefore, it appears plausible that most of the cells introduced to the column remained near the inlet, causing most of the microbial activity to occur in this region, which was shown in Chapters 2 and 3.

The dispersivity α_t of the crushed Berea sandstone in the column was calculated to be 2.0 mm, which is in the range of literature values. The dispersion coefficient for an inert tracer pulse injection in the BAM column model was about an order-of-magnitude higher than in the actual column, but it does not appear that this would significantly affect the modeling results in Chapter 5.

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APPENDIX 2**PARTITION COEFFICIENTS FOR ACETIC, PROPIONIC AND
BUTYRIC ACIDS IN A CRUDE OIL/WATER SYSTEM****Abstract**

The effects of pH, temperature and organic acid concentration on the partition coefficients for short-chain organic acids were measured in a crude oil/water system. Acetic, propionic and butyric acids, as probable substrates for microbial souring of oil reservoirs, were used in conjunction with two types of crude oil. Temperatures of 35 to 75 °C, pH values of 4.0 to 7.0 and acid concentrations of 10 to 1000 mg/L were studied. Initial naturally occurring levels of organic acids in the crude oils were also determined. pH had by far the largest effect on the partition coefficient for all three organic acids for both types of oil. At conditions normally seen in an oil reservoir (pH 5 to 7), the great percentage (85%+) of these acids were dissolved in the aqueous phase. The log of the partition coefficient K increased approximately linearly with the number of carbon atoms in the acid. It was seen that organic acids are readily available carbon sources for sulfate-reducing bacteria (SRB) at normal reservoir conditions, and that crude oil may provide a source of organic acids in a low-pH, water-flooded reservoir.

Introduction

Short-chain organic acids such as acetic acid, propionic acid and butyric acid are favored substrates for metabolism by SRB. In oil reservoirs undergoing secondary recovery (water flooding), the SRB utilize these carbon sources and dissolved sulfate to produce hydrogen sulfide (H_2S) in the phenomenon known as "souring." Since only substrates dissolved in the aqueous phase are thought to be available for bacterial metabolism, it is relevant to the understanding of souring to know how short-chain organic acids partition between the oil and water phases. In a water-flooded oil reservoir, where equilibrium can be expected between the flowing injection water and the oil (which may be stationary in a low-permeability zone), the quantity and type of organic acid available for SRB metabolism in the aqueous phase may be very important information in modeling and controlling souring.

Materials and Methods

A three-variable central composite designed experiment was used to determine the effects of pH, temperature and organic acid concentration on the partition coefficients in a crude oil/water system. Three levels of each variable were used in the design: temperatures of 35, 55 and 75 °C; pH values of 4.0, 5.5 and 7.0; and high, medium and low levels of acid concentration. The high concentration consisted of 1000 mg/L acetic acid, 500 mg/L propionic acid and 100 mg/L butyric

acid; the medium concentration was 550 mg/L acetic acid, 275 mg/L propionic acid and 55 mg/L butyric acid; and the low concentration was 100 mg/L acetic acid, 50 mg/L propionic acid and 10 mg/L butyric acid. These variables and their levels were selected to represent reservoir conditions based on input from oil company personnel sponsoring this research.

The measured experimental response was the partition coefficient for each of the three organic acids, as defined in the equation

$$K = \frac{\text{concentration in oil by mass}}{\text{concentration in water by mass}} \quad (1)$$

The acetic acid (Fisher) was ACS reagent-grade with 99.7% minimum purity. Propionic acid and n-butyric acid (Sigma) were approximately 99% pure. The aqueous phase used was a solution (#1) developed by Widdel and Pfennig (1981) for SRB, as modified for a high-salinity, low-sulfate environment (Table A2.1). All chemicals used in this solution were ACS reagent-grade manufactured by Sigma.

Table A2.1. Composition of aqueous phase used in experiments.

distilled water	970 cm ³
Na ₂ SO ₄	0.8 g
NaCl	.20 g
KCl	0.3 g
NH ₄ Cl	0.3 g
MgCL ₂ • 6 H ₂ O	3.0 g
KH ₂ PO ₄	0.2 g
CaCl ₂ • 2 H ₂ O	0.15 g

The experimental procedure involved measuring 20 cm³ of the aqueous phase, adding the proper amount of organic acids via a stock solution containing all three acids, and then adjusting the pH to the desired level (± 0.1 pH unit) with 0.1 M NaOH. This pH-adjusted aqueous phase was then added to a 50 cm³ narrow-necked reaction vessel which contained 20 cm³ of crude oil. The vessels were then stoppered, sealed and autoclaved for 20 minutes at 121 °C to sterilize the contents and eliminate the possibility of microbial activity consuming the organic acids. The vessels were incubated at the chosen temperature, and the oil and water phases were sampled after allowing the vessels to equilibrate without mixing for approximately seven days. In preliminary experiments, it was shown that equilibrium was reached within two days. Three incubators were used in this procedure so the entire experiment could be run at one time.

Aqueous-phase organic acid concentrations were determined by inverting the vessel, withdrawing 0.5 cm³ (duplicate samples) and diluting 1:10 with ultrapure water. A Dionex AI-450 ion chromatograph, which separates peaks for each of the short-chain organic acids, was used to determine the concentrations (Table A2.2). The minimum level of detection and the instrument error have both been determined to be approximately one mg/L.

The oil-phase organic acid concentration was determined by withdrawing five cm³ of oil after laying the reaction vessel on its side to allow access to the oil phase. Ten cm³ of 0.1 M Na₂CO₃ was then added to extract the acids from the oil. It was shown in separate experiments that 0.1 M Na₂CO₃ used in the following method

extracted 79% of the acetic acid, 94% of the propionic acid and 92% of the butyric acid compared to a water control. Except for acetic acid, it is believed that these extractions are within the experimental error possible with this method so no correction factors were used. If not all the organic acids were extracted from the oil during these experiments, the partition coefficients would be slightly higher than their reported values.

Table A2.2. Dionex ion chromatograph analysis specifications.

column size	AS10
pore size	4 mm
detector	conductivity
eluent	$K_2B_4O_7 \cdot 4 H_2O$
eluent conc.	3.5 mM
regenerant	$K_2B_4O_7 \cdot 4 H_2O$
regenerant conc.	100 mM
eluent flow rate	1.0 cm ³ /min.
suppressor liquid	H ₂ SO ₄
suppressor conc.	25 mM

The sample was shaken for two hours at 160 oscillations/min to allow intimate mixing of the oil and the extraction agent, then was centrifuged for 15 min at 20,000 rpm on a Sorvall RC5C centrifuge. The sample was shaken slightly to break the emulsion, poured into a separatory funnel, and the aqueous phase now containing the acids was withdrawn from the bottom of the funnel. This sample,

already diluted 1:2 by the addition of 10 cm³ of the extraction agent, was filtered and run in duplicate on the Dionex ion chromatograph.

Two different experiments were performed using the three-variable central composite design. The first experiment used crude oil supplied by ARCO from the Kuparuk field on Alaska's North Slope. The second experiment used crude oil supplied by the Idaho National Engineering Laboratory (INEL) from the Schuricht field in Wyoming. The ARCO oil was sour (high H₂S content) from a field which had undergone water flooding, while the INEL oil was sweet (low H₂S content) from a field with no water flooding. Both oils were tested for their initial level of short-chain fatty acids using the same extraction technique used for the oil phase in the incubated vessels. Liquid chromatography fractionation and elemental analysis for both oils is shown in Tables A2.3 and A2.4, respectively.

Table A2.3. Liquid chromatography fractionation (mass percent).

oil	aliphatic	aromatic	C ₅ soluble ^a	C ₅ insoluble ^b
ARCO	32.5	66.6	—	—
INEL	62.68	22.53	6.38	6.40

a. Non-pentane precipitable resins

b. Pentane precipitable asphaltics

Table A2.4. Elemental analysis (mass percent).

oil	carbon	hydrogen	nitrogen	sulfur
ARCO	85.0	9.2	0.25	1.73
INEL	82.37	11.60	0.35	3.24

Results and Discussion

The oils used in the two experiments each had very low initial levels of short-chain organic acids (Table A2.5). It was expected that the "sweet" oil which came from a non-water-flooded field (INEL) would have significantly higher acid concentrations than the ARCO oil because of less SRB activity, but this was not the case.

Table A2.5. Initial acid levels of ARCO and INEL crude oils.

oil source	ARCO	INEL
acetic acid conc.	3 mg/L	1 mg/L
propionic acid conc.	< 1 mg/L	< 1 mg/L
butyric acid conc.	< 1 mg/L	< 1 mg/L

The data from both central composite designed experiments (using the two types of crude oil) fit the same general linear model very well (Table A2.6). Variables which were significant at a 95% confidence level were pH and concentration for acetic acid, pH and temperature for propionic acid and pH for butyric acid. As shown by the very low p-values, pH was by far the most significant variable affecting the partition coefficient for all three short-chain organic acids. Data from both experiments are shown in Tables A2.7 and A2.8.

Since temperature and organic acid concentrations had only minor effects on partition coefficients, only data showing the effect of pH is presented (Figures

Table A2.6. Statistical p-values for combined experimental data from ARCO and INEL crude oils.

	R-square	temperature	pH	concentration
acetic acid	0.8281	0.3921	0.0001	0.0042
propionic acid	0.9726	0.0163	0.0001	0.9986
butyric acid	0.9745	0.0541	0.0001	0.8160

* A p-value ≤ 0.05 means that a change in the variable over the experimental range has a significant effect on the measured response (in this case, K, the partition coefficient) at a 95% confidence level.

A2.1 to A2.3). It is evident that the variables in the experiment (pH, temperature and organic acid concentration) have the same effect on the two types of oil.

The main difference is that the ARCO oil had slightly higher partition coefficients for most of the experimental conditions. Figure A2.4 shows the experimental data compared to the most comparable data which could be found in the literature (Verschueren, 1983; Thomas, 1984). A linear relationship between log K and the number of carbon atoms in the acid is seen in each case.

Conclusions

Temperature and acid concentration have only small effects on the partition coefficients for acetic, propionic and butyric acids, while pH has by far the largest effect. At conditions normally expected in an oil reservoir (pH 5 to 7), the large majority of the acids (85%+) will be dissolved in the aqueous phase. The log of the

partition coefficient K increases approximately linearly with the number of carbon atoms in the acid. These results are true for both types of crude oil tested. Since the large majority of organic acids are dissolved in the aqueous phase at normal pH, these carbon sources are readily available for metabolism by sulfate-reducing bacteria. If reservoir conditions exist at below pH 5, however, a significant quantity of organic acids is dissolved in the oil and could thus replenish depleted organic acids in the aqueous phase if a concentration equilibrium is maintained between the two phases.

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- Pfennig, N., Widdel, F., and Trueper, H.G. The dissimilatory sulfate-reducing bacteria. p. 931-932 *in* M.P. Starr, H. Stolp, H.G. Trueper, A. Balows, and H.G. Schlegel (eds.), *The Prokaryotes*. Springer-Verlag, New York.
- Verschueren, K. 1983. *In* The handbook of environmental data on organic chemicals, 2nd ed.; Van Nostrand Reinhold Company Inc., New York.
- Thomas, B.T. 1984. Determination of oil/water and octanol/water distribution coefficients from aqueous solutions from four fossil fuels. M.S. Thesis, Washington State University.

Table A2.7. Partition coefficients (K) for ARCO oil.

vessel #	pH	T°C	conc.	K (but.)	K (prop.)	K (Ac)
1	4	35	LO	0.243	0.047	0.011
2	7	35	LO	0.000	0.000	0.007
3	4	75	LO	0.484	0.095	0.036
4	7	75	LO	0.091	0.019	0.015
5	4	35	HI	0.345	0.075	0.015
6	7	35	HI	0.005	0.001	0.001
7	4	75	HI	0.316	0.075	0.016
8	7	75	HI	0.019	0.003	0.002
9	4	55	MID	0.344	0.078	0.019
10	7	55	MID	0.011	0.002	0.001
11	5.5	35	MID	0.075	0.008	0.018
12	5.5	75	MID	0.084	0.010	0.009
13	5.5	55	LO	0.144	0.022	0.008
14	5.5	55	HI	0.057	0.011	0.002
15	5.5	55	HI	0.068	0.017	0.004
16	5.5	55	MID	---	---	---
17	5.5	55	MID	0.060	0.012	0.005
18	5.5	55	MID	0.043	0.008	0.002

Table A2.8. Partition coefficients (K) for INEL oil.

vessel #	pH	T/°C	conc.	K (but.)	K (prop.)	K (Ac)
1	4	35	LO	0.241	0.056	0.020
2	7	35	LO	0.000	0.000	0.007
3	4	75	LO	0.137	0.031	0.011
4	7	75	LO	0.000	0.015	0.010
5	4	35	HI	0.218	0.051	0.011
6	7	35	HI	0.000	0.001	0.001
7	4	75	HI	0.236	0.057	0.014
8	7	75	HI	0.000	0.001	0.001
9	4	55	MID	0.265	---	---
10	7	55	MID	0.000	0.001	0.003
11	5.5	35	MID	0.029	0.007	0.002
12	5.5	75	MID	0.027	0.007	0.002
13	5.5	55	LO	0.000	0.009	0.008
14	5.5	55	HI	0.042	0.010	0.003
15	5.5	55	HI	0.026	0.008	0.003
16	5.5	55	MID	0.042	0.013	0.006
17	5.5	55	MID	0.032	0.007	0.002
18	5.5	55	MID	0.031	0.007	0.002

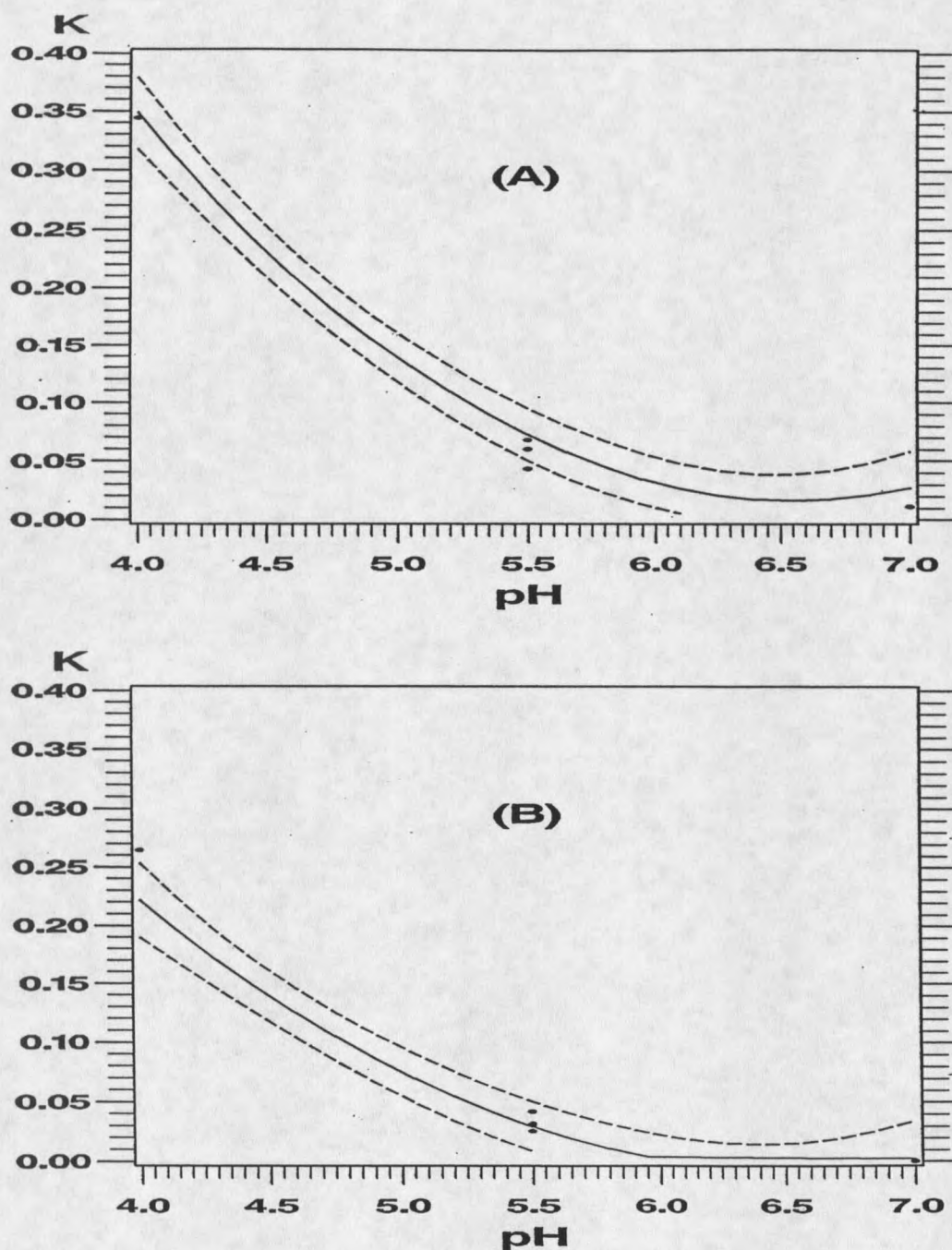


Figure A2.1. Partition coefficient K for butyric acid vs. pH, at mid-temperature and mid-concentration levels: A) ARCO oil, B) INEL oil. Data points (●) are shown along with the statistically predicted curve (—) and 95% confidence intervals (---). Shown are the data points corresponding to the mid-levels of temperature and concentration. The predicted values and prediction intervals, however, are based on data from the entire design.

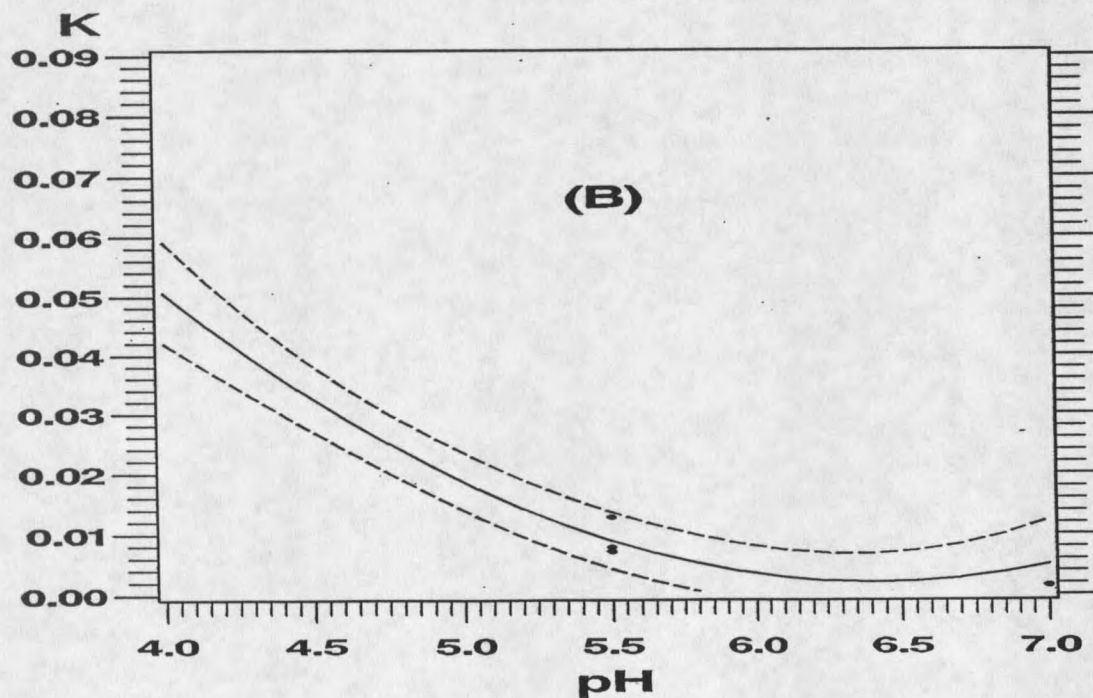
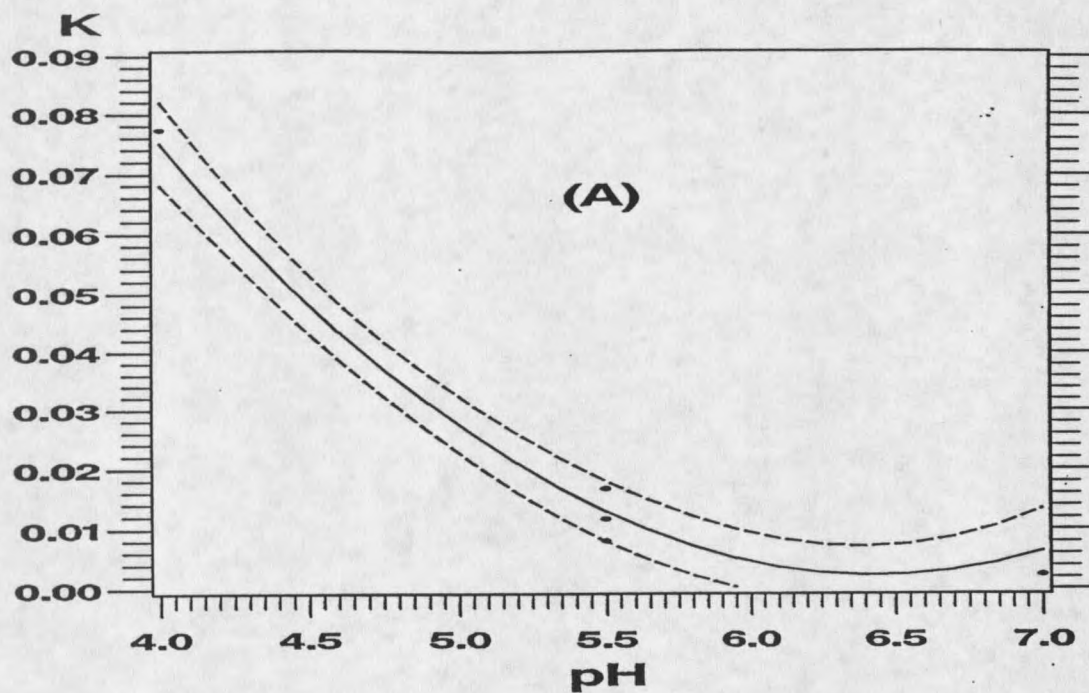


Figure A2.2. Partition coefficient K for propionic acid vs. pH, at mid-temperature and mid-concentration levels: A) ARCO oil, B) INEL oil. Data points (●) are shown along with the statistically predicted curve (—) and 95% confidence intervals (---).

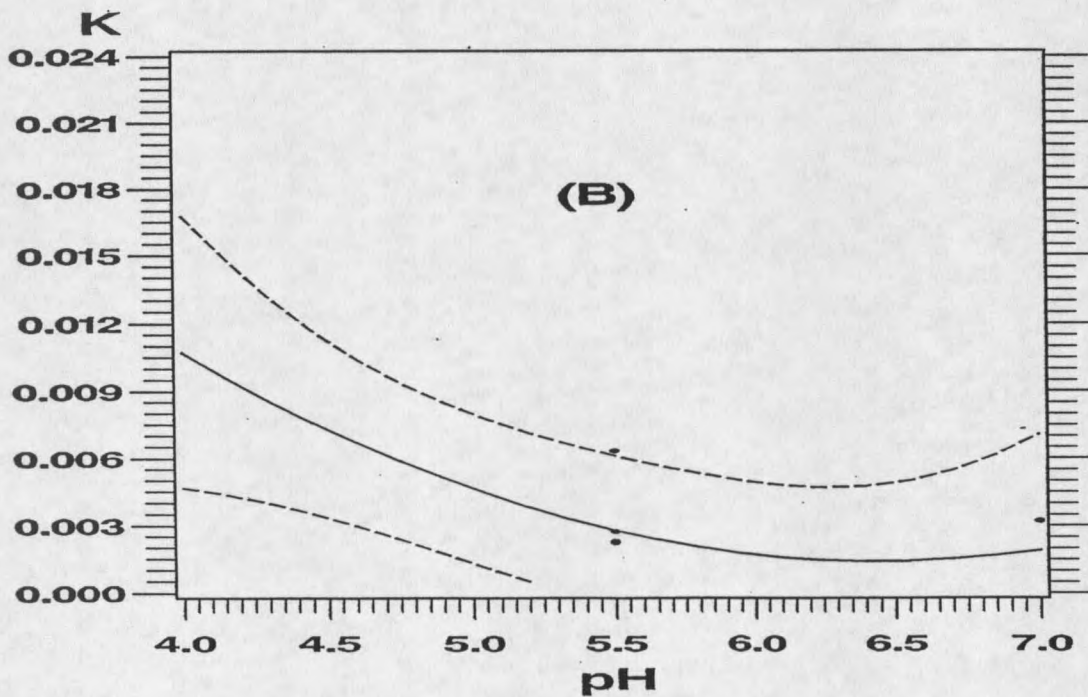
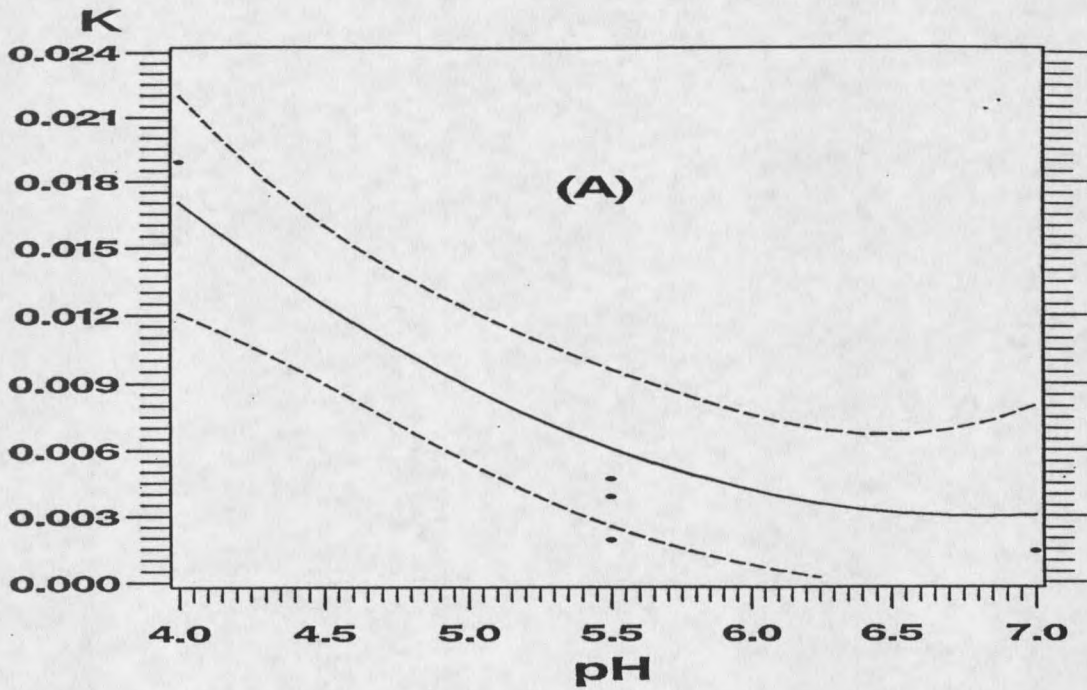


Figure A2.3. Partition coefficient K for acetic acid vs. pH, at mid-temperature and mid-concentration levels: A) ARCO oil, B) INEL oil. Data points (●) are shown along with the statistically predicted curve (—) and 95% confidence intervals (---)

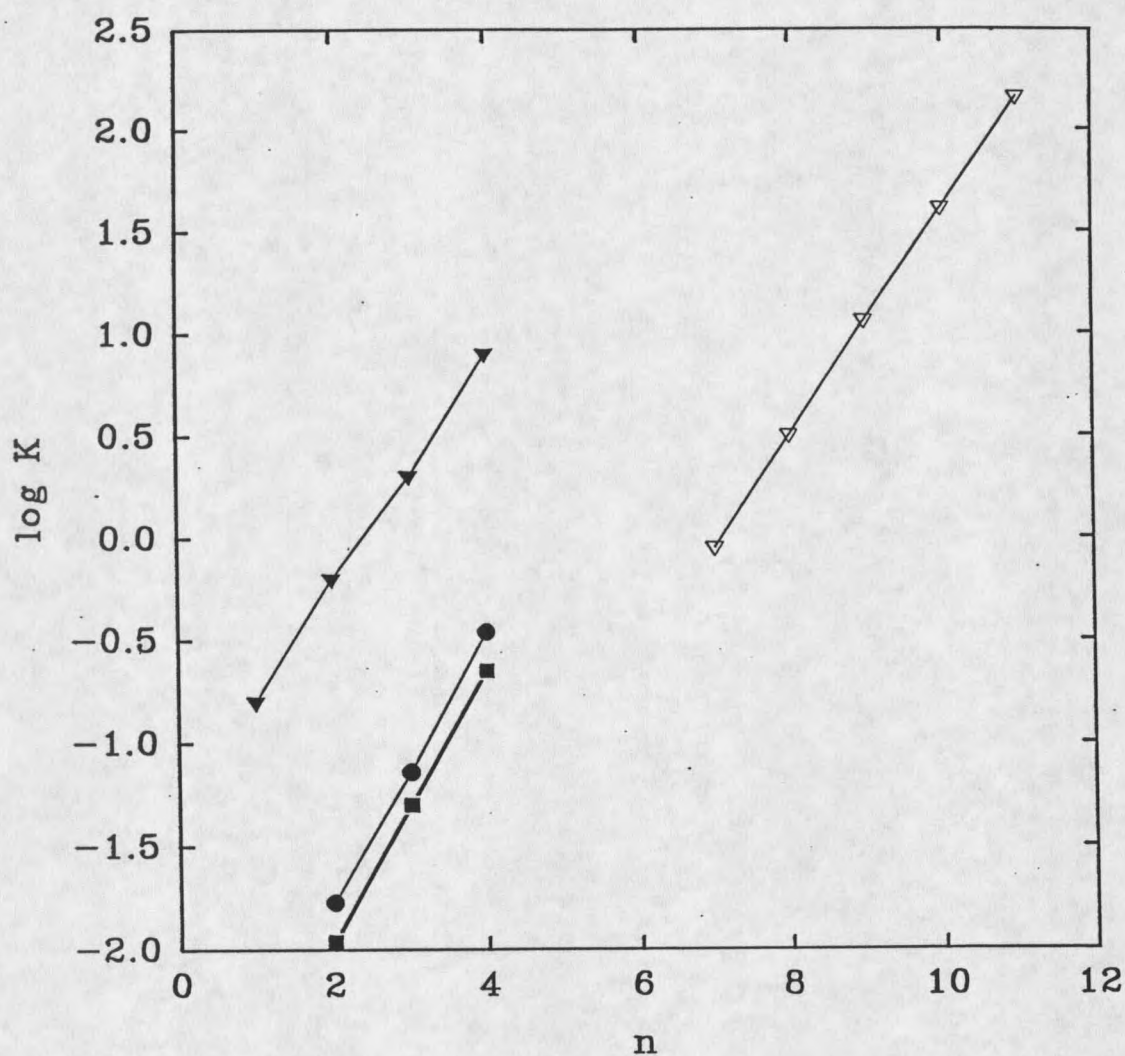


Figure A2.4. Partition coefficients for ARCO oil and INEL oil compared to relevant literature data. Log of the partition coefficient K vs. the number of carbon atoms in the organic acid. Shown are: octanol-water (∇), Oxy oil (∇), ARCO oil at pH 4, 55 °C and mid-concentration level ($\blacksquare$), and INEL oil at pH 4, 55 °C and mid-concentration level ($\bullet$).

APPENDIX 3

DATA TABLES FOR FIGURES

Chapter 2

Figure 2.3

day	H ₂ S	SO ₄ ²⁻
0	0	280
3	0	285
6	0	273
7	0	295
9	2	282
10	21	270
13	61	241
15	93	192
17	126	105

Figures 2.4 and 2.5

day	H ₂ S	cells	acetate	prop.	isobut.	<i>n</i> -but.	form.
0	0	0					
4	0	2 x 10 ⁵					
8	0	3 x 10 ⁵	220	250	205	195	220
11	0	1 x 10 ⁶	230	243	190	183	210
12	0	1.5x10 ⁶	228	192	195	150	190
21	3	3 x 10 ⁶	330	200	195	60	165
23	30	4 x 10 ⁶	415	150	200	40	130

28	73	7×10^6	455	95	196	25	112
29	82	2×10^7	520	75	190	0	105
30	76	6×10^7	555	50	150	0	62
31	58	5×10^7	560	50	140	0	55
32	62	1×10^6	450	120	220	30	125
35	95	1.5×10^7	570	60	110	0	60
37	93	7×10^6	535	120	210	0	55
39	57	2×10^7	560	70	170	0	48
45	80	1×10^7					
50	90	3×10^7					
53	76	4×10^6					
56	92	2×10^7					
57	64	3×10^5					

Figures 2.6 and 2.8

distance	H ₂ S	SO ₄ ²⁻	acetate	prop.	isobut.	n-but.	form.
0	0	279	251	176	183	175	232
5	107	135	371	84	150	9	0
10	108	131	381	83	151	3	0
15	102	136	369	83	151	5	0
20	114	112	369	62	117	0	0
30	117	78	389	44	85	0	0
40	120	56	408	30	51	0	0
50	125	74	410	37	73	0	0

Figures 2.7 and 2.9

port	H ₂ S	SO ₄ ^{s-}	acetate	prop.	isobut.	<i>n</i> -but.	form.
1	0	220	202	212	202	190	195
2	130	35	430	612	156	0	64
3	120	26	450	47	135	0	52

Figures 2.10 and 2.11

distance	H ₂ S	acetate
0	0	230
5	99,109,114	344,381,387
10	104,106,114	367,386,389
15	89,108,108	348,379,381
20	109,114,120	355,370,382
30	106,121,125	381,390,397
40	106,118,136	387,418,419
50	118,128,130	392,418,420

Figures 2.14 and 2.15

distance	H ₂ S (N. Sea)	E _h (N. Sea)	E _h (Slope #2)
0	5	-100	-100
5	137	-411	-461
10	160	-439	-447
15	160	-417	-441
20	166	-479	-444
30	153	-434	
40	163	-448	

Figure 2.12

section	biomass (Slope #1)	biomass (Slope #2)
1	7.7	11.5
2	7.6	8.5
3	3.9	9.6
4	5.0	11.9
5	5.9	8.9
6	4.8	7.7
7	4.9	10.1
8	9.0	9.1
9	2.2	6.7
10	8.0	10.6
11	6.6	10.1

Figure 2.13

section	mg FeS(S)/g sand
1	0.89
2	0.74
3	0.66
4	0.65
5	0.70
6	0.82
7	0.81
8	0.29

Chapter 3

Figure 3.2

distance	NO ₃ ⁻ (N. Sea)	NO ₂ ⁻ (N. Sea)	NO ₃ ⁻ (N. Slope #2)	NO ₂ ⁻ (N. Slope #2)
0	54	0	46.3	0
5	5	44.0	2.0	46.2
10	4	43.5	1.1	45.6
15	3	45.3	1.0	43.3
20	3	43.5	1.0	42.6
30	3	40.8	1.0	40.3
40	4	39.3	1.0	38.5
50	5	37.8	0	37.6

Figure 3.3

day	H ₂ S at distance = 5 cm
0	0
10	0
13	72
16	100
22	120
24	12
35	22
37	5
41	4

Figures 3.4 and 3.5

distance	E_h during souring (N. Slope #2)	E_h during souring (N. Sea)	E_h during NO_3^- reduction (N. Sea)	H_2S during NO_3^- reduction (N. Sea)
0	-100	-100	-100	0
5	-461	-411	-140	3.3
10	-447	-417	-45	3.0
15	-441	-439	15	2.2
20	-444	-479	75	1.6
30		-434	115	0.8
40		-448	330	0.5

Chapter 4

Figure 4.1

distance	H_2S (souring)	H_2S (pulsed NO_2^-)	H_2S w/10 ppm NO_2^-	H_2S w/12 ppm NO_2^-
0	0	0	0	0
5	107	1.5	13.0	0.7
10	108	1.0	13.5	0.7
15	102	1.0	15.1	1.7
20	114	1.5	14.8	3.9
30	117	1.6	13.3	1.8
40	120	1.2	10.9	1.0
50	125	0.7	8.4	0.8

Figure 4.2

distance	NO ₂ ⁻ w/10 ppm NO ₂ ⁻	NO ₂ ⁻ w/12 ppm NO ₂ ⁻
0	8.1	10.8
5	5.3	11.0
10	5.2	11.9
15	4.5	10.7
20	4.4	9.3
30	4.6	9.8
40	4.0	8.6
50	4.5	9.4

Figure 4.3

distance	E _h during souring	E _h w/10 ppm NO ₂ ⁻
0	-100	-100
5	-411	-340
10	-439	-372
15	-417	-264
20	-479	-365
30	-434	-337
40	-448	-345

Figure 4.4

day	H ₂ S	delta P
258	84	8.1
259	0	6.2

262	22	6.8
262	25	7.2
267	16	4.2
269	17	5.0

Figures 4.5 and 4.7

hours	H ₂ S w/glut. at distance = 5 cm	H ₂ S w/NO ₂ ⁻ at distance = 5 cm	H ₂ S w/glut. at distance = 40 cm
0	195	139	229
16	180	3	228
24	168	1	224
40	135	4	200
72	101		181
96	99		178
120	52		139
144	44		134
170	36		123
216	27		123
336	15		102

Figure 4.6

day	initial souring	souring after glut.	souring after NO ₂ ⁻
0	0	23	21
1	0	30	29
2	0	51	35
3	0	79	37

4	0	85	33
5	0	95	40
6	0	110	42
7	0	122	94
8	0	130	162
9	2	143	181
10	21	164	210
11	30	185	242
13	61		
15	93		
17	126		
20	148		
22	195		

Table 4.8

distance	NO ₂ ⁻
0	0
5	3.3
10	1.4
15	15
20	2.9
30	3.9
40	4.5
50	7

Chapter 5

Figure 5.2

distance	SO ₄ ²⁻ (original model)	H ₂ S (original model)	SO ₄ ²⁻ (revised model)	H ₂ S (revised model)	SO ₄ ²⁻ (data)	H ₂ S (data)
0	269	0	269	0	269	0
5	0	269	156	113	135	107
10	0	269	150	119	131	108
15	0	269	144	125	136	102
20	0	269	138	131	112	114
30	0	269	126	142	78	117
40	0	269	115	154	56	120
50	0	269	105	164	74	125

Figure 5.3

distance	<i>n</i> -but. (original model)	isobut. (original model)	<i>n</i> -but. (revised model)	isobut. (revised model)	<i>n</i> -but. (data)	isobut. (data)
0	174	184	174	184	174	184
5	14	20	0	170	9	150
10	14	20	0	156	3	151
15	14	20	0	143	5	151
20	14	20	0	130	0	117
30	14	20	0	104	0	85
40	14	20	0	80	0	51
50	14	20	0	56	0	73

Figure 5.4

dist.	form. (orig. mod.)	Ac (orig. mod.)	prop. (orig. mod.)	form. (final mod.)	Ac (final mod.)	prop. (final mod.)	form. data	Ac data	prop. data
0	234	230	175	234	230	175	234	230	175
5	71	435	2	0	360	102	0	371	84
10	71	435	2	0	368	92	0	381	83
15	71	435	2	0	376	82	0	369	83
20	71	435	2	0	384	72	0	369	62
30	71	435	2	0	399	54	0	389	44
40	71	435	2	0	414	36	0	408	30
50	71	435	2	0	427	19	0	410	37

Figure 5.5

distance	SO ₄ ²⁻ (original model)	H ₂ S (original model)	SO ₄ ²⁻ (revised model)	H ₂ S (revised model)	SO ₄ ²⁻ (data)	H ₂ S (data)
0	290	0	290	0	290	0
5	289	1	290	0	287	0
10	287	3	289	1	286	0
15	285	5	289	1	282	0
20	283	7	288	2	288	0
30	278	12	287	3	283	0
40	274	16	286	4	283	0
50	270	20	285	5	286	0

Figure 5.6

distance	NO ₃ ⁻ (original model)	NO ₂ ⁻ (original model)	NO ₃ ⁻ (revised model)	NO ₂ ⁻ (revised model)	NO ₃ ⁻ (data)	NO ₂ ⁻ (data)
0	54	0	54	0	54	0
5	1	53	2	43	5	44
10	0	54	0	45	4	44
15	0	54	0	45	3	45
20	0	54	0	45	3	44
30	0	54	0	45	3	41
40	0	54	0	45	4	39
50	0	54	0	45	5	38

Figures 5.7 and 5.8

dist.	Ac (orig. mod.)	i-but. (orig. mod.)	n-but. (orig. mod.)	Ac (final mod.)	i-but. (final mod.)	nbut. (final mod)	Ac data	i-but. data	n-but. data
0	218	213	204	218	213	204	218	213	204
5	198	192	201	191	199	203	194	201	193
10	200	191	294	190	199	202	194	198	194
15	203	191	187	191	199	200	195	201	196
20	206	191	180	192	199	198	190	195	193
30	212	191	165	193	199	195	197	196	195
40	218	191	151	194	199	191	192	195	197
50	224	191	137	196	199	188	189	190	194

Figures 5.9 and 5.10

days	SO_4^{2-}	H_2S	NO_2^-
0	105	164	0
0.5	160	114	11
1.0	247	37	35
1.5	278	11	44
2.0	284	6	45
2.5	285	5	45
3.0	285	5	45

Figure 5.11

distance	$K_t = 0.2$	$K_t = 0.02$	$K_t = 0.01$	data
0	0	0	0	0
5	8	0.8	0.4	0.7
10	15	1.6	0.8	0.7
15	22	2.4	1.2	0.7
20	30	3.2	1.6	3.9
30	43	4.8	2.4	1.8
40	56	6.3	3.2	1.0
50	68	7.9	4	1.8

APPENDIX 4

STANDARD CURVES AND CALCULATIONS

Standard Curves

Hydrogen Sulfide Standard Curve (12/30/92)

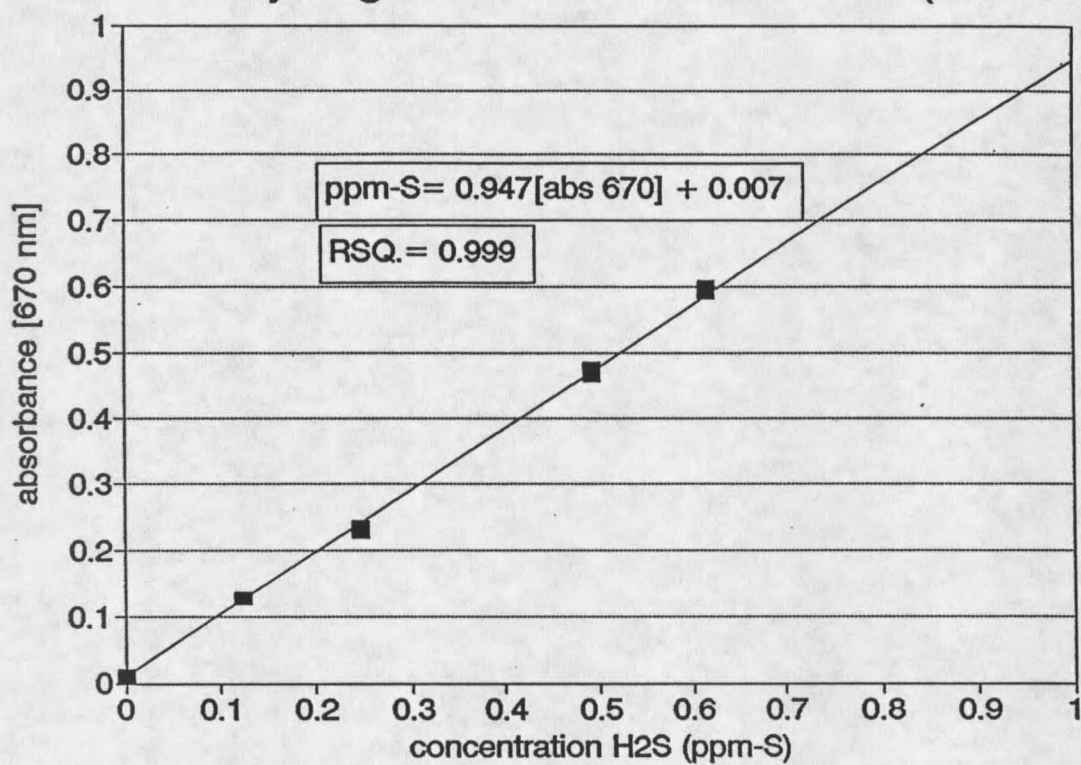


Figure A4.1. Hydrogen sulfide standard curve for the spectrophotometer.

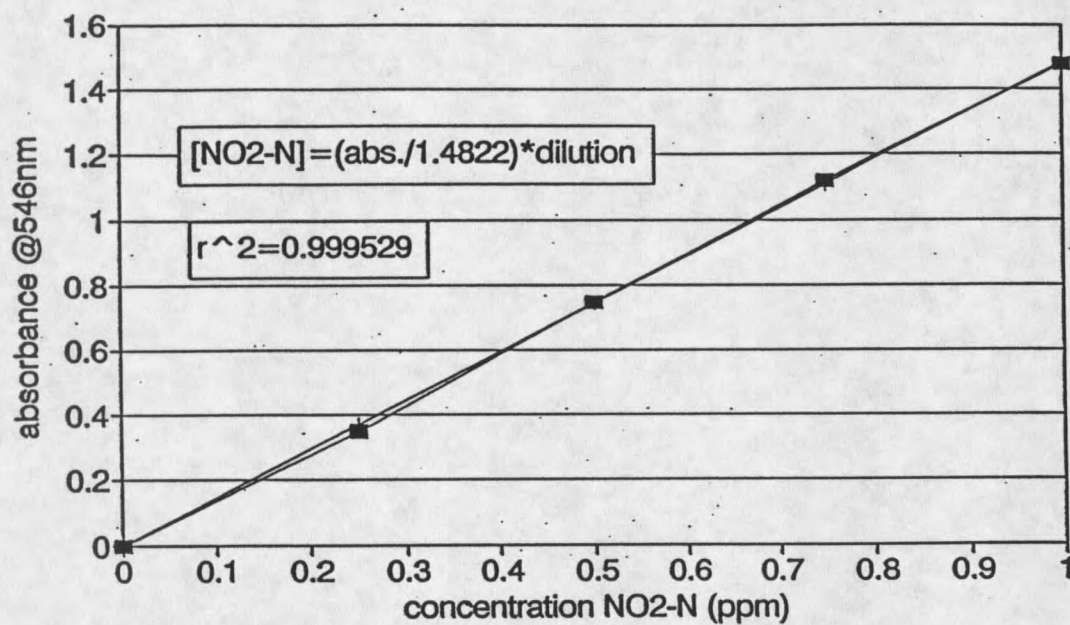


Figure A4.2. Nitrite standard curve for the spectrophotometer.

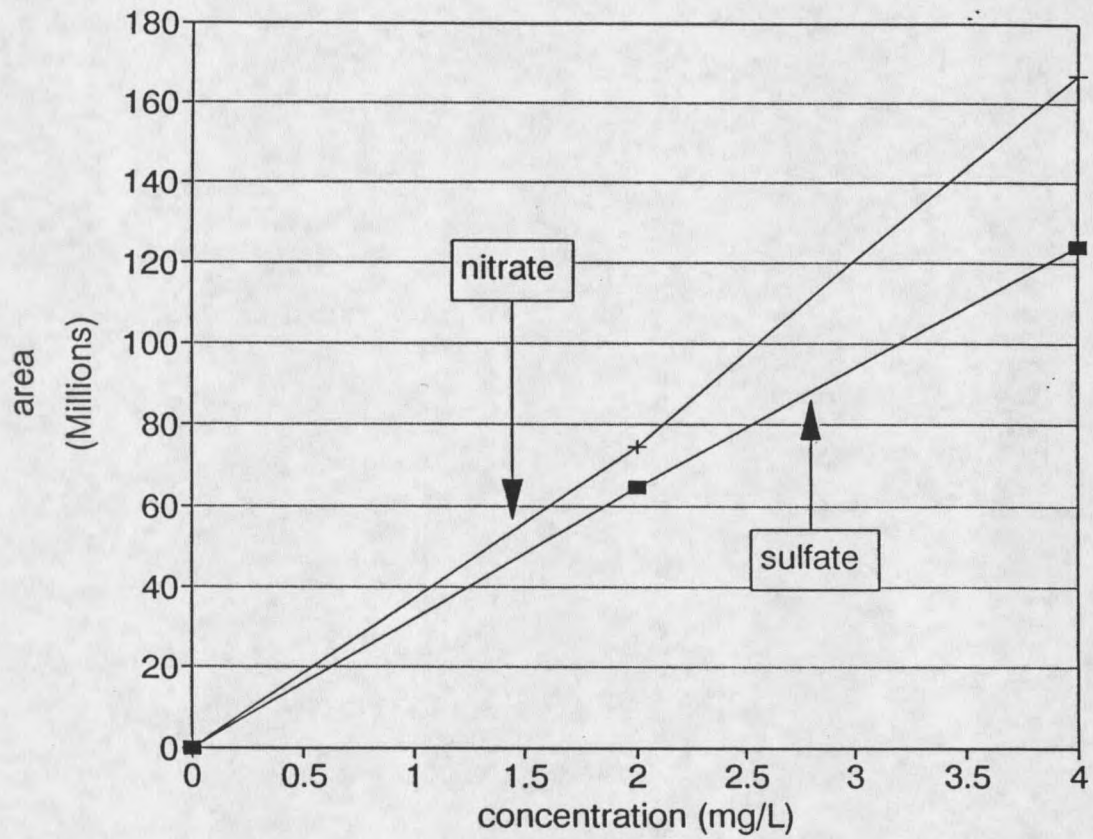


Figure A4.3. Sulfate and nitrate standard curves for the ion chromatograph.

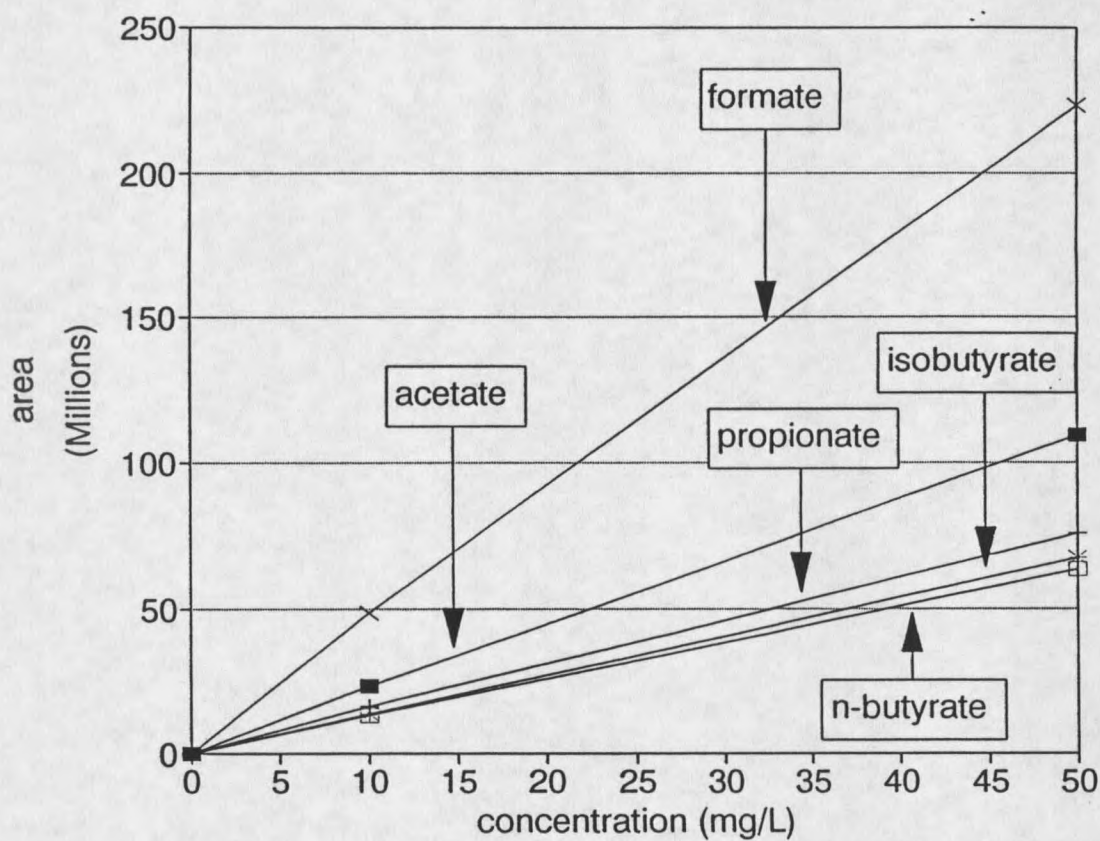


Figure A4.4. Organic acid standard curves for the ion chromatograph.

CalculationsCell Counts

for 1000x magnification:

$$\text{grid area} = 0.001936 \text{ mm}^2$$

$$\text{filter area} = \left(\frac{16.5 \text{ mm}}{2} \right)^2 \pi = 213.8 \text{ mm}^2$$

$$\text{size factor} = 213.8 / 0.001936 = 110,400$$

$$\text{cell count} = \text{average} \times \text{dilution factor} \times \text{size factor}$$

Sulfur Balance

measured iron sulfide in North Slope PW column:

$$\sim 0.7 \text{ mg FeS(S)/g sand} \times 1600 \text{ g sand} = 1.12 \text{ g FeS(S)}$$

difference between hydrogen sulfide and sulfate concentrations across the column

at steady state:

$$310 - 50 = 260 \text{ mg SO}_4^{2-}\text{(S)/L consumed}$$

$$225 - 0 = 225 \text{ mg H}_2\text{S(S)/L produced}$$

if souring for 30 days:

$$(260 - 225) \text{ mg S/L} \times 0.65 \text{ L/day} \times 30 \text{ days} \times 1 \text{ g/1000 mg} = 0.68 \text{ g FeS(S)}$$

Thus, the amount of sulfur measured in the column as iron sulfide (1.12 g)

approximately equals the amount of sulfur theoretically deposited there as iron sulfide (0.68 g).

Biofilm versus Suspended Cells

Biofilm cells (first 6 of 11 sections of the North Slope #2 column):

$$\rho_{\text{sand}} = 2.0 \text{ g/cm}^3$$

$$V_{\text{sand}} = 6/11 (50 \text{ cm}) \pi (2.75 \text{ cm})^2 = 648 \text{ cm}^3$$

$$2.0 \frac{\text{g sand}}{\text{cm}^3 \text{ sand}} \times 648 \text{ cm}^3 \text{ sand} \times \frac{10 \text{ mg biomass}}{\text{g sand}} \times \frac{1 \text{ g}}{1000 \text{ mg}} = 12.96 \text{ g dry biomass}$$

Suspended cells (first 6 sections):

$$2 \times 10^{-7} \frac{\text{cells}}{\text{ml}} \times 0.30 \frac{\mu\text{m}^3}{\text{cell}} \times \frac{1 \text{ ml}}{1 \text{ cm}^3} \times \frac{6}{11} \times 50 \text{ cm} \times \pi (2.75 \text{ cm})^2$$

$$\times 1.09 \times 10^{-12} \frac{\text{g wet cell}}{\mu\text{m}^3} \times 0.22 \frac{\text{g dry cell}}{\text{g wet cell}} = 10^{-3} \text{ g dry biomass}$$

Thus, the biomass of attached cells is about four orders-of-magnitude greater than the biomass of the suspended cells.

Biofilm Thickness

$$\rho_{\text{biomass}} = 1.07 \times 10^6 \frac{\text{g wet cell}}{\text{m}^3} \times 0.22 \frac{\text{g dry cell}}{\text{g wet cell}} = 2.35 \times 10^5 \frac{\text{g dry cell}}{\text{m}^3}$$

$$\rho_{\text{sand}} = \frac{35.2 \text{ g}}{25.0 \text{ ml}} \times 1000 \frac{\text{ml}}{\text{L}} \times 1000 \frac{\text{L}}{\text{m}^3} = 1.41 \times 10^6 \frac{\text{g sand}}{\text{m}^3}$$

$$\rho_{\text{sand particle}} = 1.41 \times 10^6 \times \frac{1}{0.50} = 2.82 \times 10^6 \frac{\text{g sand}}{\text{m}^3}$$

$$0.1198 \frac{m^3 \text{ dry cell}}{m^3 \text{ sand}} = \frac{V_2}{V_1} = \frac{4\pi(110 \times 10^{-6} m)^2 L_r}{4/3 \pi(110 \times 10^{-6} m)^3} = \frac{3 L_r}{110 \times 10^{-6} m}$$

Solving this equation for L_r yields $L_r = 4.39 \times 10^{-6} m$. Therefore, for this column with a measured porosity of 0.50 and measured biomass of 10 mg biomass/g sand, the biofilm thickness is approximately $4.4 \mu m$.

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