IMPACT OF MICROMIAL IRON REDUCTION ON RESERVOIR SOURING DURING WATERFLOODING

A Thesis in
Energy and Mineral Engineering

by

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ABSTRACT

Water-injection is widely practiced in petroleum reservoirs to improve oil recovery, maintain reservoir pressure and re-inject produced water. This practice can lead to microbial reservoir souring, which generates hydrogen sulfide via sulfate-reducing bacteria. Hydrogen sulfide adversely impacts the health and safety of employees, reduces oil quality and thus demands more processing requirements, corrodes pipeline and facilities, and plugs permeable formations. Reservoir souring costs the industry millions of dollars annually.

Laboratory experiments that have been made to study microbial souring mechanisms and growth, while published mathematical models simulate and forecast the souring process. In spite of the diverse microbial communities in oil reservoirs, current studies have been limited to selective microbes that mostly include sulfate-reducers and nitrate-reducers. One of the processes that has not yet been considered is microbial iron reduction. Current reservoir souring studies narrow the impact of iron minerals to the adsorption of hydrogen sulfide, namely through abiotic reactions to precipitate iron sulfides. Studies show that ferric iron can exist as iron oxides in natural systems within clay minerals and coating grains, a fraction of which would be bio-available for microbial reduction.

In this research, we have utilized the published results of microbial experiments conducted in upflow porous reactors to comprehend microbial processes and growth parameters. We used CrunchFlow, a reactive transport model, to simulate those processes in seawater injection for 1D homogeneous media at various ferric iron concentrations, and 2D heterogeneous media where ferric iron distribution was correlated with permeability reduction. Random heterogeneous fields were generated using a Fast Fourier Transform simulator at differing correlation lengths and Dykstra-Parson coefficients.
The analysis of microbial experiments by simulation indicate that microbial iron reduction inhibits sulfate reduction, imposing a delay on microbial souring by several pore volumes injected after seawater breakthrough. The results also show that higher concentrations of bio-available ferric iron would impose further delay on the microbial souring process in oil reservoirs. Formations with higher heterogeneity experience earlier hydrogen sulfide breakthrough than those that are more homogenous. In more heterogeneous reservoirs, the microbial reductions of sulfate and ferric iron are more isolated from one another than in homogenous reservoirs. Sulfate reduction prevails in permeable formations, while ferric iron reduction is limited to tight formations in which there are higher concentrations of iron minerals.

Our research demonstrates the benefits of understanding potential microbial iron reduction and ferric iron bio-availability in oil fields. This knowledge will enhance the prediction and forecast regarding biogenic hydrogen sulfide breakthrough, leading to improvements in the field and further developments.
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<table>
<thead>
<tr>
<th>Roman symbols</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A$</td>
<td>ratio of energy production to cell synthesis of electron-flow</td>
</tr>
<tr>
<td>$a_i$</td>
<td>ionic activity of component $i$ (mole/L)</td>
</tr>
<tr>
<td>$A_m$</td>
<td>mineral reactive surface area ($m^2$ solid phase/$m^3$ porous medium)</td>
</tr>
<tr>
<td>$A_{ow}$</td>
<td>$H_2S$ retardation factor due to oil-water phase partitioning</td>
</tr>
<tr>
<td>$B_T$</td>
<td>total biomass ($m^3/m^3$)</td>
</tr>
<tr>
<td>$C$</td>
<td>Cumulative $H_2S$ production constant (Kmole/$m^3$)</td>
</tr>
<tr>
<td>$C_i$</td>
<td>concentration of component $i$ (mole/L)</td>
</tr>
<tr>
<td>$d_g$</td>
<td>average grain diameter ($\mu m$)</td>
</tr>
<tr>
<td>$D_i$</td>
<td>diffusion/ dispersion coefficient of component $i$ ($m^2/s$)</td>
</tr>
<tr>
<td>$f_e$</td>
<td>electron-flow fraction for energy production (dimensionless)</td>
</tr>
<tr>
<td>$f_s$</td>
<td>electron-flow fraction for cell synthesis (dimensionless)</td>
</tr>
<tr>
<td>$\Delta G_a$</td>
<td>Gibb’s free energy of electron-acceptor half-reaction (KJ/ e’ eq)</td>
</tr>
<tr>
<td>$\Delta G_d$</td>
<td>Gibb’s free energy of electron-donor half-reaction (KJ/ e’ eq)</td>
</tr>
<tr>
<td>$\Delta G_{ic}$</td>
<td>Gibb’s free energy of converting substrate to pyruvate (KJ/ e’ eq)</td>
</tr>
<tr>
<td>$\Delta G_p$</td>
<td>Gibb’s free energy of pyruvate oxidation (KJ/ e’ eq)</td>
</tr>
<tr>
<td>$\Delta G_{pc}$</td>
<td>Gibb’s free energy of converting pyruvate to cell (KJ/ e’ eq)</td>
</tr>
<tr>
<td>$\Delta G_R$</td>
<td>Gibb’s free energy of intermediate-conversion (KJ/ e’ eq)</td>
</tr>
<tr>
<td>$\Delta G_S$</td>
<td>Gibb’s free energy of synthesis reaction (KJ/ e’ eq)</td>
</tr>
<tr>
<td>$k$</td>
<td>permeability in Carmen-Kozeny correlation ($m^2$)</td>
</tr>
<tr>
<td>$k$</td>
<td>kinetic rate constant in chemical reactions (mole/$m^2/s$)</td>
</tr>
<tr>
<td>$K_1$</td>
<td>maximum reducible sulfate (fraction)</td>
</tr>
<tr>
<td>$K_2$</td>
<td>effective rate of nutrient supply (dimensionless)</td>
</tr>
<tr>
<td>$K_3$</td>
<td>sulfate reduction in function of temperature (dimensionless)</td>
</tr>
<tr>
<td>$K_4$</td>
<td>DOC consumed per one mole of reduced sulfate</td>
</tr>
<tr>
<td>$k_{50}$</td>
<td>log of permeability at 50% ($m^2$)</td>
</tr>
<tr>
<td>$k_{84.1}$</td>
<td>log of permeability value at 84.1% ($m^2$)</td>
</tr>
<tr>
<td>$K_{eq}$</td>
<td>reaction equilibrium constant (dimensionless)</td>
</tr>
<tr>
<td>$K_i$</td>
<td>half-saturation coefficient of inhibitory compound (mole/g)</td>
</tr>
<tr>
<td>$K_i$</td>
<td>local hydraulic conductivity (m/day)</td>
</tr>
<tr>
<td>$K_{H_2S}^{bw}$</td>
<td>portioning of $H_2S$ between phase $j$ and water (dimensionless)</td>
</tr>
<tr>
<td>$k_{max}$</td>
<td>maximum growth rate (mole/$m^3$-SRB/day or mole/$m^2$-FeRB/s)</td>
</tr>
<tr>
<td>$K_S$</td>
<td>half-saturation coefficient of substrate (mole/L)</td>
</tr>
<tr>
<td>$K_{TEA}$</td>
<td>half-saturation coefficient of electron-acceptor (mole/L)</td>
</tr>
<tr>
<td>$L$</td>
<td>Length of 1D media ($m$)</td>
</tr>
<tr>
<td>$M_j$</td>
<td>molecular weight of phase $j$ (g/mole)</td>
</tr>
<tr>
<td>$n$</td>
<td>energy-transfer efficiency exponent (dimensionless)</td>
</tr>
<tr>
<td>$N_m$</td>
<td>total number of mineral reactions in a system</td>
</tr>
<tr>
<td>$N_r$</td>
<td>total number of aqueous reactions in a system</td>
</tr>
<tr>
<td>$P_r$</td>
<td>reservoir pressure (atm)</td>
</tr>
</tbody>
</table>
\( P_{H_2S} \) = cumulative \( H_2S \) production (Kmole/m²)

\( R \) = overall microbial reaction

\( R_a \) = electron-acceptor half-reaction

\( R_{ads} \) = \( H_2S \) adsorption affinity into solid phase (dimensionless)

\( R_c \) = cell-synthesis half-reaction

\( R_d \) = electron-donor half-reaction

\( R_e \) = microbial energy reaction

\( R_H \) = production rate of \( H_2S \) production in the water phase (Kmole/m³.s)

\( r_i^j \) = adsorption of the component \( i \) into phase \( j \) (dimensionless)

\( R_m \) = rate of a mineral reaction (mole/s)

\( R_r \) = rate of an aqueous reaction (mole/s)

\( R_s \) = microbial synthesis reaction

\( R_{TST} \) = reaction rate in TST (mole/s)

\( S_j \) = pore volume fraction of phase \( j \)

\( S_j^* \) = bulk volume fraction of phase \( j \)

\( t \) = time (day or seconds)

\( t_D \) = dimensionless time

\( u_j \) = Darcy-velocity of phase \( j \) (m/day)

\( V_{DP} \) = Dykstra-Parson coefficient (dimensionless)

\( V_{fracture} \) = fracture volume in chalk formation (volumes)

\( v_{ir} \) = stoichiometric coefficient of component \( i \) in aqueous reaction \( r \)

\( v_{im} \) = stoichiometric coefficient of component \( i \) in mineral reaction \( m \)

\( v_x \) = velocity in \( x \)-direction (m/s)

\( \Delta x \) = width of \( H_2S \) source in mixing-zone model (m)

\( x_D \) = distance (dimensionless)

\( Y \) = growth yield (g-biomass/ mole-\( S \) or g-biomass/ mole-TEA)

**Greek letters:**

\( \alpha \) = dispersivity (m)

\( \beta \) = middle-line slope of trilinear sulfate consumption approximation

\( \varepsilon \) = microbial energy-transfer efficiency (dimensionless)

\( \lambda \) = correlation length (m)

\( \mu \) = microbial specific growth rate (1/s)

\( \mu_{Fe} \) = mean of ferric iron concentration (g/mole)

\( \mu_{logK} \) = hydraulic conductivity global mean (m/day)

\( \mu_{max} \) = microbial maximum specific growth rate (1/hour)

\( \sigma_{logK} \) = standard deviation of hydraulic conductivity (m/day)

\( \sigma_{Fe} \) = standard deviation of ferric iron concentration (mole/g)

\( \sigma_{Fe}^2 \) = variance of ferric iron concentration (mole/g)²

\( \tau_b \) = time constant of microbial reaction in mixing-zone model (s)

\( \rho \) = correlation coefficient (dimensionless)

\( \rho_j \) = density of phase \( j \) (Kg/m³)

\( \varnothing \) = porosity (fraction)
### Abbreviations:

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1D</td>
<td>one-dimensional</td>
</tr>
<tr>
<td>2D</td>
<td>two-dimensional</td>
</tr>
<tr>
<td>3D</td>
<td>three-dimensional</td>
</tr>
<tr>
<td>B</td>
<td>biomass</td>
</tr>
<tr>
<td>BC</td>
<td>bio-complex intermediate</td>
</tr>
<tr>
<td>DOC</td>
<td>dissolved organic content</td>
</tr>
<tr>
<td>e' eq</td>
<td>electron equivalent</td>
</tr>
<tr>
<td>FeRB</td>
<td>iron-reducing bacteria</td>
</tr>
<tr>
<td>FFT</td>
<td>Fast Fourier Transform</td>
</tr>
<tr>
<td>I</td>
<td>inhibitory compound</td>
</tr>
<tr>
<td>IAP</td>
<td>ion activity product</td>
</tr>
<tr>
<td>NRB</td>
<td>nitrate-reducing bacteria</td>
</tr>
<tr>
<td>NR-SOB</td>
<td>nitrate-reducing, sulfide-oxidizing bacteria</td>
</tr>
<tr>
<td>sal</td>
<td>salinity</td>
</tr>
<tr>
<td>S</td>
<td>substrate</td>
</tr>
<tr>
<td>SI</td>
<td>saturation index</td>
</tr>
<tr>
<td>SRB</td>
<td>sulfate-reducing bacteria</td>
</tr>
<tr>
<td>SSA</td>
<td>specific surface area</td>
</tr>
<tr>
<td>SW</td>
<td>seawater</td>
</tr>
<tr>
<td>TEA</td>
<td>terminal electron acceptor</td>
</tr>
<tr>
<td>TST</td>
<td>transition state theory</td>
</tr>
<tr>
<td>P</td>
<td>biogenic product</td>
</tr>
<tr>
<td>PDF</td>
<td>probability density function</td>
</tr>
<tr>
<td>PWRI</td>
<td>produced water re-injection</td>
</tr>
<tr>
<td>PVI</td>
<td>pore volume injected</td>
</tr>
<tr>
<td>WS</td>
<td>well-spacing</td>
</tr>
<tr>
<td>VFA</td>
<td>volatile fatty acid</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

I begin in the Name of Allah, The Beneficent, The Merciful. The last two years have been a start of unexpected blessing. I am dedicating this work to my beloved wife Fatima and my beautiful daughter Kawthar. They are the blessing of my life that kept me surrounded with love and support. I cannot also forget my lovely family back home, specially my mother that she never forgot me in her prayers and kept me in her mind and heart.

I would like to take this opportunity to express my appreciation to my academic advisers, Dr. Johns and Dr. Li Li, for their continuous support and guidance. I would like to thank Hang Wen for his endless help and support in the time of need. I express sincere gratitude to my sponsor, Saudi Aramco, for giving this opportunity and supporting me along the way.

Mohammed A. AlSaffar

University Park, Pennsylvania

August, 2017
Chapter 1

Introduction

1.1 Microbial Reservoir Souring in Oil Fields

Water injection has become an integral practice in oil fields, helping to sustain their productivity and increasing hydrocarbon recovery. Injection serves to maintain reservoir pressure when natural drives, if present, become exhausted, and helps to sweep and displace untapped oil volumes. The relative abundant and low cost of water compared to other fluids makes it more feasible to utilize in oil fields, especially in offshore fields where the injection of seawater is more economically attractive. The seawater injected into oil reservoirs usually contains high sulfate concentrations, between 1850 and 3245 mg/L (Tyrie et al. 1993, Khatib 1997); this could increase the risk of stimulating the growth of sulfate-reducing bacteria (SRB). These bacteria have been identified to be the primary cause of reservoir souring, in which hydrogen sulfide (H\textsubscript{2}S) concentrations in reservoir fluids increase rapidly.

H\textsubscript{2}S is a toxic compound that endangers the health and safety of employees (Table 1-1), and that plays destructive roles in the oil industry with significant associated costs and reduced revenue. The oil and gas market demands low concentrations of H\textsubscript{2}S, below 4 mg/L, at which sour crudes would have lower economic value and require further refining to remove H\textsubscript{2}S (Hilyard 2012, Kelland 2014). Furthermore, the presence of SRB and biogenic H\textsubscript{2}S is believed to be the leading cause of microbial corrosion in oil pipelines and facilities (Figure 1-1), which accounts for 15-30\% of corrosion incidents and results in annual financial losses of approximately $100 million, without including losses associated with interrupting operations and remedial jobs (Dunsmore et al. 2006, Beech et al. 2007). In addition, it may be necessary to upgrade existing wells’ equipment and
installations to cope with the corrosiveness of H₂S gas, which would cost tens to hundreds of thousands of dollars per well (Khatib 1997). In some cases, aggressive localized microbial souring in shale formations could lead to the premature failure of coil-tubing units used in hydraulic fracturing (Seal et al. 2015). SRB could also cause significant damage to permeability. Experiments conducted on core samples show permeability reductions by 16-33% within five to 11 days of SRB activity (Rosnes et al. 1991).

In addition to biocides, several mitigation methods have been applied in oil fields to encounter reservoir souring. One method is to reduce sulfate concentrations in injected seawater by using sulfate removal technologies, or injecting aquifer and produced waters instead of seawater (Cavallaro et al. 2005, Robinson et al. 2010, Evans et al. 2015). Another method is to add nitrate into injected water, prior to or after reservoir souring takes place (Kuijvenhoven et al. 2006, Zhu et al. 2016). Nitrate could stimulate nitrate-reducing bacteria (NRB) and nitrate-reducing, sulfide-oxidizing bacteria (NR-SOB) that compete with SRBs, leading to a reduction in sulfide concentrations. Also, the reduction of nitrate generates nitrite, which is toxic to SRBs and thus inhibits their growth. However, these mitigation methods have not been universally successful in preventing reservoir souring. Sulfate reduction technologies are costly and likely to be unfeasible in offshore fields, while lowering sulfate concentrations helps to maintain low H₂S production but does not effectively prevent reservoirs from souring. Several nitrate injection trials have been unsuccessful. Nitrate could also contribute to corrosion and to the formation of elemental sulfur (Evan 2008, Voordouw et al. 2009, Johnson et al. 2017).

The current need for water injection and the rising challenges of microbial souring demand a better understanding of the reservoir-related factors that lead to its generation. This requires further exploration of more effective methods to mitigate reservoir souring and to improve the reliability of simulation results. Our research focuses on one aspect of these reservoir factors that can impose a significant impact on microbial souring in oil fields.
Table 1-1: Health symptoms and hydrogen sulfide effects of hydrogen sulfide exposure.

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00011- 0.00033</td>
<td>Typical background concentrations</td>
</tr>
<tr>
<td>0.01-1.5</td>
<td>Odor threshold (when rotten egg smell is first noticeable to some). Odor becomes more offensive at 3-5 ppm. Above 30 ppm, odor described as sweet or sickeningly sweet.</td>
</tr>
<tr>
<td>2-5</td>
<td>Prolonged exposure may cause nausea, tearing of the eyes, headaches or loss of sleep. Airway problems (bronchial constriction) in some asthma patients.</td>
</tr>
<tr>
<td>20</td>
<td>Possible fatigue, loss of appetite, headache, irritability, poor memory, dizziness.</td>
</tr>
<tr>
<td>50-100</td>
<td>Slight conjunctivitis (gas eye) and respiratory tract irritation after 1 hour. May cause digestive upset and loss of appetite.</td>
</tr>
<tr>
<td>100</td>
<td>Coughing, eye irritation, loss of smell after 2-15 minutes (olfactory fatigue). Altered breathing, drowsiness after 15-30 minutes. Throat irritation after 1 hour. Gradual increase in severity of symptoms over several hours. Death may occur after 48 hours.</td>
</tr>
<tr>
<td>100-150</td>
<td>Loss of smell (olfactory fatigue or paralysis).</td>
</tr>
<tr>
<td>200-300</td>
<td>Marked conjunctivitis and respiratory tract irritation after 1 hour. Pulmonary edema may occur from prolonged exposure.</td>
</tr>
<tr>
<td>500-700</td>
<td>Staggering, collapse in 5 minutes. Serious damage to the eyes in 30 minutes. Death after 30-60 minutes.</td>
</tr>
<tr>
<td>700-1000</td>
<td>Rapid unconsciousness, knockdown or immediate collapse within 1 to 2 breaths, breathing stops, death within minutes.</td>
</tr>
<tr>
<td>1000-2000</td>
<td>Nearly instant death</td>
</tr>
</tbody>
</table>

Source: U.S. Occupational Safety and Health Administration (www.osha.gov/SLTC/hydrogensulfide/hazards.html)
1.2 The Role of Iron-Bearing Minerals

The presence of SRB and reducible sulfate concentration does not always result in reservoir souring. Some production wells produce significant amounts of H₂S while others in the same field maintain sweet oil production. Some wells may contain SRBs in their fluids and still produce sweet oil, while others produce significant H₂S concentrations without detecting SRBs (Herbert et al. 1985, Khatib 1997). Moreover, the breakthrough of H₂S in production wells usually takes place after producing several pore volumes of injected water (Herbert et al. 1985, Sunde et al. 1993, Tyrie et al. 1993). One factor that can delay biogenic H₂S production, in addition to phase-partitioning between oil and water, is the presence of iron-bearing minerals.

Figure 1-1: Conceptual model of microbial-corrosion by SRB (From Cord-Ruwisch et al. 1987).
Iron-bearing minerals are known in oil fields for their capability to react with H₂S, which results in the precipitation of iron-sulfides. The reactive iron in these minerals may be ferrous (Fe²⁺) or ferric (Fe³⁺); fully-oxidized iron is more abundant in natural systems (Ligthelm et al. 1991, Worden et al. 2003, Johnson et al. 2017). Most oil fields evaluate the adsorption capacity of H₂S by the amount of siderite (FeCO₃) in formations (Kuijvenhoven et al. 2006, Burger et al. 2009, Zhu et al. 2016). Other scavenging minerals include hematite (Fe₂O₃), magnetite (Fe₃O₄), goethite (FeOOH), iron-containing silicates and iron-rich clays. The amount of these minerals varies in sandstone formations (Table 1-2), while the scavenging capacities of carbonate reservoirs are in most cases insignificant. However, the experimental measurements of H₂S adsorption capacity, using crushed rocks and core plugs, are largely unreliable and overestimated. Thus, more reliable estimates are often attempted throughout the process of history-matching of produced fluids (Evans 2008).

One aspect of iron-bearing minerals has not been considered by current reservoir souring studies, namely the stimulation of ferric iron reduction by iron-reducing bacteria (FeRB). The presence of FeRB has been established in several oil fields (Semple et al. 1987, Nazina et al. 1995, Slobodkin et al. 1999). Although FeRBs are capable of metabolizing soluble and non-soluble iron oxides (Greene et al. 1997), some studies have suggested that insoluble ferric iron is more bio-available due to the presence of organic acids in petroleum fluids, which act as a ligand to mobilize iron oxides (Lovley et al. 1994, Schmitt et al. 1996).

There are two viable methods through which microbial iron reduction could impact reservoir souring. The first method involves FeRB outcompeting and inhibiting SRB by up to 90% (Lovley et al. 1987). The second method involves microbial processes that occur successively via either FeRB or SRB being the primary reducers, through which ferric iron is reduced before sulfate, producing stable ferrous iron concentrations (García-Balboa et al. 2010). In both of these methods, the formation of ferrous iron would help to precipitate more H₂S, while biogenic H₂S would further
reduce ferric iron through abiotic reactions. Studies have shown that 5-20% of total ferric iron content in iron-rich formations is bio-reducible, which corresponds to concentrations of 2.0-2.5 µmol/g (Kostka et al. 2002, Li et al. 2009, Ko et al. 2016). However, the bio-availability of ferric iron in oil fields has not been extensively evaluated; thus, the influence of FeRB requires further evaluation (Magot et al. 2000, Peters et al. 2007).

Table 1-2: The range of iron-containing minerals in sandstone reservoirs in the North Sea (Ligthelm et al. 1991).

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Percent by Volume of Sandstone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotite (Fe-bearing silicate)</td>
<td>0.00-0.33</td>
</tr>
<tr>
<td>Iron-rich clay</td>
<td>1.33-5.00</td>
</tr>
<tr>
<td>Hematite</td>
<td>0.00-0.33</td>
</tr>
<tr>
<td>Siderite</td>
<td>0.00-0.33</td>
</tr>
<tr>
<td>Pyrite</td>
<td>0.00-2.67</td>
</tr>
</tbody>
</table>

1.3 The Role of Reservoir Permeability

There are three aspects by which reservoir permeability could impact the souring process: bacterial mobility, H₂S transport in thief zones and scavenging capacities. Several studies have suggested that the mobility of bacteria, which has a diameter of approximately 1 µm, could be restricted by the pore-throat size of the porous media (Herbert et al. 1985, Evans 2008). Higher permeability values with larger pore-throats could help transport SRBs and thus expand their activities beyond injection wells. In addition, higher permeability could also expedite the breakthrough of biogenic H₂S generated near injection wells, where permeability may vary spatially and thief zones may exist between producers and injectors (Figure 1-2).

The degree of permeability variation within oil reservoirs can be estimated using the heterogeneity index suggested by Dykstra and Parson (1950) (Equation 1-1):
\[ V_{DP} = \frac{k_{50} - k_{84.1}}{k_{50}}, \]  

Eq. 1-1

where \( V_{DP} \) is the Dykstra-Parson coefficient, \( k_{50} \) is the log of permeability value at 50%, and \( k_{84.1} \) is the log of permeability at 84.10% (one standard deviation). \( V_{DP} \) has a value between 0 and 1, where zero represents an ideally-homogenous reservoir, and unit a perfectly-heterogeneous reservoir. Moreover, laboratory measures from some oil fields have shown that adsorption capacity increases with permeability reduction (Table 1-3). These measurements indicate a correlation between the presence of iron-bearing minerals and permeability reduction. However, current studies do not evaluate the potential impact of trends, or how the distribution of iron-bearing minerals could impact reservoir souring in heterogeneous formations through abiotic and biotic processes.
Figure 1-2: Conceptual illustration of the impact of permeability variations on biogenic H$_2$S breakthrough and microbial transport (from Evans et al. 2015).

Table 1-3: Permeability and corresponding H$_2$S adsorption capacity for oil reservoirs in the North Sea (Sunde et al. 1993).

<table>
<thead>
<tr>
<th>Reservoir</th>
<th>Permeability (mD)</th>
<th>Adsorption Capacity (µg H$_2$S/ g reservoir rock)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5200</td>
<td>14</td>
</tr>
<tr>
<td>A</td>
<td>510</td>
<td>350</td>
</tr>
<tr>
<td>A</td>
<td>0.4</td>
<td>19600</td>
</tr>
<tr>
<td>B</td>
<td>1300</td>
<td>5</td>
</tr>
<tr>
<td>B</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>C</td>
<td>8200</td>
<td>550</td>
</tr>
<tr>
<td>C</td>
<td>740</td>
<td>1950</td>
</tr>
</tbody>
</table>
1.4 Research Objectives

Recently-published studies have not considered the impact of microbial iron reduction on reservoir souring, which can be investigated through the correlation between heterogeneity and the mineralogy of oil reservoirs. The objectives of this research are as follows:

1. Use laboratory experiments to understand and calibrate the biotic chemical reactions of sulfate and ferric iron reductions that are involved in reservoir souring, and utilize these reactions to conduct reservoir simulation analyses.
2. Study the impact of bio-available ferric iron concentrations on biogenic H$_2$S breakthrough and development in homogenous reservoirs with seawater injection.
3. Evaluate the role of reservoir heterogeneity and the correlated iron distribution on microbial reductions of iron and sulfate and reservoir souring development in seawater injection projects.

1.5 Thesis Structure

Chapter 2 presents the literature review in five sections. The first section provides a brief background on oil reservoir microbiology and the processes associated with reservoir souring. The second section discusses the mathematical models used to express microbial reactions and growth. The third section illustrates the experimental methods to characterize bacterial growth and metabolism. The fourth section describes the basic mathematical models developed to understand microbial processes, while the fifth section summarizes the most important aspects of the literature review.

Chapter 3 discusses the methodology used in this study. This chapter summarizes the procedures and results of the microbial experiments, which were required in this research to obtain
microbial reactions. The next section describes the configurations of 1D and 2D waterflooding simulations, for both homogenous and heterogeneous reservoirs. In addition, this chapter describes the abiotic chemical reactions considered.

Chapter 4 presents and discusses the simulation results of the microbial experiments. The first section analyzes a microbial experiment with unexpected souring results, where these results are explained using physical hypotheses. The next section discusses the results of a microbial experiment that are less uncertain in terms of occurring microbial metabolisms. The results of these experiments are compared to identify critical parameters.

Chapter 5 presents and discusses the results of waterflooding simulations. The results of the 1D homogenous simulations are analyzed, followed by those of the 2D heterogeneous simulations. Finally, chapter 6 presents the main conclusions of the study and potential areas for future research.
Chapter 2

Literature review

2.1 Introduction to Reservoir Microbiology

Microbial communities commonly found in petroleum reservoirs can be classified into two groups: temperature tolerant bacteria and metabolism bacteria. The temperature gradient in oil reservoirs caused by cooling of injected water is believed to be one of the factors that stimulate microbial activity. Most microbes function better at relatively low temperatures; thus, it was presumed that deep and hot oil reservoirs such as those in the North Sea would be immune to microbial reservoir souring (Maxwell et al. 2005). However, several of these reservoirs produced sour crudes, which highlighted the need for understanding the relationship between reservoir temperatures and microbial activities.

Most microbes have been introduced into reservoirs through drilling operations and water injection is likely to be mesophilic, while those microbes that are indigenous to reservoirs are mostly thermophilic (Herbert et al. 1985, Magot et al. 2000). Mesophilic microbes can survive in low-temperature environments (< 50 °C); thus, they usually inhabit low-temperature reservoirs and sectors of oil fields where the reservoir temperature has been reduced by water injection. Thermophilic microbes, however, tolerate higher temperatures (up to 90 °C), which allows them to exist in relatively high-temperature reservoirs and at further distances from injection wells (Figure 1-2). The activity of indigenous thermophilic microbes is stimulated by injected water, which holds higher concentrations of the species required for their growth. Thermophilic SRBs are stimulated by the high sulfate concentrations found in seawater.

The anoxic nature of oil reservoirs and the lack of dissolved oxygen in injected water create an environment in which bacteria use anaerobic respiration for energy and growth. There are five
main metabolic bacterial cultures that exist within oil reservoirs (Figure 2-1): SRB, FeRB, NRB (sometime called denitrifying bacteria), methanogenic bacteria, and fermentative bacteria. While some microbes compete for common compounds essential to their metabolism, some microbes depend on others to sustain their growth. For example, SRB cannot efficiently oxidize hydrocarbon components for growth; thus, they depend on fermentative bacteria to degrade oil components into organic volatile fatty acids (VFAs) that SRB can more efficiently metabolize (Herbert et al. 1985, Magot et al. 2000, Callback et al. 2013). The bio-products of SRB respiration in oil fields are largely H₂S, CO₂ and acetate. A second reaction can occur following SRB respiration, during which methanogenic bacteria oxidizes the acetate produced by SRB to methane. Methanogenic bacteria can also utilize CO₂ and H₂ to produce methane. The activities of NRB and FeRB are contingent on the availability of nitrate and ferric iron, respectively, which are usually found in limited concentrations in initial reservoir conditions. Thus, the injection of nitrate is essential to stimulate microbes that outcompete SRB. In addition, NR-SOB can oxidize biogenic sulfide rather than VFA to sulfate.

Recent advancements in the DNA analysis of microbes have helped to improve the characterization of the diversities of their metabolism, and may lead to a better understanding of microbial problems and proper mitigation plans. These analyses have also led to the further consideration of prokaryotes in oil reservoirs, including both bacteria and archaea (for simplicity, these prokaryotes will hereafter be referred to as ‘bacteria’). DNA analyses have also revealed the capability of these microbes to utilize multiple energy sources in their anaerobic respiration (sulfate, nitrate and iron). For example, Nalco Champion™ started an initiative to develop a database containing the analysis of many microbial oil samples (Geissler et al. 2014-2016). Figure 2-2 illustrates the metabolic breakdown of microbial cultures in oil fields that experience H₂S problems.
The availability of sulfate in injected water and the common utilization of nitrate for souring treatment results in increasing the populations of their reducers in oil fields. Both sulfate and nitrate are aqueous species; thus, they are more accessible for microbial reduction compared to ferric iron. However, there may be a shift in the dominant bacterial cultures within oil reservoirs based on spatial heterogeneity and ferric iron distribution, and microbial iron reduction could in this way have an increased influence on reservoir souring. In the presence of multiple energy sources, microbial reductions would be more dependent on the thermodynamics of bacterial growth. These will be discussed in the next section.
Figure 2-1: Conceptual model of main microbial metabolisms in oil reservoirs (from Wolicka et al. 2010).
2.2 Thermodynamics of Bacterial Growth

2.2.1 Development of Microbial Reactions

Bacterial mass is composed mostly of carbon, oxygen, hydrogen, nitrogen and phosphorus. Biomass can also include traces of iron, sulfur and other elements. In general, bacteria need relatively small amounts of nitrogen and phosphorus for their growth, and the availability of these elements has not been extensively evaluated in oil reservoirs (Magot et al. 2000). The relative proportion of elements can be experimentally measured based on the dry biomass, or empirically...
estimated based on the oxygen demand. However, a commonly-used empirical formula for biomass is C$_5$H$_7$O$_2$N; this formula will be utilized throughout this research.

During their growth process, bacteria utilize the flow of electrons for energy production and cell synthesis that can be expressed based on three half-reactions: Electron-acceptor (R$_a$), electron-donor (R$_d$) and cell synthesis (R$_c$). The electron-acceptor reactions describe the reduction in oxidation state of energy sources such as sulfate and ferric iron, which are known as terminal electron acceptors (TEAs). Table 2-1 shows examples of TEA half-reactions that are common in natural systems and relevant to oil fields; they are here ranked based on their Gibb’s standard free energy ($\Delta G^0$). The reactions that produce higher energy would be more preferable for microbial growth, and the corresponding TEAs would be consumed first either in sequential metabolisms or through bio-competition. Based on this, microorganisms would always prefer aerobic respiration that utilizes oxygen as TEA. As oil reservoirs are usually anoxic environments, microorganisms utilize anaerobic respiration to maintain their growth. Table 2-1 shows that ferric iron would result in higher energy than sulfate; thus, microbial iron reduction would conceptually dominate microbial souring. However, this process would be influenced by the accessibility of the TEAs, at which sometime ferric iron could be more limited than sulfate.

In order to establish the electron flow needed to reduce these TEAs, substrates are oxidized to donate electrons, as illustrated by donor half-reactions. These substrates are also the source of the organic carbon required for microbial synthesis. In the case of oil reservoirs, these substrates are largely VFAs generated by fermentative bacteria. Table 2-2 shows some examples of the substrates utilized by microorganisms. These substrates can be completely oxidized to CO$_2$ and water, or they can be incompletely oxidized to simpler organic compounds such as acetate and butanol.
Cell-synthesis reactions describe the development of biomass and specify the nitrogen source in the environment for microbial growth. A common source for nitrogen in natural systems is ammonia; thus, the half-cell synthesis reaction can be expressed as (Reaction 2-10):

$$\frac{1}{4} \text{HCO}_3^- + \frac{1}{20} \text{NH}_4^+ + \frac{6}{5} \text{H}^+ + \bar{\varepsilon} = \frac{1}{20} \text{C}_5\text{H}_7\text{N}_2\text{O}_2 + \frac{13}{20} \text{H}_2\text{O}$$

Reaction 2-10

Table 2-1: TEA reactions relevant to oil reservoirs. Ranking based on Gibb’s standard free energy at pH 7.0 (Rittmann et al. 2001).

<table>
<thead>
<tr>
<th>Reaction number</th>
<th>Reduction couple</th>
<th>Half-reaction</th>
<th>$\Delta G_d^{0'}$ (KJ/ e⁻ eq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-1</td>
<td>O₂/H₂O</td>
<td>$\frac{1}{4} \text{O}_2 + \text{H}^+ + \bar{\varepsilon} = \text{H}_2\text{O}$</td>
<td>-78.72</td>
</tr>
<tr>
<td>2-2</td>
<td>Fe³⁺/Fe²⁺</td>
<td>$\text{Fe}^{3+} + \bar{\varepsilon} = \text{Fe}^{2+}$</td>
<td>-74.27</td>
</tr>
<tr>
<td>2-3</td>
<td>NO₃⁻/N₂</td>
<td>$\frac{1}{5} \text{NO}_3^- + \frac{6}{5} \text{H}^+ + \bar{\varepsilon} = \frac{1}{10} \text{N}_2 + \frac{3}{5} \text{H}_2\text{O}$</td>
<td>-72.20</td>
</tr>
<tr>
<td>2-4</td>
<td>NO₃⁻/NO₂⁻</td>
<td>$\frac{1}{2} \text{NO}_3^- + \text{H}^+ + \bar{\varepsilon} = \frac{1}{2} \text{NO}_2^- + \frac{1}{2} \text{H}_2\text{O}$</td>
<td>-41.65</td>
</tr>
<tr>
<td>2-5</td>
<td>SO₄²⁻/H₂S</td>
<td>$\frac{1}{8} \text{SO}_4^{2-} + \frac{5}{4} \text{H}^+ + \bar{\varepsilon} = \frac{1}{8} \text{H}_2\text{S} + \frac{1}{2} \text{H}_2\text{O}$</td>
<td>20.85</td>
</tr>
<tr>
<td>2-6</td>
<td>CO₂/CH₄</td>
<td>$\frac{1}{8} \text{CO}_2 + \text{H}^+ + \bar{\varepsilon} = \frac{1}{8} \text{CH}_4 + \frac{1}{4} \text{H}_2\text{O}$</td>
<td>23.53</td>
</tr>
</tbody>
</table>

Table 2-2: Some common VFAs utilized by microbes as electron donors and their Gibbs’ free energy at pH 7.0 (Rittmann et al. 2001).

<table>
<thead>
<tr>
<th>Reaction number</th>
<th>Substrate</th>
<th>Half-reaction</th>
<th>$\Delta G_d^{0'}$ (KJ/ e⁻ eq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-7</td>
<td>Acetate</td>
<td>$\frac{1}{4} \text{HCO}_3^- + \frac{1}{8} \text{H}^+ + \bar{\varepsilon} = \frac{1}{8} \text{CH}_3\text{COO}^- + \frac{1}{2} \text{H}_2\text{O}$</td>
<td>27.40</td>
</tr>
<tr>
<td>2-8</td>
<td>Lactate</td>
<td>$\frac{1}{4} \text{HCO}_3^- + \frac{7}{6} \text{H}^+ + \bar{\varepsilon} = \frac{1}{12} \text{CH}_3\text{CHOHCOO}^- + \frac{1}{2} \text{H}_2\text{O}$</td>
<td>32.29</td>
</tr>
<tr>
<td>2-9</td>
<td>Pyruvate</td>
<td>$\frac{3}{10} \text{HCO}_3^- + \frac{6}{5} \text{H}^+ + \bar{\varepsilon} = \frac{1}{10} \text{CH}_3\text{COCOO}^- + \frac{3}{5} \text{H}_2\text{O}$</td>
<td>35.09</td>
</tr>
</tbody>
</table>
A fraction of the electron-flow will be utilized to produced energy, while the rest will be utilized for synthesis. Based on this, the overall biochemical reaction (R) is expressed as (Eq. 2-1, Eq. 2-2):

$$R = f_e R_a + f_s R_c - R_d$$

Eq. 2-1

$$f_e + f_s = 1.0,$$

Eq. 2-2

where $f_e$ is the ratio of electrons used for energy production, and $f_s$ is cell synthesis. In addition, energy ($R_e$) and synthesis ($R_s$) reactions can be formulated as follows (Eqs. 2-3, 2-4):

$$R_e = R_a - R_c$$

Eq. 2-3

$$R_s = R_c - R_d.$$  

Eq. 2-4

Furthermore, $f_s$ can be utilized to express the true yield in the unit of cell electron equivalent/donor electron equivalent (McCarty 2007). Also, $f_e$ describes the conversion of electrons into bio-species, which are the final products resulting from energy generation. Based on this, the $f_e$ units are the electron equivalent of bio-products/electron equivalent of an electron donor.

2.2.2 Bacterial Growth Yield

Accurate estimations of microbial yields are essential to produce experimental results, which are approached through understanding the thermodynamics of microbial metabolism and changes in Gibb’s energy due to electron flow. Figure 2-3 demonstrates the utilization of electrons for energy production and synthesis, which is based on the thermodynamic electron equivalent models (TEEMs) developed by Rittmann et al. (2001) and McCarty (2007). In order to utilize electrons for synthesis, microorganisms must convert the organic-carbon source into an
intermediate component prior to generating the cellular structure, which is represented by pyruvate in Figure 2-3. The Gibb’s free energy of the synthesis reaction ($\Delta G_s$) is calculated as follows (Eqs. 2-6, 2-7):

$$\Delta G_s = \frac{\Delta G_{ic}}{\varepsilon^n} + \frac{\Delta G_{pc}}{\varepsilon}$$

Eq. 2-6

$$\Delta G_{ic} = \Delta G_p - \Delta G_d.$$  

Eq. 2-7

The energy-transfer efficiency ($\varepsilon$) for anaerobic microorganisms ranges from 0.4 to 0.7, and is assumed to be 0.6 in most environmental applications. The exponent $n$ is used to assist the energy-transfer efficiency in the intermediate conversion ($\Delta G_{ic}$), for which it has a value of +1 if the conversion is endothermic and a value of -1 if it is exothermic. The energy associated in converting the intermediate component to a cellular structure ($\Delta G_{pc}$) is estimated at 18.8 kJ/e\' eq, at which the cell empirical formula is C$_5$H$_7$O$_2$N and ammonia is the nitrogen source.

In order to complete bacterial synthesis, (A) equivalents of the electron donor must be oxidized to produce the needed energy. This value is calculated as follows (Eq. 2-8):

$$A = -\frac{\Delta G_s}{\varepsilon \Delta G_r}$$

Eq. 2-8

The Gibb’s free energy of the energy reaction ($\Delta G_s$) is calculated from the electron-donor ($\Delta G_d$) and electron-acceptor ($\Delta G_a$) half-reactions (Eq. 2-9):

$$\Delta G_s = \Delta G_a - \Delta G_d.$$  

Eq. 2-9

Because (A) corresponds to the ratio between the electron fraction used for synthesis and that used for energy, it can be utilized to calculate $f_e$, as follows (Eq. 2-10):

$$A = \frac{f_e}{1 - f_e}$$

Eq. 2-10
Most experiments report the true yield (Y) in the units of g cells/mole substrate or g cells/mole TEA. Thus, Y can be calculated based on the molecular weight of cellular empirical formulae and stoichiometric coefficients in the overall reaction (R).

Figure 2-3: Conceptual illustration of thermodynamic electron model equivalent models (TEEMs) for bacterial yield approximations.
2.2.3 Dual-Monod Growth Model

Microbial metabolism is a process in which substrates are converted into biogenic products and biomass. The role of microorganisms can be described as that of a catalyst that accelerates reduction and oxidization reactions. This process can be defined as (Reaction 2-10):

\[ \frac{k_1}{k_{-1}} \text{ Biomass + Substrate} \Leftrightarrow \text{Bio-complex} \Rightarrow \text{Product + Biomass}, \]

Reaction 2-10

where \( k \) terms are here reversible and irreversible rate constants. A similar reaction can be constructed in terms of TEA. The process can be described mathematically using the Michaelis-Menton (1913) and Monod (1949) rate laws. The derivation of these laws commences with estimating the accumulation rates of the biogenic product (P) and intermediate bio-complex (BC) (Eqs. 2-11, 2-12):

\[ \frac{d[P]}{dt} = k_2[BC] \quad \text{Eq. 2-11} \]

\[ \frac{d[BC]}{dt} = k_1[S][B] - (k_{-1} + k_2)[BC], \quad \text{Eq. 2-12} \]

where \([B]\) and \([S]\) are the biomass and substrate concentrations, respectively. The total biomass concentration \([B_T]\) is the summation of \([B]\) and \([BC]\) (Eq. 2-13):

\[ [B_T] = [B] + [BC] \quad \text{Eq. 2-13} \]

By assuming an accumulation rate of zero for the bio-complex, its concentration can be estimated as follows (Eq. 2-14):

\[ [BC] = \frac{k_1[S][B]}{k_{-1} + k_2} \quad \text{Eq. 2-14} \]

and by substituting Equation 2-13 into 2-14 (Eq. 2-15, Eq 2-16):
\[ [BC] = \frac{[S][B_T]}{K_s + [S]} \quad \text{Eq. 2-15} \]

\[ K_s = \left(\frac{k_{-1} + k_2}{k_1}\right) \quad \text{Eq. 2-16} \]

Finally, the substitution of Equation 2-15 into Equation 2-11 yields (Eq. 2-17, Eq. 2-18):

\[ \frac{d[P]}{dt} = k_{max}[B_T]\left(\frac{[S]}{K_s + [S]}\right) \quad \text{Eq. 2-17} \]

\[ k_{max} = k_2, \quad \text{Eq. 2-18} \]

where \( k_{max} \) is the maximum growth, and has units of mole/m³-biomass/day in this investigation.

Equation 2-17 can be expanded to consider the limitations of TEA (\( K_{TEA} \) and \([TEA]\)) and the inhibiting species (\( K_I \) and \([I]\)) by including their respective hyperbolic terms (Eq. 2-19):

\[ \frac{d[P]}{dt} = k_{max}[B_T]\left(\frac{[S]}{K_s + [S]}\right)\left(\frac{[TEA]}{K_{TEA} + [TEA]}\right)\left(\frac{K_I}{K_I + [I]}\right). \quad \text{Eq. 2-19} \]

Equation 2-19 is known as the dual-Monod equation. The half-saturation coefficients (\( K_s, K_{TEA}, K_I \)) are the concentrations of species at half \( k_{max} \), and they can be estimated from experimental results, as illustrated in Figure 2-4. In the case of ferric iron, where TEA is in a mineral form instead of an aqueous form, the respective hyperbolic term can be replaced by a reactive surface area (m² solid phase/m³ porous medium). This leads to a modification of the units for the \( k_{max} \) to mole/m²-biomass/s (Druhan et al. 2013). However, many experimental publications and simulation studies have expressed growth rates using the unit of 1/time (\( \mu \)); thus, the Monod equation can be re-expressed by dividing Equation 2-17 by \([B_T]\) (Eq. 2-20):

\[ \mu = \mu_{max}\left(\frac{[S]}{K_s + [S]}\right) \quad \text{Eq. 2-20} \]
2.3 Experimental Methods Used to Characterize Microbial Properties

The utilization of microbial experimentation requires a basic understanding of the growth phases of microorganisms. Growth consists of four phases: Lag, log, stationary and decline (Figure 2-5). In the lag phase, microorganism concentrations are at minimum levels, as they are introduced to a relatively new growth environment. After the adaptation of the microorganisms to the new environment, they begin to multiply and rapidly increase their concentrations in the log phase. After depleting the available species needed for growth, the microorganisms reach their maximum concentration during the stationary phase, at which their decay and generation rates are equal. The rate of bacterial decay is impacted by the toxicity imposed by bio-products and changes in acidity.
levels. The decline phase is reached when the decay rate of microorganism exceeds that of generation.

Figure 2-4: The phases of microbial growth experienced in laboratory experiments (Cappuccino et al. 2008).

Several experimental methods can be utilized to characterize bacterial growth and yield, the uptake of species and the generation of bio-products, and the limiting factors of microbial metabolism. This section explains the most common and relevant methods to this research, and discusses the main experimental results conducted to study microbial reservoir souring. The discussion focuses on the basic aspects of microbial reservoir souring utilized throughout our research, and it highlights the limitations of the current studies.
2.3.1 Basic Reactor Experiments

In basic reactors, microorganisms are cultured in an environment in which they are well-mixed with the species they require for growth. The basic reactor can be a closed system (batch), in which microorganisms undergo the four phases of growth, or can be an open system (chemostat), in which the culturing medium is continuously circulated to sustain the log phase for longer periods. The composition of the culturing medium would include the substrate and TEA, in addition to some other species needed for bacterial growth. Common culturing recipes are those developed by Postgate (1979) for SRB growth. Table 2-3 shows an example of these culturing media. The composition of culturing media can be modified by adding and removing needed substrates and TEAs. The experiments can be conducted utilizing an isolated microbial strain for more specific analysis, or can utilize mixed cultures retrieved from an environment of interest.

Table 2-3: The chemical composition of Postgate Medium B used for SRB growth (Postgate 1979).

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration (g/L)</th>
<th>Medium description</th>
</tr>
</thead>
<tbody>
<tr>
<td>KH₂PO₄</td>
<td>0.5</td>
<td>1.00 L of tap water</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>1.0</td>
<td>Adjusted pH between 7.0 and 7.5</td>
</tr>
<tr>
<td>CaSO₄</td>
<td>1.0</td>
<td>Precipitates always present</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Sodium lactate</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>Yeast extract</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Thioglycolic acid</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>FeSO₄·7H₂O</td>
<td>0.5</td>
<td></td>
</tr>
</tbody>
</table>

Basic reactors are utilized to calculate the growth yield and approximate stoichiometric coefficients, in order to characterize bacterial thermodynamics. Experiments can be utilized to estimate the growth rate and half-saturation coefficients for the Monod equation. Basic reactors are
also useful to study the influence of abiotic factors, such as temperature, pH, salinity and the toxicity of certain products, on microbial metabolism. Due to the well-mixed culturing environment, the growth rates in basic reactors are 1-5 orders of magnitude higher than those experienced in porous media (Boa et al. 2014).

In the case of reservoir souring applications, basic reactors have been utilized to evaluate potential substrates for SRB in oil fields. Some experiments have evaluated the consumption rate of main VFAs and organic materials by SRB, and have developed empirical formulae of microbial growth based on the most consumed substrate (Sugai et al. 2014). Lillebø et al. (2010) evaluated the utilization of heavy and light crudes by SRB for different water-cuts; their results showed that SRB growth is more efficient in heavier crudes and with higher water-cuts. These results reveal that SRB metabolism is more dominant in the aqueous phase than the oil phase. Also, their analysis showed that high fractions of light compounds inhibit SRB growth, for which they suggested that more water-soluble components in light crudes could be more toxic for SRB. Tanji et al. (2014) evaluated SRB growth in different mixtures that contained alkane, VFAs and crude oil. The results showed that microbial sulfide production is higher in crude oil mixtures than alkanes, for which the oxidation of aromatic oil components would be the primary contributor of VFAs needed for SRB growth. The fractions of aromatic oil components are higher in heavy crudes than light, which is consistent with the results obtained by Lillebø et al. (2010).

In regard to microbial iron reduction, basic reactors have been utilized to evaluate microbial metabolism on various iron-bearing minerals found in natural systems. In addition to FeRB, several SRB strains have been found capable of reducing ferric iron without sulfate, and the addition of sulfate further stimulates iron reduction (Coleman et al. 1993, Lovley et al. 1993, Li et al. 2004-2006, Kwon et al. 2014). Experiments have shown that aqueous ferric iron is metabolized faster than in minerals, where iron mobilization by organic acids could have an impact on increasing the bio-availability of ferric iron. In addition, iron reduction is more efficient in minerals that are
poorly crystalized, which have large reactive surface areas. In the case of clay minerals, 20% of structural ferric iron is bio-available for microbial reduction as an FeOOH equivalent (Kostka et al. 2002), while iron reduction is more efficient at the edges of clay minerals, where ferric iron is more accessible than in the interior layers (Liu et al. 2012). These experiments highlight the potential of microbial iron reduction in oil reservoirs, where such potential sources of ferric iron can impact microbial souring and H₂S breakthrough. However, FeRB experiments associated with oil reservoirs are limited in the literature, and they do not evaluate the influence of ferric iron microbial reduction on reservoir souring.
2.3.2 Biofilm Reactor Experiments

Biofilm reactors are useful to assist microbial growth in natural systems, where microorganisms adhere and attach themselves to surfaces to form biofilms (Figure 2-5). A typical biofilm reactor consists of an upflow column packed with either sand grains or glass beads to form a porous medium; several sampling ports may be placed along the length of the column. Figure 2-6 shows a classic setup of a biofilm reactor for SRB experiments. The culturing media used in the biofilm reactors are similar to those described earlier, while microbes are usually induced at the beginning of the experiment, after they reach their log phase in a basic reactor. This approach aims to minimize the impact of the lag phase in the column. The biofilm reactors can be utilized to characterize microbial growth and thermodynamics similar to basic reactors, while some studies have utilized biofilm reactors to understand microbial transport and attachment in porous media.

After concluding the experiments, the porous media can be retrieved to estimate biomass attachment. One approach is to slice the porous column into several sections and dry them at a temperature of 75 °C for four hours, and then heat them at higher temperatures of between 400 and 500 °C for four hours in order to remove organic materials (Chen et al. 1994). The difference in weight before and after removal of organic materials is presumed to be the total weight of the biomass. However, such an approach has a high level of inaccuracy, due to the possible decomposition of biomass at high temperatures, the loss of water in the biofilms themselves, and the loss of volatile suspended solids within the biofilms (Middleton et al. 1977). In addition, some of the measured mass may be the result of abiotic precipitates, including carbonates and iron sulfides in the case of SRB and FeRB.

Biofilm reactors have been extensively utilized to evaluate microbial reservoir souring and mitigation methods. These mitigation methods include injecting biocides and stimulating bio-competitive microbes that reduce nitrate and perchlorate. Most of these experiments continuously
inject potential substrates that are affiliated with oil reservoirs into the reactors (Chen et al. 1993-1996, Reinsel et al. 1996, Greene et al. 2003, Crigoryan et al. 2008, Bernardez et al. 2012, Engelbrektson et al. 2014, Xue et al. 2015). Few experiments have utilized residual oil as a substrate source for SRB, with or without the presence of oil-degrading microbes (Myhr et al. 2002, Callbeck et al. 2013, da Silva et al. 2014). This shows that oil-degrading microbes are essential for SRB to supply the VFAs required for their growth, as SRB is unable to efficiently utilize oil components. SRB reduces the VFAs to acetate; thus, an increase in the acetate concentration would be an indication of microbial souring in oil fields. However, some SRB could start utilizing the biogenic acetate when sulfate concentrations are high. It is common for souring experiments to experience a delay in H₂S breakthrough after injecting several pore volumes, which has often been assumed to occur due to the reaction with aqueous iron in the medium and potential iron-bearing minerals. In addition, some studies have speculated regarding the possibility of trapped H₂S gas inside the reactor (Chen et al. 1993-1996). Moreover, the geochemical compositions of sand used in those experiments were not extensively characterized regarding their iron content; thus, the impact of microbial iron reduction was not considered during the analysis of the results of those experiments.

To the best of our knowledge, no experiments have yet been conducted in biofilm reactors that evaluate the potential impact of microbial iron reduction on reservoir souring in oil fields.

However, a number of experiments have been conducted to evaluate microbial iron reduction in iron-rich natural systems. These experiments have included those conducted to evaluate the microbial reduction of sulfate and iron at a uranium-contaminated site near Rifle, Colorado (Li et al. 2009), and to study the fractioning of sulfur isotopes (Druhan et al. 2013). Another example is an experiment conducted by Ko et al. (2015) to study the reduction of iron-bearing minerals in a managed aquifer recharge process. Druhan et al. (2013) experiment will be used in this research.
Table 2-4 shows the ranges of microbial growth and thermodynamic parameters relevant to this research. These parameters include the growth yield and half-saturation coefficients for microbial sulfate and iron reductions. Bacterial growth could also be impacted by pH, salinity and temperature. However, no appropriate mathematical models exist that establish the relationship between these factors and microbial metabolism; thus, different microbes have different responses and tolerance levels to stressful conditions (Barton et al. 2007). In general, bacterial growth yields may vary by an order of magnitude based on their conditions and growth phase (Rittmann et al. 2001). Furthermore, the inhibiting half-saturation coefficients of SRB have been estimated based on field-scale environmental studies.

Figure 2-6: Electron micrograph of bacterial biofilm generated inside a porous media (from Bass et al. 1997).
Figure 2-7: Classic setup of an upflow biofilm reactor, which is used to study microbial reservoir souring by Chen et al. (1994).
Table 2-4: Experimental limits of bacterial thermodynamics and growth parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Lower limit</th>
<th>Upper limit</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Y^{SRB}$</td>
<td>g biomass/mole substrate</td>
<td>1.51</td>
<td>30.60</td>
<td>Cappenberg et al., 1975 Middleton et al., 1977 Widdel et al., 1981 Traore et al., 1981-1983 Ingvorsen et al., 1984 Okabe et al., 1992</td>
</tr>
<tr>
<td></td>
<td>g biomass/mole TEA</td>
<td>8.30</td>
<td>14.30</td>
<td>Badziong et al., 1978 Ingvorsen et al., 1984 Reis et al., 1992</td>
</tr>
<tr>
<td>$Y^{FeRB}$</td>
<td>g biomass/mole substrate</td>
<td>3.80</td>
<td>18.50</td>
<td>Cord-Ruwisch et al., 1998 Esteve-Núñez et al., 2005</td>
</tr>
<tr>
<td></td>
<td>g biomass/mole TEA</td>
<td>0.073</td>
<td>0.20</td>
<td>Kostka et al., 2002 Esteve et al., 2005</td>
</tr>
<tr>
<td>$K_s^{SRB}$</td>
<td>mole/L</td>
<td>1.57E-5</td>
<td>4.23E-3</td>
<td>Cappenberg et al., 1975 Middleton et al., 1977 Ingvorsen et al., 1984 Gupta et al., 1994 Chen et al., 1994</td>
</tr>
<tr>
<td>$K_s^{FeRB}$</td>
<td>mole/L</td>
<td>5.2E-4</td>
<td>Not available</td>
<td>Liu et al., 2001</td>
</tr>
<tr>
<td>$K_{TEA}^{SRB}$</td>
<td>mole/L</td>
<td>4.8E-6</td>
<td>9.00E-4</td>
<td>Ingvorsen et al., 1984 Okabe et al., 1992 Pallud et al. 2006</td>
</tr>
<tr>
<td>$K_f^{SRB}$</td>
<td>mole/g</td>
<td>8.0E-7</td>
<td>5.0E-6</td>
<td>Wang et al., 2003 Yabusaki et al., 2007</td>
</tr>
</tbody>
</table>
2.4 Simulation Models of Reservoir Sourcing

Before the development of souring simulation models, reservoir studies had been directed towards evaluating the factors contributing to reservoir souring, and there had therefore not been any significant attempts to predict and estimate H$_2$S breakthrough (Herbert et al. 1985). The primary presumption was that H$_2$S would breakthrough in production wells with injected water, and the delay in its breakthrough would correspond to a finite scavenging capacity of iron-bearing minerals. However, the rising challenges and inconsistent field observations in the North Sea demanded an improved assessment of reservoir souring, leading to the development of the three 1D models developed to simulate microbial souring between an injector-producer pair. These three models have been utilized in several oil field studies, and have become the basis of recent reservoir simulators. This section provides a brief summary of the concept and main features of these models. In addition, this section describes recent developments in reservoir souring simulations. The main mathematical expressions of these models are included in the Appendix.

2.4.1 Mixing Zone Model (Ligthelm et al. 1991)

The mixing zone model assumes that reservoir souring occurs in the mixing zone between injected seawater and formation water, where SRB is transported along the aqueous phase. The mixing zone here contains the sulfate and VFAs needed for SRB metabolism, which results in the generation of an H$_2$S source. The thickness of the H$_2$S source depends on reservoir mixing and the rate of the souring reaction. As the H$_2$S source moves with the mixing zone, H$_2$S is adsorbed by iron-bearing minerals and partitioned between residual oil and water, which imposes a delay in H$_2$S breakthrough. The reservoir souring is presumed to occur under isothermal and isobaric conditions, and it assumes a constant injection velocity between the producer and the injector based on the
advection front. This model does not consider biomass growth during sulfate metabolism. The results in this model demonstrate that H₂S production commences with a sudden increase in concentration after seawater breakthrough, followed by a gradual decline as the production of the mixing zone continues.

### 2.4.2 Biofilm Model (Sunde et al. 1993)

Unlike the presumed results of the mixing zone model, H₂S production continues to gradually increase after its breakthrough in production wells. To take these oil field observations into consideration, the biofilm model assumes that SRB is largely attached to formations and grows to a greater extent near injection wells; thus, the H₂S source would be closer to the injectors than producers. Similarly to the previous model, the biofilm model assumes incompressible flow and isothermal conditions. This model considers a single-phase flow of water without residual oil, and accounts for H₂S adsorption by iron-bearing minerals. SRB growth is estimated using the Michaelis-Menton model (1913). This model also accounts for the limitation of nitrogen, phosphorus, sulfate and organic carbon.

### 2.4.3 Thermal Viability Shell (TVS) model (Eden et al. 1993)

Unlike the previous two models, the TVS model considers the influence of temperature gradient on microbial reservoir souring. This gradient is imposed by injecting cold seawater into hot oil reservoirs. This model utilizes experimental results and statistical techniques to constrain microbial souring to the thermal limits of mesophilic and thermophilic SRB. The model correlates H₂S production with sulfate reduction, without considering species limitations and microbial growth. Similar to the biofilm model, TVS assumes that the H₂S source would be located near to
the injection wells, where temperatures are relatively lower, and that late H₂S breakthrough would be due to a lag between the water and the cold fronts. This model neither considers H₂S partitioning between phases nor formation adsorption by iron-bearing minerals.

### 2.4.4 Algorithm for History-Matching of Reservoir Souring

This model was developed to simulate reservoir souring in the Ekofisk field in the North Sea (Burger et al. 2005). The reservoir of interest is a naturally-fractured chalk, and is discretized in the model using equal-size volume elements. These volume elements contain a fracture, chalk matrix, oil and connate water. The H₂S partition is estimated using Henry’s law and the Peng-Robinson equation of state. It is assumed that SRB would be active in fractures only at temperatures below 80ºC; thus, this model constrains SRB mobility via permeability and the growth by temperature gradient. The algorithm estimates sulfate production by using field data to arrive at coefficients of maximum sulfate reduction and effective rate of nutrient supply. In addition, a third coefficient could be estimated from experiments to describe the impact of temperature on the sulfate reduction efficiency (Burger et al. 2006). In the case of the Ekofisk field, the model assumes a limited sulfate concentration in early volumetric elements, due to the expected precipitation of CaSO₄. This algorithm has also been used to forecast microbial souring in the presence of siderite, which is assumed to react with H₂S through surface and internal core reactions (Burger et al. 2009). Furthermore, this model has been utilized to assist the bio-competition between SRB and NRB based on potential substrates and simple stoichiometric relationships (Burger et al. 2013).
2.4.5 UTCHEM

Delshad et al. (2009) incorporated reservoir souring into UTCHEM, in which microbial growth is estimated using Monod kinetics that considers both attached biomass and free-floating bacteria. The model attempts to estimate temperature gradient based on thermal properties and the partition of biogenic H₂S between oil and water. In addition, UTCHEM estimates H₂S adsorption imposed by iron-bearing minerals based on adsorption capacity, fluid and rock densities, and formation porosity. The model was initially validated by reproducing the results of the mixing zone, biofilm and TVS models (Farhadinia et al. 2010). UTCHEM also has been utilized to reproduce the experimental results in a biofilm reactor, and evaluate microbial souring and nitrate treatment in oil reservoirs (Haghshenas et al. 2012).

Although the study by Haghshenas et al. considered heterogeneous permeability distribution, they assumed a constant spatial porosity. Thus, the distribution of iron minerals was insufficiently incorporated. In a recent study, a correlation was incorporated into UTCHEM that expresses the impact of temperature, salinity and pH on \( k_{\text{max}} \) in Monod kinetics (Hosseininoosheri et al. 2017). However, the correlation was limited to the optimal growth conditions of a specific microbial strain.

2.4.6 Other Models

Other commercially available reservoir souring models include SourMax, H₂S Model, Dynamic TVS, REVEAL and SourSim®RL (Johnson et al. 2017). These simulation models utilize the concepts of the three souring models of the North Sea oil fields described earlier. The published studies of reservoir souring simulation models have a common theme of neglecting to consider the biotic impact of ferric iron on microbial souring; thus, they only consider the abiotic impact of H₂S.
precipitation. Furthermore, these studies do not consider the correlation between permeability reduction and iron-bearing minerals in heterogeneous media, which could have abiotic and biotic impacts on reservoir souring development and H₂S breakthrough.

2.5 Summary

Petroleum reservoirs accommodate diverse microbial cultures that vary in their metabolism and tolerance to stressful environments. The degree of diversity is largely dependent on the availability of TEAs. The difference in TEA concentrations could initiate bio-competition between microbes and enhanced the growth of one metabolic culture over another. Based on bacterial thermodynamics, microbial metabolism would be preferential toward TEAs that supply more energy, thus inhibiting the microbial reductions of lower energy sources. Ferric iron has the potential to supply more energy compared to other potential sources of anaerobic respiration; however, this can also be impacted by its bio-availability from iron-bearing minerals in natural systems. Microbial yield and reactions depend mainly on the efficient distribution of electron-flow between energy production and synthesis. Microbial growth can be expressed using dual-Monod kinetics that consider the limitation of substrates, TEAs and inhibiting species. Microbial metabolism can be experimentally studied using basic and biofilm reactors. These experiments are beneficial for approximating the microbial yield, stoichiometric coefficients, growth rate and half-saturation coefficients. In the case of oil field reservoirs, these experiments revealed that the primary source of substrates for SRB is the microbial degradation of oil to VFAs, and that iron-bearing minerals have the potential to be a more preferential source of energy in iron-rich systems. The majority of biofilm-reactor experiments that utilize sand for porous media have experienced a delay in H₂S breakthrough after injecting several pore volumes, which resembles oil field observations. The characterization of this delay and the reasoning behind it are limited to the
assumption of abiotic reactions with iron and trapped H₂S gas. Finally, published studies of reservoir souring simulation are limited to the microbial actives that involve aqueous TEAs of sulfate and nitrate; thus, they neither account for microbial iron reduction nor describe the correlation between permeability reduction and iron-bearing minerals in heterogeneous media.
Chapter 3
Methodology

3.1 Mass Conversation

The results of experimental and waterflooding simulations were obtained using the CrunchFlow reactive transport model (Steefel et al. 2009), in which aqueous species are partitioned into primary and secondary species. The conversation of mass for species can be expressed as (Eq. 3-1):

\[
\frac{\partial (\phi C_i)}{\partial t} = \nabla \cdot (\phi D_i \nabla C_i) - \nabla \cdot (\phi u C_i) - \sum_{r=1}^{N_r} v_{ir} R_r - \sum_{m=1}^{N_m} v_{im} R_m,
\]

Eq. 3-1

where \( \phi \) is porosity (fraction), \( C_i \) is the concentration of species \( i \) (mol/L), \( t \) is time (seconds), \( D_i \) is the diffusion/dispersion coefficient (m\(^2\)/s), \( u \) is the Darcy-flow velocity (m/s), \( N_r \) is the total number of aqueous reactions involving species \( i \), \( v_{ir} \) is the stoichiometric coefficient of the species in an aqueous reaction, \( R_r \) is the rate of an aqueous reaction, \( N_m \) is the total number of mineral reactions involving species \( i \), \( v_{im} \) is the stoichiometric coefficient of the species in a mineral reaction, and \( R_m \) is the rate of a mineral reaction. The aqueous and mineral reactions are discussed in the next section.

Microbial reactions (R) are developed using Equation 2-1 (section 2.2.1), that is throughout the process of matching the experimental results presented in the next section. The half-reaction of TEA (R\(_a\)) varies based on reduced species (ferric iron or sulfate). The half-reaction of electron-donor (R\(_d\)) varies based on oxidized VFAs (lactate or acetate), and if they are completely oxidized to CO\(_2\) or to a shorter-chain VFA. The synthesis half-reaction (R\(_s\)) is Reaction 2-10, as we are assuming that ammonium is the nitrogen source in all microbial reactions. The fraction of electron flow that is utilized for energy production (\( f_e \)) will be calibrated for using experimental results. The
microbial yields are calculated from the developed microbial reactions, and they are validated based on the experimental limits in table 2-4.

Dual-Monod model (Eq. 2-19) is utilized to describe microbial growth and the accumulation of biomass. The half-saturation coefficients ($K_s$, $K_{\text{TEA}}$ and $K_l$) are calibrated using experimental results presented in the next sections, and they will be constrained by the experimental limits established in table 2-4. Also, experimental results are utilized to calibrate the maximum growth rates ($k_{\text{max}}$) and the initial volume fractions of biomass for both SRB and FeRB. In natural systems, cell density would have a range between $10^4$ to $10^7$ cells/mL, which will occupy a volume fraction of $10^{-6}$ m$^3$/m$^3$ (Li et al. 2009). However, initial biomass concentration could vary in column experiments based on initial inoculation and growth conditions, in addition to possible bio-competitions between microbial species that lead to different cell densities. In this research, the assumptions of initial biomass volume fractions are constrained between $10^{-8}$ to $10^{-4}$ m$^3$/m$^3$, a range that is within two orders of magnitude lower or greater than the value typically found in natural systems. Based on that, calibrating the maximum growth rates depend on the assumption of initial biomass of SRB and FeRB.

Constraining microbial yield and growth parameters as described previously aimed to maintain reasonable adjustment of these parameters to match experimental data. In addition, these parameters were varied within their physical ranges to identify the most sensitive ones. Experimental data were matched using logical and physical speculations, and they might be matched with different valid assumptions.
3.2 Abiotic Chemical Reactions

Table 3-1 shows the instantaneous aqueous speciation reactions considered in this research. This study assumed that the instantaneous aqueous speciation of ferrous and ferric irons is insignificant; thus, the microbial reduction of ferric iron and the precipitation of FeS would be more dominant in natural systems. In addition, this research did not consider the impact of charged surface sites and ion exchange reactions.

Table 3-2 shows the mineral reactions considered in this research. The dissolution and precipitation rates of these minerals were assumed to follow the rate law derived from the Transition State Theory (TST; Eq. 3-2; Lasaga 1998):

\[ R_{TST} = A_m k \left(1 - \frac{IAP}{K_{eq}}\right), \quad \text{Eq. 3-2} \]

where \( k \) represents the reaction rate constant (mole/m\(^2\)/s), \( A \) is the reactive surface area (m\(^2\) solid phase/m\(^3\) porous medium), and IAP is the ion activity product. In experiment 2, magnesite (Reaction 3-18) was utilized to be consistent with the presumptions made by Druhan et al. 2013. However, this reaction was replaced with dolomite in the homogenous and heterogeneous simulations (Reaction 3-19), because dolomite is more relevant to oil reservoirs than magnesite. Several studies have suggested that SRB may be associated with the deposition of dolomite (Howe* et al. 2016). In addition, this research did not consider the precipitation of siderite (FeCO\(_3\)); thus, the experiments indicated that the precipitations of calcite (Reaction 3-17) and iron sulfide (Reaction 3-20) were more dominant (Li et al. 2004).
Table 3-1: Instantaneous aqueous speciation reactions considered in simulation cases and their equilibrium constants (Li et al. 2010, Druhan et al. 2013).

<table>
<thead>
<tr>
<th>Reaction Number</th>
<th>Aqueous Reactions</th>
<th>Log(K_{eq})</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-1</td>
<td>H$_2$O ⇌ H$^+$ + OH$^-$</td>
<td>-14.00</td>
</tr>
<tr>
<td>3-2</td>
<td>HCO$_3^-$ + H$^+$ ⇌ CO$_2$(aq) + H$_2$O</td>
<td>6.34</td>
</tr>
<tr>
<td>3-3</td>
<td>CO$_3^{2-}$ + 2H$^+$ ⇌ CO$_2$(aq) + H$_2$O</td>
<td>16.70</td>
</tr>
<tr>
<td>3-4</td>
<td>CaOH$^+$ + H$^+$ ⇌ Ca$^{2+}$ + H$_2$O</td>
<td>12.90</td>
</tr>
<tr>
<td>3-5</td>
<td>CaCO$_3$(aq) + 2H$^+$ ⇌ Ca$^{2+}$ + H$_2$O + CO$_2$(aq)</td>
<td>13.40</td>
</tr>
<tr>
<td>3-6</td>
<td>CaSO$_4$(aq) ⇌ Ca$^{2+}$ + SO$_4^{2-}$</td>
<td>-2.10</td>
</tr>
<tr>
<td>3-7</td>
<td>CaCl$^+$ ⇌ Ca$^{2+}$ + Cl$^-$</td>
<td>0.70</td>
</tr>
<tr>
<td>3-8</td>
<td>CaHCO$_3^+$ + H$^+$ ⇌ Ca$^{2+}$ + H$_2$O + CO$_2$(aq)</td>
<td>5.30</td>
</tr>
<tr>
<td>3-9</td>
<td>MgCO$_3$(aq) + 2H$^+$ ⇌ Mg$^{2+}$ + H$_2$O + CO$_2$(aq)</td>
<td>13.70</td>
</tr>
<tr>
<td>3-10</td>
<td>MgCl$^+$ ⇌ Mg$^{2+}$ + Cl$^-$</td>
<td>0.14</td>
</tr>
<tr>
<td>3-11</td>
<td>MgSO$_4$(aq) ⇌ Mg$^{2+}$ + SO$_4^{2-}$</td>
<td>-2.41</td>
</tr>
<tr>
<td>3-12</td>
<td>S$^{2-}$ + 2H$^+$ ⇌ H$_2$S(aq)</td>
<td>19.90</td>
</tr>
<tr>
<td>3-13</td>
<td>HS$^-$ + H$^+$ ⇌ H$_2$S(aq)</td>
<td>6.98</td>
</tr>
<tr>
<td>3-14</td>
<td>NH$_3$(aq) + H$^+$ ⇌ NH$_4^+$</td>
<td>9.24</td>
</tr>
<tr>
<td>3-15</td>
<td>CH$_3$COOH(aq) ⇌ H$^+$ + CH$_3$COO$^-$</td>
<td>-4.75</td>
</tr>
<tr>
<td>3-16</td>
<td>Lactic acid(aq) ⇌ H$^+$ + Lactate$^-$</td>
<td>-3.86</td>
</tr>
</tbody>
</table>
As discussed in the literature review, the oxidation of residual oil components to organic acids by fermentative bacteria is the primary source of substrates for SRB. The concentration of VFAs in formation water can range from 50-3300 mg/L (Collins 1975, Khatib et al. 1997, Kuijvenhoven et al. 2006). To simplify this process, this study utilized an artificial mineral reaction that produced VFA concentrations within those observed in oil fields (Reaction 3-23):

\[
\text{CH}_3\text{COONa}_\text{(s)} \rightleftharpoons \text{CH}_3\text{COO}^- + \text{Na}^+. \quad \text{Reaction 3-23}
\]

Acetate in the reaction represented the total concentration of VFAs, while sodium was chosen due to its nonreactive nature in an aqueous environment. The saturation index (SI) of this reaction can be expressed as (Eq. 3-3):

Table 3-2: Mineral precipitation and dissolution reactions considered in simulation cases and their kinetic properties.

<table>
<thead>
<tr>
<th>Reaction number</th>
<th>Mineral reactions</th>
<th>Log((k)) (mol/m²/s)</th>
<th>Log((K_{\text{eq}}))</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-17</td>
<td>(\text{CaCO}_3\text{(s)} + H^+ \rightleftharpoons \text{Ca}^{2+} + \text{HCO}_3^-)</td>
<td>-5.2</td>
<td>1.85</td>
<td>Li et al., 2009</td>
</tr>
<tr>
<td>3-18</td>
<td>(\text{MgCO}_3\text{(s)} + H^+ \rightleftharpoons \text{Mg}^{2+} + \text{HCO}_3^-)</td>
<td>-6.3 (column)</td>
<td>2.29</td>
<td>Li et al., 2009</td>
</tr>
<tr>
<td>3-19</td>
<td>(\text{CaMg(CO}_3\text{)}_2\text{(s)} + 2\text{H}^+ \rightleftharpoons \text{Ca}^{2+} + \text{Mg}^{2+} + 2\text{HCO}_3^-)</td>
<td>-7.53</td>
<td>2.51</td>
<td>Palandri et al., 2004</td>
</tr>
<tr>
<td>3-20</td>
<td>(\text{Fe}^{2+} + \text{H}<em>2\text{S}</em>\text{(aq)} \rightleftharpoons \text{FeS}_\text{(am)} + 2\text{H}^+)</td>
<td>-9.0</td>
<td>3.50</td>
<td>Li et al., 2009</td>
</tr>
<tr>
<td>3-21</td>
<td>(\text{Fe(OH)}<em>3\text{(s)} + \frac{1}{2} \text{H}<em>2\text{S}</em>\text{(aq)} + 2\text{H}^+ \rightleftharpoons \text{Fe}^{2+} + \frac{1}{2} \text{S}</em>\text{(s)} + 3\text{H}_2\text{O})</td>
<td>-5.0 (column)</td>
<td>-19.60</td>
<td>Poulton et al., 2004; Li et al., 2009</td>
</tr>
<tr>
<td>3-22</td>
<td>(\text{C}_5\text{H}_7\text{O}<em>2\text{N}</em>\text{(s)} \rightleftharpoons \text{C}_5\text{H}_7\text{O}<em>2\text{N}</em>\text{(aq)})</td>
<td>-2.0</td>
<td>-15.00</td>
<td>Druhan et al., 2013</td>
</tr>
</tbody>
</table>
where $a$ is the ionic activity of species in the reaction. When SI = 0, the reaction reaches equilibrium; when SI < 0, the reaction proceeds to dissolution; when SI > 0, the reaction proceeds to precipitation. By applying the division-subtraction log rule, Equation 3-3 can be expressed as (Eq. 3-4):

$$SI = \log_{10} \left( \frac{1AP}{K_{eq}} \right) = \log_{10} \left( \frac{a_{Na^+} a_{CH_3COO^-}}{a_{Na^+} a_{CH_3COO^-}} \right)_{\text{actual}},$$

$$\text{Eq. 3-3}$$

Because the log value of ionic activity products is negative, and to avoid the interference of Na$^+$ in formation and injected water with Reaction 3-23, this research assumed a value of $\log_{10}(K_{eq})$ that approached zero. This approach ensured that SI is always negative and that Reaction 3-23 always proceeds toward dissolution. In this investigation, the log($k$) of Reaction 3-23 was assumed at -6.0 mole/m$^2$/s.

### 3.4 Microbial Experiments Described in the Literature

Two microbial experiments are considered in this research; both were conducted in biofilm reactors. The first experiment utilized an isolated SRB strain common to environmental studies. The results of this experiment showed a deviation from the expected stoichiometry observed in a basic reactor, while there was a significant delay in H$_2$S breakthrough. This research analyzed the causes behind these results, and investigated the possibility of microbial iron reduction in this experiment. The second experiment was based on multiple publications that studied microbial activities in an environmental project, in which there was abundant bio-available ferric iron to
stimulate FeRB in addition to SRB. This section provides a brief description of these experiments and their key results.

3.4.1 Experiment 1: Microbial Sourcing by Isolated SRB Strain

This experiment was conducted by Chen et al. (1994); the objective was to study microbial reservoir souring in an upflow porous column with a length of 50 cm and a diameter of 5.5 cm (Figure 2-7). The column accommodated six sampling ports along its length. The reactor was filled with washed sea sand, and the average porosity was estimated at 37%. An isolated strain of SRB (*Desulfovibrio desulfuricans*) was cultured in a batch reactor using Postgate Medium B (Table 2-3), and microbes were inoculated into the reactor when they reached their log phase. The experiment consisted of three runs at different rates and concentrations. Lactate and sulfate were injected at concentrations of 130 and 900 mg/L, respectively, and the pore velocity was maintained at 2.74 cm/h. Based on chemostat experiments, microbes were expected to oxidize lactate to acetate.

The breakthrough results of lactate, H$_2$S and sulfate are shown in Figure 3-1. Details regarding acetate breakthrough throughout the experiment were not reported. The results showed a significant delay in H$_2$S breakthrough, while the stoichiometric results were inconsistent with those observed in the chemostat. The stoichiometric ratios of oxidized lactate to biogenic H$_2$S were generally higher in the column, while the ratios of lactate to acetate were generally lower. The formation of black precipitates of FeS were observed early in the experiment, which confirmed the presence of iron in the medium. However, the concentrations of iron species were not quantified, and the geochemical composition of the sand was not characterized in the experiment. At the conclusion of the experiment, the column was sliced into 14 sections to estimate biomass attachment; most of the biomass was found attached near the inlet.
This experiment is used in this research to evaluate the deviation in results between the packed-sand column and the chemostat experiments. Furthermore, this research evaluated the potential of ferric iron reduction to impose a delay in H₂S breakthrough.

Figure 3-1: Breakthrough results of lactate, acetate, sulfate and H₂S in a microbial column experiment by Chen et al. (1994).
3.4.2 Experiment 2: Dissimilatory Iron and Sulfate Reduction

This experiment was conducted by Druhan et al. (2013) to study the fractioning of sulfur isotopes imposed by microbial and abiotic reductions. The porous column used in this experiment had a diameter of 10 cm and a length of 100 cm, and it accommodated five sampling ports along its length. The experiment was conducted using sediments from the Old Rifle aquifer in Colorado. The sediments were retrieved from a uranium-contaminated site, and several studies have indicated that acetate injection stimulates microbial iron reduction followed by sulfate reduction (Li et al. 2009-2010, Bao et al. 2014). The column has an average porosity of 30%, and the injection rate was kept constant to maintain a pore velocity of 15 cm/day. Table 3-3 shows the initial and injected concentrations of the main species in this experiment.

The results of this experiment were simulated using CrunchFlow (Steefel et al. 2009), in which the chemical reactions and the bio-availability of ferric iron were based on previous studies (Li et al. 2009). The main reported results included the concentrations of ferrous iron, acetate, sulfate and H$_2$S (Figure 3-2).

This experiment is used in this research to compare its results to those obtained from the first experiment (Chen et al. 1994). As the results of this experiment would be associated with fewer speculations compared to that described previously, the microbial growth and thermodynamic parameters obtained from this experiment were utilized later in waterflooding simulations.
Table 3-3: Initial and injected concentrations of main species in a column experiment by Druhan et al. (2013).

<table>
<thead>
<tr>
<th>Species</th>
<th>Initial concentration (mg/L)</th>
<th>Injected concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.2</td>
<td>8.2</td>
</tr>
<tr>
<td>SiO$_2$(aq)</td>
<td>21.03</td>
<td>21.03</td>
</tr>
<tr>
<td>Na$^+$</td>
<td>243.69</td>
<td>505.78</td>
</tr>
<tr>
<td>K$^+$</td>
<td>17.48</td>
<td>61.78</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>212.42</td>
<td>212.42</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>131.46</td>
<td>131.46</td>
</tr>
<tr>
<td>SO$_4^{2-}$</td>
<td>845.3</td>
<td>845.33</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>106.35</td>
<td>106.35</td>
</tr>
<tr>
<td>CO$_2$(aq)</td>
<td>396.09</td>
<td>396.09</td>
</tr>
<tr>
<td>Acetate</td>
<td>0.00</td>
<td>575.64</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>18.04</td>
<td>18.04</td>
</tr>
<tr>
<td>Br$^-$</td>
<td>0.00</td>
<td>105.47</td>
</tr>
</tbody>
</table>

Figure 3-2: Breakthrough results of sulfate, H$_2$S, acetate and ferrous iron in Druhan et al. (1994).
3.5 Waterflooding Simulations

3.3.1 Homogenous Simulations (1D)

The objective of the 1D homogenous simulations was to understand the impact of high bio-available ferric iron concentrations on microbial reservoir souring. The results were analyzed based on H\textsubscript{2}S breakthrough, biomass growth and the precipitation of the main minerals. The simulations were constructed as an injector-producer pair, with a constant differential pressure between the two wells to maintain a constant pore velocity of 1 m/day. The wells were 100 m apart with a grid size of 1.0 m; thus, the dispersivity was assumed to be 0.5 m. Furthermore, the permeability ($k$) was assumed to be $7.00\times 10^{-13}$ m$^2$ ($\approx 709$ mD), and the porosity was estimated based on the Carmen-Kozeny correlation (Eq. 3-5; Fitts 2002):

$$k = \frac{\theta^3}{(1 - \theta)^2} \left( \frac{d_g^2}{180} \right), \quad \text{Eq. 3-5}$$

where the grain diameter ($d_g$) was assumed to be constant at 500 $\mu$m.

Table 3-4 shows the compositions of injected seawater and formation water used in the simulations. Although the average sulfate concentrations in formation water ranges from 210-1170 mg/L (Collins 1975), the primary sulfate source that stimulates SRB in oil reservoirs is the injected seawater. Furthermore, several studies have speculated that the source of sulfate in formation water could be from minerals, such as the abiotic oxidation of sulfide (Hutcheon 1993). Based on this hypothesis, this research did not consider the impact of sulfate concentration in formation water. In addition, the concentrations of aqueous iron species in injected and produced waters were presumed to be insignificant. Because no studies were found that evaluated the nitrogen source for SRB in oil reservoirs, we assumed a relatively high concentration of ammonium in the injected water. In this way, we attempted to avoid ammonium limitation in microbial reactions while
making the simulations. Ammonium can reach a maximum concentration of 3300 mg/L in produced water (Collins 1975).

Table 3-5: The compositions of formation water (Collins 1975) and injected seawater (Haynes 2017) used in waterflooding simulations.

<table>
<thead>
<tr>
<th>Species</th>
<th>Formation water concentration (mg/L)</th>
<th>Seawater concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.0</td>
<td>8.2</td>
</tr>
<tr>
<td>Na⁺</td>
<td>4,700.0</td>
<td>12,146.0</td>
</tr>
<tr>
<td>K⁺</td>
<td>170.0</td>
<td>410.0</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>8,600.0</td>
<td>208.0</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>2,000.0</td>
<td>2,094.0</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>0.0</td>
<td>3643.0</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>92,700.0</td>
<td>22,360.0</td>
</tr>
<tr>
<td>CO₂(aq)</td>
<td>112.0</td>
<td>116.0</td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>24.0</td>
<td>1,000.0</td>
</tr>
<tr>
<td>Br⁻ (tracer)</td>
<td>0.0</td>
<td>78.0</td>
</tr>
<tr>
<td>Fe³⁺</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Fe²⁺</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>SiO₂(aq)</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

3.5.2 Heterogeneous Simulations (2D)

The primary objective of the heterogeneous simulations was to evaluate the potential role of the correlation between permeability reduction and iron-bearing minerals, and characterize the impact on reservoir souring development by stimulating microbial iron reduction. The simulation model is 90 x 90 m with a grid block size of 1.5 x 1.5 m, resulting in a total of 3600 blocks. The longitudinal dispersivity was assumed to be 0.75 m while the transverse dispersivity was assumed
to be 0.075 m. The simulations were constructed with an injector-producer pair that resembled a quarter of a five-spot pattern as is commonly used in oil fields (Craig 1971). The wells operated at a constant rate of 0.5 L/s (≈ 272 bbl/day); thus, the differential pressure between the two wells was constant. The compositions of formation water and injected seawater were the same as those used in the homogenous simulations (Table 3-5).

A Fast Fourier Transform (FFT) simulator was utilized to generate random permeability fields based on random seeds (Jennings 2000). The 2D permeability fields were generated at different correlation lengths (λ) that were on the order of the well-spacing (WS), and at different Dykstra-Parson coefficients (V_{DP}). The correlation lengths in the x and y directions were assumed to be equal. In order to determine a correlation between the permeability reduction and ferric iron concentration, it was assumed that iron distribution would follow a Gaussian distribution truncated at zero. A probability model was used to calculate the local mean (μ_{Fe,i}) and variance (σ_{Fe,i}^2) of the ferric iron content (Eq. 3-6, Eq. 3-7; Li et al. 2010):

\[μ_{Fe,i} = μ_{Fe} + ρ \frac{σ_{Fe}}{σ_{logK}} (logK_i - μ_{logK})\]  
\[σ_{Fe,i}^2 = σ_{Fe}^2(1.0 - ρ^2).\]  

The global mean of hydraulic conductivity (μ_{logK}, m/day) was calculated from the permeability value used in the homogenous simulation, the local mean (logK_i) corresponded to the permeability of grid block, and the standard deviation (σ_{logK}) varies based on V_{DP}. Furthermore, the global mean (μ_{Fe}) and standard deviation (σ_{Fe}) are assumed at 0.13 µmole/g, which represents 20% of ferric iron in Berea sandstone (Fleischer 1962). The reason of considering the mineralogy of Berea sandstone is due to its wide utilization in petroleum studies, and to avoid exaggerated results imposed by those concentrations of iron-rich formations (2.0–2.5 µmole/g), which may not be representative of oil reservoirs. The correlation coefficient (ρ) between hydraulic conductivity and
ferric iron is set arbitrarily at −0.8. Furthermore, the porosity values are calculated using equation 3-5. The spatial distribution of permeability, porosity and ferric iron for the heterogeneous fields are shown in Figures 3-3, 3-4 and 3-5, respectively. The results of each simulation case were obtained by averaging the outcomes of 25 random realizations, which were generated using a FTT simulator at the same $\lambda$ and $V_{DP}$.

Figure 3-3: Permeability distributions at different correlation lengths ($\lambda$) on the order well-spacing (WS), and at different Dykstra-Parson coefficients ($V_{DP}$).
Figure 3-4: Porosity distributions at different correlation lengths ($\lambda$) on the order well-spacing (WS), and at different Dykstra-Parson coefficients ($V_{DP}$).
Figure 3-5: Distributions of bio-available ferric iron (m³/m³) at different correlation lengths (λ) on the order well-spacing (WS), and at different Dykstra-Parson coefficients (V_{DP}).
Chapter 4

History Matching and Sensitivity Analysis of Experiments

4.1 Experiment 1: The Influence of Substrates and TEAs on Microbial Sourcing

This section analyzes and simulates the results of the experiment performed by Chen et al. (1994). The first three sub-sections focus on the impact of microbial yields and the microbial utilization of potential substrates and TEA in the column. The fourth sub-section addresses the impact of the parameters in the dual-Monod model on microbial processes in the experiment.

4.1.1 First Hypothesis: SRB Oxidizes Lactate Only

Following the initial presumption of the Chen et al. (1994), we first assumed that SRB did not reduce the biogenic acetate in the column, and that lactate is the only substrate that the microbe oxidizes for electron flow. Based on that, $R_4$ in Equation 2-1 can be expressed as (Reaction 4-1):

$$\frac{1}{4}CH_3COO^- + \frac{1}{4}HCO_3^- + \frac{5}{4}H^+ + \bar{e} = \frac{1}{4}CH_3CHOHCOO^- + \frac{1}{2}H_2O.$$  \hspace{1cm} \text{Reaction 4-1}

In the case of SRB, sulfate was the TEA ($R_s = \text{Reaction 2-5}$) and ammonia was presumed to be the nitrogen source ($R_c = \text{Reaction 2-10}$). Table 4-1 shows the overall microbial reactions for different $f_c$ fractions, which reflect different microbial yields, as discussed previously. Large $f_c$ fractions result in the allocation of electron flow for energy production more than for synthesis, thus decreasing the stoichiometric coefficients of biomass. In contrast, the stoichiometric coefficients of sulfate and H$_2$S increase with the $f_c$ fraction. The pairs of lactate-acetate, sulfate-H$_2$S, ammonia-
biomass all had molar ratios of one in all reactions, because these species are dependent on one another in their respective half-reactions.

Figure 4-1 shows the simulation results of the experiment by Chen et al. (1994) using the microbial reactions in Table 4-1. The increase in $f_e$ fractions restricts biomass generation, resulting in longer transient periods prior to reaching the limiting conditions of SRB growth. That is, the species involved in microbial reactions require longer periods before reaching steady-state conditions. These initial results indicated that lactate was limited in this experiment, while sulfate concentration was unlimited and could provide the potential for further reduction. The results were consistent with stoichiometric coefficients of the reactions in Table 4-1, where the molar ratios of the consumed lactate and sulfate concentrations to the generated acetate and $\text{H}_2\text{S}$ concentrations were one. In addition, the maximum $\text{H}_2\text{S}$ concentration increased with $f_e$. Thus, the sulfate concentration decreased as it was further reduced.

However, the limitation of lactate did not fulfil the potential of sulfate reduction imposed by the experimental results, leading to the generation of significantly less $\text{H}_2\text{S}$ concentrations in the simulation results. The incremental increase in $f_e$ fractions illustrates that allocating more of the electron flow towards energy production is in itself insufficient to produce more $\text{H}_2\text{S}$. These results raise the possibility of an additional substrate compensating for the lactate limitation, allowing microbes to reduce more sulfate and increase the concentration of biogenic $\text{H}_2\text{S}$ at breakthrough.
Table 4-1: Microbial SRB reactions that oxidize lactate to acetate at different $f_e$.

<table>
<thead>
<tr>
<th>Reaction Number</th>
<th>Microbial Reactions</th>
<th>$f_e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-2</td>
<td>CH$_3$CHOHCOO$^-$ + 0.4 SO$_4^{2-}$ + 0.04 NH$_4^+$ $\Rightarrow$ 0.04 C$_5$H$_2$O$<em>2$N$</em>{(aq)}^{SRB}$ + 0.4 H$_2$S + CH$_3$COO$^-$ + 0.8 HCO$_3^-$ + 0.12 OH$^- + 0.08$ H$^+$</td>
<td>0.80</td>
</tr>
<tr>
<td>4-3</td>
<td>CH$_3$CHOHCOO$^-$ + 0.45 SO$_4^{2-}$ + 0.02 NH$_4^+$ $\Rightarrow$ 0.02 C$_5$H$_7$O$<em>2$N$</em>{(aq)}^{SRB}$ + 0.45 H$_2$S + CH$_3$COO$^-$ + 0.9 HCO$_3^-$ + 0.06 OH$^- + 0.04$ H$^+$</td>
<td>0.90</td>
</tr>
<tr>
<td>4-4</td>
<td>CH$_3$CHOHCOO$^-$ + 0.475 SO$_4^{2-}$ + 0.01 NH$_4^+$ $\Rightarrow$ 0.01 C$_5$H$_7$O$<em>2$N$</em>{(aq)}^{SRB}$ + 0.475 H$_2$S + CH$_3$COO$^-$ + 0.95 HCO$_3^-$ + 0.03 OH$^- + 0.02$ H$^+$</td>
<td>0.95</td>
</tr>
</tbody>
</table>

Figure 4-1: Simulation results of breakthrough of lactate, acetate, sulfate and H$_2$S in Chen et al. (1994) experiment, assuming that SRB oxidizes lactate only. Simulations are at different SRB-lactate yield (reactions in table 4-1).
4.1.2 Second Hypothesis: SRB Oxidizes Lactate and Biogenic Acetate

One potential substrate that microbes may utilize in the column would be the biogenic acetate. Although the consideration of acetate was outside the scope of this study, its oxidation by SRB is often reported in experimental studies and environmental applications (Middleton et al. 1977, Widdel et al. 1977-1981, Ingvorsen et al. 1993, Li et al. 2010). Some studies highlight that reservoir souring is associated with high acetate concentrations, because SRBs are recognized to oxidize long-chain VFA and generate more acetate as a result (Chen et al. 1996, Kilian et al. 2015). In addition, some field observations have indicated that acetate oxidation is unfavorable for SRB but is favorable for NRB, which raises some challenges in planning bio-competition treatments (Grigoryan et al. 2008). Such presumptions of the SRB oxidation of acetate may result in neglecting to consider its oxidation by SRB to enhance sulfate reduction. However, Callbeck et al. (2013) observed in their column experiments that some SRB would adapt to oxidize the biogenic acetate at unlimited sulfate concentrations. Furthermore, there are several reasons why SRB would be restricted from utilizing acetate in a basic reactor, resulting in the discrepancy that the Chen et al. (1994) highlighted between the results obtained and those obtained from the packed-sand column. One of these reasons could be a higher level of toxicity in the basic reactor due to high concentrations of biogenic species. For example, experiments conducted in a basic reactor show that SRB growth can be completely inhibited when H₂S reaches high concentrations of between 230 and 547 mg/L, while the increase in pH can lead to an unfavorable environment for SRB growth (Badzio et al. 1978, Reis et al. 1992). The increase in pH is caused by the generation of hydroxide by SRB, as indicated in the microbial reactions. However, the pH level at equilibrium conditions
would depend mainly on the aqueous speciation of H$_2$S into HS$^-$/S$_2^-$ and H$^+$ (Millero 1986), as well as its precipitation as metal sulfides in natural systems.

In order to evaluate the impact of potential acetate oxidation, the reactions presented in Table 4-2 were constructed using Reaction 2-7 as $R_d$. Figure 4-2 shows the simulation results at different SRB-acetate yields and at a constant SRB-lactate yield. These results reveal that acetate oxidation has the significant potential to increase the electron-flow and reduce larger amounts of sulfate to H$_2$S. The impact of $f_e$ fractions is consistent with what has previously been described, and it is consistent with the results presented in Figure 4-3 for SRB-lactate reactions. Furthermore, these results show that a change in $f_e$ for SRB-lactate has less impact on H$_2$S generation than a change in SRB-acetate. The $f_e$ fractions were higher in SRB-lactate reactions than those in SRB-acetate, meaning that SRB-lactate here produced less biomass than SRB-acetate.

Based on the simulation results, experimental data can be matched with SRB-lactate and SRB-acetate reactions with $f_e$ fractions of 0.90 and 0.65, respectively. Their microbial yields can be calculated from the overall reactions as 2.26 g biomass/mole-substrate (5.02 g-biomass/mole-TEA) and 15.82 g-biomass/mole-substrate (24.34 g-biomass/mole-TEA) for SRB-lactate and SRB-acetate, respectively. These values are consistent with the experimental measurements presented in Table 2-4.
Table 4-2: Microbial SRB reactions that oxidize acetate at different $f_e$.

<table>
<thead>
<tr>
<th>Reaction Number</th>
<th>Microbial Reactions</th>
<th>$f_e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-5</td>
<td>$\text{CH}_3\text{COO}^- + 0.28 \text{H}^+ + 0.55 \text{SO}_4^{2-} + 0.18 \text{NH}_4^+ \Rightarrow 0.18 \text{C}_5\text{H}_7\text{O}<em>2\text{N}</em>{(aq)}^\text{SRB} + 0.55 \text{H}_2\text{S} + 1.1 \text{HCO}_3^- + 0.54 \text{OH}^-$</td>
<td>0.55</td>
</tr>
<tr>
<td>4-6</td>
<td>$\text{CH}_3\text{COO}^- + 0.44 \text{H}^+ + 0.65 \text{SO}_4^{2-} + 0.14 \text{NH}_4^+ \Rightarrow 0.14 \text{C}_5\text{H}_7\text{O}<em>2\text{N}</em>{(aq)}^\text{SRB} + 0.65 \text{H}_2\text{S} + 1.3 \text{HCO}_3^- + 0.42 \text{OH}^-$</td>
<td>0.65</td>
</tr>
<tr>
<td>4-7</td>
<td>$\text{CH}_3\text{COO}^- + 0.60 \text{H}^+ + 0.75 \text{SO}_4^{2-} + 0.10 \text{NH}_4^+ \Rightarrow 0.10 \text{C}_5\text{H}_7\text{O}<em>2\text{N}</em>{(aq)}^\text{SRB} + 0.75 \text{H}_2\text{S} + 1.5 \text{HCO}_3^- + 0.30 \text{OH}^-$</td>
<td>0.75</td>
</tr>
</tbody>
</table>

Figure 4-2: Simulation results of breakthrough of lactate, acetate, sulfate and $\text{H}_2\text{S}$ in Chen et al. (1994) experiment, assuming that SRB oxidizes lactate and acetate. Simulations are at different SRB-acetate yield (reactions in table 4-2), where $f_e$ of SRB-lactate is 0.90 (reaction 4-3).
4.1.3 Third Hypothesis: Dissimilatory Iron Reduction

In contrast to the experimental data, the simulation results showed the earlier breakthrough of H$_2$S. This required an assessment to evaluate the potential causes of late H$_2$S breakthrough. It is unlikely that SRB growth experienced a lag phase in the column, because microbes reached their log phase prior to their inoculation. Moreover, identical species concentrations were utilized in the column to those used in the basic reactor. One explanation for these findings concerns the reaction between H$_2$S and potential iron in the system. Chen et al. (1994) acknowledge the presence of iron in the column, where black precipitations were presumed to be iron sulfide. However, the potential...
sources of iron and the mechanism of its reaction with \( \text{H}_2\text{S} \) were outside the scope of this study. One potential source was the injection of ferrous iron, indicated by the composition of the utilized Postgate medium in Table 2-3. To evaluate such a possibility, Figure 4-4 shows the impact of injected ferrous iron on \( \text{H}_2\text{S} \) breakthrough, where the precipitation mechanism was governed by Reaction 3-20. The results show that concentrations between 10.43 and 15.43 mg/L of ferrous iron were sufficient to describe the delay in \( \text{H}_2\text{S} \) breakthrough. However, such conditions would decrease the concentration of \( \text{H}_2\text{S} \) at steady-state conditions, which suggests the potential for further sulfate reduction to increase \( \text{H}_2\text{S} \) concentration. This would in turn result in further deviation in simulation results from experimental findings as the sulfate concentration further decreases at breakthrough. In later publications, Chen et al. (1994, 1996) acknowledged that their experiments would have a typical feed concentration of 7.5 \( \mu \text{mol/L} \) of ferrous iron (0.42 mg/L), which is significantly lower than those considered in Figure 4-4.

![Figure 4-4](image)

Figure 4-4: The effect of potential ferrous iron concentrations on the breakthrough of biogenic \( \text{H}_2\text{S} \) in experiments by Chen et al. (1994).
Another potential source of iron would be the mineralogy of the porous media. Although the experiment utilized washed sea sand, the geochemical composition may not have been completely purified to silica, and thus the sand may still have been coated with some iron oxide (Clarke 1924). The amount of iron oxide would have varied depending on the sand source and the geological environment. As discussed in previous chapters, the presence of iron oxides can have abiotic and biotic impacts on microbial souring, resulting in a late H$_2$S breakthrough.

To examine such potential in the column, sensitivity analyses were conducted to evaluate the concentrations of iron oxides that could cause the observed delay in H$_2$S breakthrough. The abiotic reaction between iron oxides and H$_2$S is governed by Reaction 3-21. To simplify the complexity of substrate utilization, we assumed that microbes would completely oxidize lactate while reducing ferric iron for energy (R$_d$ = Reaction 2-8). In addition, we assumed that microbes would utilize iron (III) oxide-hydroxide (FeOOH) for energy. Thus, R$_a$ is described as (Reaction 4-8):

$$\text{FeOOH} + 3 \text{H}^+ + \bar{e} = \text{Fe}^{2+} + 2 \text{H}_2\text{O}.$$  

Reaction 4-8

Table 4-3 shows the overall microbial reactions of dissimilatory iron reduction at different $f_c$ fractions. Similar to SRB reactions, higher $f_c$ fractions would allocate more of the electron flow towards FeOOH reduction and energy production; thus, less biomass would be generated. Sensitivity analyses were initiated by assuming a $K_t$ of 1.8 $\mu$mol/g (Li et al. 2010), and volume fractions of FeOOH and Fe(OH)$_3$ at 5.50E-5 m$^3$/m$^3$ each.

Figure 4-5 shows the simulation results considering the microbial reactions in Table 4-3. Because high $f_c$ fractions restrict biomass generation, the microbial iron reduction would be slow and was thus extended for longer periods prior to reaching the limiting growth conditions of FeRB. This led to an extended inhibitory impact on SRB, imposing a further delay in H$_2$S breakthrough.
In addition, delaying the microbial reduction of sulfate slowed the oxidation of lactate; thus, their breakthrough curves shifted to the left as $f_e$ increased in the FeRB reactions. These results indicate that FeRB must oxidize a lower amount of substrate compared to SRB. The growth yield of FeRB reactions in Table 4-3 ranges from 19.94 g-biomass/mole-substrate (2.37 g-biomass/mole-TEA) to 5.65 g-biomass/mole-substrate (2.37 g-biomass/mole-TEA), which are within the acceptable range of the experimental limits (Table 2-4). The minor discrepancy observed between our yield estimates and those in Table 2-4 was largely due to our assumption of complete lactate oxidation, which would alter the amount of electron flow and organic carbon. However, because FeRB demands low lactate concentrations and because the lactate reduction to acetate has a molar ratio of one, the biogenic acetate associated with FeRB would be found at negligible concentrations. The breakthrough curves of acetate also shifted to the left as $f_e$ of dissimilatory iron reduction increased. This was due to the inhibition of SRB that is responsible for both the generation and consumption of acetate. The simulation results show that the experimental data could be better matched with FeRB Reaction 4-10.
Table 4-3: Microbial FeRB reactions assuming complete oxidation of lactate at different $f_e$

<table>
<thead>
<tr>
<th>Reaction Number</th>
<th>Microbial Reactions</th>
<th>$f_e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-9</td>
<td>FeOOH$_{(s)}$ + 1.866 H$^+$ + 0.0500 NH$_4^+$ + 0.166 CH$_3$CHOHCOO$^-$ $\Rightarrow$ Fe$^{2+}$ + 0.0500 C$_5$H$_7$O$<em>2$N$</em>{(aq)}^{FeRB}$ + 0.250 HCO$_3^-$ + 1.650 H$_2$O</td>
<td>0.50</td>
</tr>
<tr>
<td>4-10</td>
<td>FeOOH$_{(s)}$ + 1.855 H$^+$ + 0.0330 NH$_4^+$ + 0.138 CH$_3$CHOHCOO$^-$ $\Rightarrow$ Fe$^{2+}$ + 0.0330 C$_5$H$_7$O$<em>2$N$</em>{(aq)}^{FeRB}$ + 0.250 HCO$_3^-$ + 1.600 H$_2$O</td>
<td>0.60</td>
</tr>
<tr>
<td>4-11</td>
<td>FeOOH$_{(s)}$ + 1.848 H$^+$ + 0.0210 NH$_4^+$ + 0.119 CH$_3$CHOHCOO$^-$ $\Rightarrow$ Fe$^{2+}$ + 0.0210 C$_5$H$_7$O$<em>2$N$</em>{(aq)}^{FeRB}$ + 0.250 HCO$_3^-$ + 1.564 H$_2$O</td>
<td>0.70</td>
</tr>
</tbody>
</table>

Figure 4-5: Simulation results of breakthrough of lactate, acetate, sulfate and H$_2$S in Chen et al. (1994) experiment, assuming dissimilatory iron reduction in the column. Simulations are at different FeRB yield (reactions in table 4-3).
4.1.4 Impact of Dual-Monod Parameters

The previous section illustrated how the thermodynamics of microbial reactions define the concentrations of species involved in microbial reactions at steady-state conditions. Prior to steady-state conditions, the accumulation rates of biogenic products are defined by parameters in the dual-Monod model (Eq. 2-19). The accumulation rate is proportional to the total concentration of biomass and $k_{\text{max}}$. However, this accumulation rate is inversely proportional to $K_s$, $K_{\text{TEA}}$ and the concentration of inhibiting species. This section illustrates these relationships through the calibration of the respective parameters for the experiments conducted by Chen et al. (1994), addressing both sulfate and ferric iron reductions. The calibration of the parameters in the dual-Monod equation is a dynamic more than sequential process; thus, the change in one parameter may force the adjustment of another.

In calibrating the initial biomass in the column, we assumed that SRB and FeRB would have initially equal volume fractions. Figure 4-6 illustrates the impact of the initial SRB concentration on breakthrough curves. In our approach, the initial SRB volume fractions is mainly calibrated based on the maximum concentrations of lactate and sulfate at early breakthrough, which defines the starting points of microbial processes. Increasing the initial volume fraction of SRB results in lowering these starting points at early breakthrough, resulting in reaching the limiting growth conditions earlier. Therefore, the breakthrough curves of acetate and H$_2$S more to the left. Figure 4-7 shows the influence of the initial FeRB volume fraction on the simulation results, which resembled that of SRB. The increase in FeRB volume fractions stimulated the reduction of iron oxides; thus, the concentration of the inhibiting species of microbial souring decreased rapidly. The hyperbolic term of inhibition in dual-Monod equation would converge towards unity faster, resulting in earlier sulfate reduction and H$_2$S breakthrough. The experimental data were matched with the initial volume fractions of $1.0\text{E}-5 \text{ m}^3/\text{m}^3$ for both SRB and FeRB.
Figure 4-6: Impact of initial SRB concentration on the breakthrough of lactate, acetate, sulfate and $\text{H}_2\text{S}$ in Chen et al. (1994) experiment.

Figure 4-7: Impact of initial FeRB concentration on the breakthrough of lactate, acetate, sulfate and $\text{H}_2\text{S}$ in Chen et al. (1994) experiment.
The calibration of the microbial sulfate reduction involves SRB-lactate and SRB-acetate, in which the most recently introduced microbes are dependent on the earlier-introduced microbes to produce the substrate needed for their growth. Figure 4-8 illustrates the influence of $k_{\text{max}}$ for SRB-lactate on the souring process. The decrease in $k_{\text{max}}$ would restrict the intake of lactate and sulfate, constraining the generation of H$_2$S and acetate. Furthermore, Figure 4-9 shows the $K_{\text{TEA}}$ for SRB-lactate with values of a maximum order of magnitude of -3 presenting an efficient match to the experimental data. $K_{\text{TEA}}$, with larger orders of magnitude, would impose a significant delay on this microbial process, shifting the breakthrough curves toward longer times. Similar outcomes were obtained for $K_s$ (Figure 4-10), where the maximum sufficient order of magnitude was -4.

![Graphs showing the impact of SRB-lactate maximum growth rate on the breakthrough of lactate, acetate, sulfate and H$_2$S in Chen et al. (1994) experiment.](image-url)
Figure 4-9: Impact of $K_{TEA}$ for SRB-lactate on the breakthrough of lactate, acetate, sulfate and $H_2S$ in Chen et al. (1994) experiment.

Figure 4-10: Impact of $K_s$ for SRB-lactate on the breakthrough of lactate, acetate, sulfate and $H_2S$ in Chen et al. (1994) experiment.
SRB-acetate determines the potential of further sulfate reduction, which mainly influences the part of the breakthrough curve that lies beyond the inflection point. This inflection point corresponds to lactate limitation in the column. Figure 4-11 illustrates the impact of the $k_{\text{max}}$ for SRB-acetate on the simulation results, where it reflects the growth rate anticipated to accumulate H$_2$S sufficiently to match the experimental data. The breakthrough curve for acetate reflected that of H$_2$S. A higher $k_{\text{max}}$ influences the consumption of both lactate and acetate, for which the generated SRB biomass is capable of oxidizing both substrates. Similarly to SRB-lactate, the increase in $k_{\text{max}}$ for SRB-acetate would stimulate sulfate reduction and H$_2$S would breakthrough earlier. The calibrated $k_{\text{max}}$ for SRB-acetate was smaller than that for SRB-lactate, which was consistent with the dependence of the first group of microbes on the second to generate their substrates. Figures 4-12 and 4-13 show the impact of $K_s$ and $K_{\text{TEA}}$, respectively, on SRB-acetate, which resembled that for SRB-lactate. The $K_s$ and $K_{\text{TEA}}$ of SRB-acetate had a greater impact on acetate oxidization, in addition to the breakthrough curves of sulfate and H$_2$S beyond the inflection point. The maximum orders of magnitude for $K_{\text{TEA}}$ and $K_s$ in SRB-acetate processes were -3 and -4, respectively, which were consistent with those obtained for SRB-lactate.
Figure 4-11: Impact of SRB-acetate maximum growth rate on the breakthrough of lactate, acetate, sulfate and H$_2$S in Chen et al. (1994) experiment.

Figure 4-12: Impact of K$_{TEA}$ for SRB-acetate on the breakthrough of lactate, acetate, sulfate and H$_2$S in Chen et al. (1994) experiment.
Because we assumed that both FeRB and SRB would have the same initial volume fractions, calibration of $K_I$ was required to account for the inhibition of both SRB-lactate and SRB-acetate by microbial iron reduction. Figure 4-14 shows the simulation results at differing $K_I$ concentrations. These results resembled those of different initial FeRB concentrations, as discussed earlier. The increase in $K_I$ would initially delay the sulfate reduction and lactate oxidation, leading to a delay in the generation and oxidation of biogenic acetate. In turn, $H_2S$ breakthrough would be further delayed.

Figure 4-13: Impact of $K_s$ for SRB-lactate on the breakthrough of lactate, acetate, sulfate and $H_2S$ in Chen et al. (1994) experiment.
The calibration of microbial iron reduction requires reasonable estimations of the ferric iron concentrations available for bio-reduction, which would impose the inhibition of microbial souring. Figure 4-15 shows the simulation results at different FeOOH concentrations, showing that the minimum concentration of ferric iron needed to impose SRB inhibition would be 5.5E-5 m³/m³. Lower volume fractions of FeOOH are insufficient to inhibit SRB growth, leading to earlier H₂S breakthrough. In addition, the bio-availability of FeOOH depends on its specific surface area (SSA), which would determine the reactive surface area with FeRB and compensate for the hyperbolic TEA term used to describe the microbial reduction of aqueous species. Most iron oxides are poorly crystalline within natural systems, and would have a SSA < 100 m²/g (Cornell et al. 2003). Figure 4-16 shows the impact of the SSA on simulation results, in which the experimental results are better matched with 79.8 m²/g. Higher SSA would increase the bio-availability of ferric iron and enhance its reduction, which would shorten the period of SRB inhibition and result in
earlier H2S breakthrough. As described in the literature review, the utilization of a reactive surface in a dual-Monod equation requires adjusting the units of $k_{\text{max}}$ to mol/m$^2$-FeRB/s. Figure 4-17 illustrates that the experimental results are better matched with a $\log_{10}(k_{\text{max}})$ of -4.55, where higher values would enhance the reduction of ferric iron and advance microbial souring. Figure 4-18 shows the simulation results of varying $K_s$ values for FeRB, which were consistent with the results obtained for SRB reactions. The experimental results were better matched with a $K_s$ of 1.0E-3 mol/L.

![Graphs showing breakthrough of lactate, acetate, sulfate and H2S]

Figure 4-15: Impact of initial FeOOH concentration on the breakthrough of lactate, acetate, sulfate and H2S in Chen et al. (1994) experiment.
Figure 4-16: Impact of specific surface area (SSA) in microbial iron reduction on the breakthrough of lactate, acetate, sulfate and H2S in Chen et al. (1994) experiment.

Figure 4-17: Impact of FeRB maximum growth rate on the breakthrough of lactate, acetate, sulfate and H2S in Chen et al. (1994) experiment.
Table 4-4 summaries the dual-Monod parameters of SRB microbial reactions, while Table 4-5 summarizes those of dissimilatory iron reactions. The values of these parameter fell within the experimental limits presented in Table 2-4. Although the simulation results showed lower sulfate concentrations at steady-state conditions than the experimental data, these results achieved a sufficient match with H₂S breakthrough. As per SRB microbial reactions, sulfate and H₂S should have a molar ratio of one. The discrepancy between the simulation results regarding sulfate breakthrough and the experimental measurements indicates the possibility of an additional source of sulfate in the experiment, such as mineral dissolution and ion exchange reactions. Such results could also indicate that the sea sand had not been completely purified to silica after being washed, allowing the presence of other minerals to affect the outcomes of the microbial experiments. However, the discrepancy in results would not have had a major impact on our analysis of the souring process in the column, as the growth environment had an abundant sulfate concentration.

Figure 4-18: Impact of Kₘ for FeRB on the breakthrough of lactate, acetate, sulfate and H₂S in Chen et al. (1994) experiment.
Therefore, the limitation of substrate concentrations was the critical component of the microbial processes in this experiment. This is illustrated in Figure 4-19, which assumes higher injected concentrations of sulfate in the experiment.

Table 4-4: Parameters for SRB microbial reactions that are used to match Chen et al. (1994) experimental results

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Reaction Number</th>
<th>( f_e )</th>
<th>( K_{max} ) (mole/mole-SRB/Day)</th>
<th>( K_s ) (mole/L)</th>
<th>( K_{TEA} ) (mole/L)</th>
<th>( K_I ) (mole/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate</td>
<td>4-3 (table 4-1)</td>
<td>0.90</td>
<td>61.9</td>
<td>1.0E-5</td>
<td>1.0E-3</td>
<td>1.8E-6</td>
</tr>
<tr>
<td>Acetate</td>
<td>4-6 (table 4-2)</td>
<td>0.65</td>
<td>13.9</td>
<td>1.0E-4</td>
<td>1.0E-3</td>
<td>1.8E-6</td>
</tr>
</tbody>
</table>

Table 4-5: Parameters for FeRB microbial reaction that is used to match Chen et al. (1994) experimental results

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Reaction Number</th>
<th>( f_e )</th>
<th>( \log_{10}(K_{max}) ) (mole/m²-FeRB/s)</th>
<th>( K_s ) (mole/L)</th>
<th>SSA (m²/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate</td>
<td>4-10 (table 4-3)</td>
<td>0.60</td>
<td>-4.55</td>
<td>1.0E-3</td>
<td>79.8</td>
</tr>
</tbody>
</table>
Microbes in this experiment appeared to modify their metabolism based on substrate limitations, and possibly based on the abundance of TEAs. Analyzing the geochemical composition of the porous media would assist in the characterization of the potential of additional TEAs within minerals, which would reduce the level of uncertainty in analyzing experimental results. In this experiment, a better characterization of potential sources of iron would improve the analysis and characterization of H₂S breakthrough, while evaluating the mineral content of the porous media would aid in incorporating mineral dissolution and ion exchange reactions that could alter the concentrations of critical species.

Figure 4.19: Impact of Kₛ for FeRB on the breakthrough of lactate, acetate, sulfate and H₂S in Chen et al. (1994) experiment
4.2 Experiment 2: FeRB and SRB Bio-Competition in an Iron-Rich Environment

This section discusses and summarizes the results presented by Druhan et al. (2013). This discussion neither covers the calibrations of all dual-Monod parameters nor the yields of microbial reactions, as these are similar to what has already been discussed in a previous section. The calibrated values of those parameters are shown in Tables 4-5 and 4-6. However, this section addresses the calibration of the initial concentrations of FeRB, SRB, bio-available ferric iron, and Kf.

Table 4-5: The parameter of the FeRB microbial reaction used to match the experimental results of Druhan et al. (2013). The units of the $\log_{10}(K_{\text{max}})$ was mol/m$^2$-FeRB/s.

<table>
<thead>
<tr>
<th>Reaction Number</th>
<th>Microbial Reaction</th>
<th>$\log_{10}(K_{\text{max}})$</th>
<th>$K_s$ (mole/L)</th>
<th>SSA (m$^2$/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-12</td>
<td>$\text{FeOOH}_\text{(s)} + 0.208 \text{CH}_3\text{COO}^- + 1.925 \text{H}^+ + \text{0.033 NH}_4^+ \Rightarrow \text{Fe}^{2+} + 0.033 \text{C}_5\text{H}_7\text{O}<em>2\text{N}</em>{\text{aq}}^{\text{FeRB}} + 0.25 \text{HCO}_3^- + 1.60 \text{H}_2\text{O}$</td>
<td>-8.2</td>
<td>1.0E-5</td>
<td>79.8</td>
</tr>
</tbody>
</table>

Table 4-6: The parameter of SRB microbial reaction used to match Druhan et al. (2013) experimental results. The unit of $K_{\text{max}}$ is mole/mole-SRB/Day.

<table>
<thead>
<tr>
<th>Reaction Number</th>
<th>Microbial Reaction</th>
<th>$K_{\text{max}}$</th>
<th>$K_s$ (mole/L)</th>
<th>$K_{\text{TEA}}$ (mole/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-13</td>
<td>$\text{CH}_3\text{COO}^- + 0.872 \text{H}^+ + 0.92 \text{SO}_4^{2-} + \text{0.032 NH}_4^+ \Rightarrow \text{0.92 H}_2\text{S} + 1.84 \text{HCO}_3^- + 0.032 \text{C}_5\text{H}_7\text{O}<em>2\text{N}</em>{\text{aq}}^{\text{SRB}} + 0.096 \text{OH}^-$</td>
<td>58.0</td>
<td>5.0E-3</td>
<td>79.8</td>
</tr>
</tbody>
</table>
Figure 4-20 shows the simulation results at different initial volume fractions of SRB, in which the match to the experimental data was obtained with 1.0E-7 m³/m³. Similarly to the observations detailed in the previous section, the increase of SRB concentration enhances the uptake of substrate and TEA, leading to the generation of increased amounts of biogenic H₂S. In addition, this increase in H₂S generation reduces the concentration of ferrous iron at breakthrough, as these two species react to form FeS precipitates. Furthermore, Figure 4-21 illustrates the sensitivity of the results toward initial FeRB volume fractions. Unlike the previous section, the analysis of this experiment was not constrained by the same volume fractions of SRB and FeRB. The experimental data was matched with an initial volume fraction of 5.0E-5 m³/m³ of FeRB. The increase in FeRB volume fraction resulted in a significant increase in ferrous iron concentration; however, this did not impact the acetate concentration. This was because FeRB demands relatively little substrate compared to SRB, a finding which was consistent with the results of the first experiment. In addition, increasing the FeRB concentration had a minor impact on H₂S breakthrough, as all simulation results concluded that unlimited ferrous iron is required for FeS precipitation. Therefore, the breakthrough of H₂S in this case would be determined by the properties of the abiotic reaction of FeS. Because the initial FeRB concentration in this experiment outcompetes SRB, the microbial reduction of sulfate would be inhibited by the difference in initial biomass concentration. This was consistent with the observations in the previous section, where changes in K₁ and initial FeRB concentrations would have an identical impact on simulation results. Therefore, such an approach forms a substitute for the need of an inhibition hyperbolic term in the dual-Monod equation. These findings are illustrated by the simulation results in Figure 4-22. The initial concentration of FeOOH was estimated at 7.6E-4 m³/m³, as indicated by the results in Figure 4-23. The increase in FeOOH concentration stimulated microbial iron reduction, which resulted in the further consumption of acetate. In addition, higher ferrous iron concentrations increase the potential of FeS precipitation, as it reacts with a more biogenic H₂S.
Figure 4-20: Sensitivity analysis of initial SRB volume fraction and its impact on the breakthrough of sulfate, H$_2$S, acetate and ferrous iron in Druhan et al. (2013) experiment.

Figure 4-21: Sensitivity analysis of initial FeRB volume fraction and its impact on the breakthrough of sulfate, H$_2$S, acetate and ferrous iron in Druhan et al. (2013) experiment.
Figure 4-22: Sensitivity analysis of $K_i$ and its impact on the breakthrough of sulfate, $H_2S$, acetate and ferrous iron in Druhan et al. (2013) experiment.

Figure 4-23: Sensitivity analysis of initial FeOOH volume fraction and its impact on the breakthrough of sulfate, $H_2S$, acetate and ferrous iron in Druhan et al. (2013) experiment.
Finally, the difference in acetate concentration between the simulation results and the experimental data likely occurred due to its adsorption onto the porous media, as the presence of iron oxides provides positively-charged surface sites within formations. Figure 4-24 illustrates that approximately 152.84 mg/L of acetate could be adsorbed in the column; thus, this adsorption would have a minor impact on the breakthrough of other species. This resembled the discrepancy in sulfate concentration in the previously-discussed experiment, where the contribution of mineral content in the porous media was the likely reason for the increased sulfate concentration at breakthrough.

Figure 4-24: Sensitivity analysis of injected acetate concentration and its impact on the breakthrough of sulfate, H₂S, acetate and ferrous iron in Druhan et al. (2013) experiment.
4.3 Comparison Between Experimental Results

Although the simulations of both experiments differed in their approach towards the bio-competition between FeRB and SRB, their simulation results had significant similarities. While the results of the first experiment concluded a higher $k_{max}$ for FeRB than the second experiment, the $K_s$ for FeRB was two orders of magnitude lower in the results of the second experiment than the first. Both presumptions were within the experimental limits and valid per the dual-Monod model, in which an increase in one parameter results in a decrease in another when calibrating for the experimental results. This illustrates that assumptions made regarding initial biomass concentrations are not critical in simulating microbial activities for reservoir souring, as their growth can be constrained by the half-saturation coefficients and growth rate. Furthermore, the simulating results for both experiments showed that the estimations made for the microbial yields were more critical, as microbial reactions defined the limiting conditions and steady-state concentrations of biogenic species. However, initial biomass concentrations and their growth rates could have more importance when evaluating formation damage, when the development and distribution of bio-clogging play significant roles. Furthermore, growth parameters could vary based on the properties of microbes and their growth environment. In the first experiment, the Chen et al. (1994) utilized *D. desulforicans*, which is an SRB strain that is widely utilized in microbial and environmental studies. This bacterial strain has also been found to be capable of reducing ferric iron both in aqueous solutions and from iron oxides (Coleman et al. 1993, Lovley et al., Li et al. 2006). In contrast, the microbes used in the second experiments were presumed to be mixed cultures that were native to the formations from which the sediments were retrieved.

The first experiment was conducted under limited substrate conditions, in which higher pore-volumes were injected to reach near steady-state conditions. However, Chen et al. (1994) held reservations regarding this and other experiments (Chen et al. 1993-1996), as they were conducted
with relatively less PVI compared to other microbial experiments conducted on core samples. This is the reason referred to by Chen et al. for the insignificant permeability reduction observed in the columns. It should be noted that the main objective of those other experiments was the evaluation of bio-clogging, and that those experiments were conducted within a range of three to > 2000 PVI (Shaw et al. 1985, Raiders et al. 1986-1989, Geesey et al. 1987, MacLeod et al. 1988, Rosnes et al. 1991). However, several studies have considered evaluating reservoir souring in oil fields within a range of eight to 20 PVI (Sunde et al. 1993, Tyrie et al. 1993, Evans et al. 2008, Farhadinia et al. 2010). In comparison, the microbial experiment performed by Druhan et al. (2013) was not conducted for a sufficient length of time to reach near steady-state conditions. The experiment reached a maximum concentration of H$_2$S within 1 ppm. These results indicate that ferrous iron production remained large enough to precipitate most of the biogenic H$_2$S, and that FeRB metabolism was more significant than those of SRB. Most oil fields consider a critical H$_2$S concentration based on an exposure limit of 10 ppm, while further increase in concentration requires proper planning to separate H$_2$S from the produced fluids (Chilingar et al. 2013, Kelland et al. 2014). Figure 4-25 shows the simulation results from the experiment by Druhan et al. (2013), which was extended until steady state conditions were reached, and which revealed a considerable H$_2$S breakthrough at approximately 26 PVI. The first experiment experienced an earlier breakthrough of H$_2$S, which was expected as the estimated FeOOH fraction was one order of magnitude smaller than the fraction observed in the second experiment, and that both experiments shared the same estimate of SSA. In addition, the results in Figure 4-25 indicate limited sulfate conditions, which allowed the complete reduction of injected sulfate to H$_2$S. The second peak in ferrous iron concentration corresponds to the stimulation of ferric iron reduction further from the inlet, where it has already been depleted by FeRB. However, ferrous iron concentration then decreases rapidly as SRB becomes more dominant near the inlet, where the SRB oxidize larger amounts of acetate and generate higher H$_2$S concentrations to precipitate ferrous iron.
In both experiments, Reaction 3-22 was used to describe the attachment of biomass in the simulation results. This approximation reaction presumes that most microbes quickly attach on grains within porous media, which resembles the biofilm model of reservoir souring described in the literature review. This presumption of this reactions is also consistent with observations in column experiments, where microbial growth is more dominant near inlets at which both substrates and TEA’s are more available. Thus, most of TEA’s and substrates are consumed near inlets. However, microbial attachment and transport could be impacted by the negative charge on their surfaces. Microbes could alter this negative charge to reduce the repulsion force with formations of negative zeta potential, while the presence of iron-oxides provide positivity charged sites that microbes compete for with other negatively charged species (Dong et al. 2014). Current reservoir studies do not consider models that could account for the difference in zeta potential between microbes and formations, and how it might impact biomass distribution in heterogeneous systems and reservoir souring development. In general, SRB metabolism reduces the redox potential of aqueous solutions as more ions are consumed, thus they alter the differences in zeta potentials in oil reservoirs (Bernardez et al. 2012).
Figure 4.25: Results of an extended simulation run of Druhan et al. (2013) experiment to estimate H$_2$S breakthrough.
Chapter 5

1D and 2D Simulations at Field Scale

5.1 The Impact of the Ferric Iron Concentration (1D)

In the homogenous simulations, we utilized the microbial reactions obtained from the experimental results published by Druhan et al. (2013) (Tables 4-5 and 4-6). However, we assumed an initial volume fractions of 1.0E-6 m³/m³ for each SRB and FeRB; thus, we assumed that K₁ was 1.8 µmole/g. These estimates of the initial biomass fell within the typical cell density found in natural sediments (Li et al. 2010). Figure 5-1 shows the breakthrough curves of acetate, sulfate, H₂S and tracer. These results showed that acetate concentrations limited the reduction of sulfate, which resulted in a maximum H₂S concentration of approximately 250 mg/L. The results revealed that increasing iron concentrations does not impact the maximum concentration of H₂S at steady-state conditions, which was consistent with our analysis of the experimental results that bound H₂S concentrations by the stoichiometry of microbial reactions. However, the increase in FeOOH volume fraction would shift the breakthrough curves of species more toward the right. The inhibition of SRB would reduce acetate reduction, as FeRB demands lower amounts of substrates for their growth, and it will delay the reduction of sulfate to H₂S. As a result, the breakthrough of H₂S would be delayed by approximately five PVI after the breakthrough of tracer, for an initial FeOOH volume fraction of 1.0E-6 m³/m³. Moreover, a further increase in FeOOH by one order of magnitude, which is within the estimated concentration in the first experiment, would aggressively change H₂S breakthrough by more than 50 PVI (results not shown). Thus, the estimation of bio-available ferric iron should be carefully evaluated based on formation mineralogy and the reactive surface areas of these potential iron-bearing minerals, in order to achieve a reliable assessment of reservoir souring.
Figure 5-2 illustrates the spatial growth of biomass in a homogenous case with an initial FeOOH volume fraction of 1.0E-6 m³/m³. The volume fractions of SRB and FeRB increase as further pore volumes are injected. The distribution of FeRB was fairly uniform across the spatial domain, which was consistent with the homogenous distribution of ferric iron. SRB growth, however, was more dominant near the injection source, where sulfate concentrations in seawater would be significantly higher than in formation water. Furthermore, the growth of SRB was of higher significance than that of FeRB, as sulfate is more accessible in the aqueous phase and at higher concentrations compared to those of bio-available ferric iron.
Figure 5-3 shows the spatial development of iron and sulfur minerals in a homogenous case with an initial FeOOH volume fraction of 1.0E-6 m³/m³. There was a small decrease in FeOOH volume fractions as more pore volumes were injected, and it had a fairly uniform distribution that was consistent with the FeRB growth described previously. Furthermore, the spatial development of Fe(OH)₃ reflected the SRB growth. That was because Fe(OH)₃ reacts with biogenic H₂S (Reaction 3-21) to form ferrous iron and metal sulfur, leading areas with high SRB growth to have
lower volume fractions of Fe(OH)$_3$. This also resulted in spatial developments of FeS$_{\text{am}}$ and S$_{\text{s}}$ that were consistent with those concluded for SRB and Fe(OH)$_3$. In addition, a larger amount of ferrous iron was generated by the microbial reduction of FeOOH, which in turn resulted in increased precipitation of FeS$_{\text{am}}$. 

Microbial metabolisms generate significant concentrations of carbon dioxide; thus, its dissolution in aqueous solutions is expected to precipitate carbonate minerals. These minerals can be affiliated with barium, calcium, magnesium and iron. As discussed in chapter 3, this research considered the precipitation of calcite and dolomite, and Figure 4-29 illustrates the spatial development of these carbonate minerals. The precipitations of calcite increased as more pore
volumes were injected, and they were more dominant near the injection source due to the high calcium concentrations in seawater and the more dominant SRB growth. Our model indicated that dolomite tends to dissolve upon injection of higher pore volumes, because magnesium has a higher solubility in aqueous solutions compared to calcium.

Figure 5-4 compares the growth of SRB and FeRB in homogenous media with different initial volume fractions of FeOOH, after 4 PVI. These results were consisted with the presumptions of the dual-Monod model. Thus, FeRB would grow to a higher extent within environments with higher FeOOH volume fractions, while SRB would grow to a lower extent and be more inhibited within such environments. In results, those environments where SRB growth is more inhibited would experience less precipitations of metal sulfur and iron sulfide, and that Fe(OH)$_3$ abiotic reduction would be less effective as shown in figure 5-5.
Figure 5-4: The spatial growth of SRB and FeRB in 1D homogenous media with initial FeOOH volume fractions of $1.0 \times 10^{-6}$ and $1.0 \times 10^{-7} \text{ m}^3/\text{m}^3$, that is after 4 PVI.
Figure 5-5: The spatial development of iron and sulfur bearing minerals in 1D homogenous media with initial FeOOH volume fractions of 1.0E-6 and 1.0E-7 m³/m³, that is after 4 PVI.
Figure 5-6 shows the impact of ferric iron concentrations on H$_2$S breakthrough in our 1D homogenous simulations, which was based on a 10-mg/L cutoff that defined the typical exposure limit in oil fields. The results showed that higher iron concentrations increased the delay in the breakthrough of biogenic H$_2$S, deviating the breakthrough time further from that of the tracer. Although FeOOH concentrations are within those found in natural systems, based on the experimental results we simulated and the values reported for iron-rich natural systems, oil fields are unlikely to experience such a large delay in H$_2$S breakthrough (Figure 5-6). Unlike our 1D simulations, oil reservoirs are expected to have a heterogeneity index between 0.4 and 0.8, and their heterogeneity would be also impacted by the correlation length between producer and injectors. Thus, the ferric iron concentration would vary spatially based on permeability distribution across those reservoirs. The impact of spatial heterogeneity on oil reservoirs will be discussed in the next section.

![Figure 5-6: The breakthrough of H$_2$S and tracer verses the FeOOH volume fractions in 1D homogenous simulations. The breakthrough was based on a 10-mg/L cutoff for both species.](image-url)
5.2 The Impact of Reservoir Heterogeneity (2D)

As discussed in chapter 3, heterogeneous permeability fields are generated based on differing Dykstra-Parsons coefficients ($V_{DP} = \{0.4, 0.6, 0.8\}$) and correlation lengths that are, on the order of the well-spacing, ($\lambda = \{0.3 \times WS, 1.0 \times WS, 3.0 \times WS\}$). Increasing $V_{DP}$ would increase the heterogeneity of these fields, while increasing the correlation lengths would enhance the clustering of low and high permeability bodies (Figure 3-3). The porosity fields were estimated using the Carmen-Kozeny correlation (Eq. 3-2; Figure 3-4), and the ferric iron distributions were correlated with permeability reductions (Eq. 3-3; Figure 3-5). The simulations were constructed with an injector-producer pair that was a quarter of a five-spot pattern, in which the wells were operated at a constant differential pressure. For each simulation case, 25 random realizations of permeability fields were generated; thus, the analysis was based on their averaged breakthrough curves. Similarly to the homogenous case, the initial FeRB and SRB fractions were each assumed to be $1.0E-6$ m$^3$/m$^3$.

The breakthrough of tracer in heterogeneous fields was the reference to characterize the delay in H$_2$S breakthrough. Because all heterogeneous simulations were constrained by the same injection-production rate, which sustained constant differential pressure between the wells, the breakthrough curves of the tracer were identical in all heterogeneous fields (Figure 5-7). Figure 5-8 shows the breakthrough curves of H$_2$S in heterogeneous fields, revealing that decreasing $V_{DP}$ imposes further delay on H$_2$S breakthrough compared to that of the tracer. The increase in correlation length did not impact the delay of the H$_2$S breakthrough for a constant $V_{DP}$; however, it did lead to slight decrease in H$_2$S concentration as it reached a steady-state condition. This decrease in H$_2$S concentration became slightly more significant as $V_{DP}$ increased. The increase in H$_2$S concentration in heterogeneous fields corresponded to the decrease in sulfate and acetate concentrations consumed by microbial metabolism (Figure 5-9 and Figure 5-10, respectively).
Figure 5-7: The breakthrough curves of tracer for heterogeneous fields compared to a homogenous field (black). The Dykstra-Parsons coefficients (V_{DP}) of heterogeneous fields are 0.40 (red), 0.60 (blue) and 0.80 (green). The correlation lengths (\lambda) are 0.3 (square), 1.0 (circle) and 3.0 (triangle) times well-spacing (WS).
Figure 5-8: The breakthrough curves of $\text{H}_2\text{S}$ for heterogeneous fields compared to a homogeneous field (black). The Dykstra-Parsons coefficients ($V_{DP}$) of heterogeneous fields are 0.40 (red), 0.60 (blue) and 0.80 (green). The correlation lengths ($\lambda$) are 0.3 (square), 1.0 (circle) and 3.0 (triangle) times well-spacing (WS).
Figure 5-9: The breakthrough curves for sulfate in heterogeneous fields compared to a homogenous field (black). The Dykstra-Parsons coefficients ($V_{DP}$) of heterogeneous fields are 0.40 (red), 0.60 (blue) and 0.80 (green). The correlation lengths ($\lambda$) are 0.3 (square), 1.0 (circle) and 3.0 (triangle) times well-spacing (WS).
Figure 5-10: The breakthrough curves for acetate in heterogeneous fields compared to a homogenous field (black). The Dykstra-Parsons coefficients ($V_{DP}$) of heterogeneous fields are 0.40 (red), 0.60 (blue) and 0.80 (green). The correlation lengths ($\lambda$) are 0.3 (square), 1.0 (circle) and 3.0 (triangle) times well-spacing (WS).
The increase in reservoir heterogeneity was associated with the presence of more highly permeable zones, owing to a lower content of iron-bearing minerals that microbes could metabolize for energy. These permeable zones would be more favorable for SRB growth as they are less inhibited by microbial iron reduction; thus, biogenic H$_2$S breakthrough would breakthrough earlier in more heterogeneous fields. Figure 5-11 demonstrates the increase of SRB growth with $V_{dp}$, revealing it was more dominant in zones with higher permeabilities and lower FeOOH volume fractions. Furthermore, the increase in correlation length enhanced the clustering of SRB biomass, which was consistent with the spatial distribution of permeability and FeOOH.

Furthermore, Figure 5-12 illustrates the spatial growth of FeRB in heterogeneous fields. The increase in SRB growth within high permeable zones coincides with lower FeRB volume fractions, as these would contain less ferric iron needed for their growth. These zones would experience less bio-competition between SRB and FeRB. Likewise, FeRB is more dominant in low-permeability zones, where they outcompete and inhibit SRB. The increase in reservoir heterogeneity would result in more isolation between FeRB and SRB communities, as the growth environment of those microbes would be constrained by the permeability and corresponding content of iron-bearing minerals. Thus, the spatial influence of FeRB on inhibiting SRB growth would be less effective as reservoir heterogeneity increases.

Figure 5-13 shows the development of calcite in heterogeneous fields. These results show that calcite concentrations are significantly higher near injection wells, which was consistent with the high concentrations of calcium in injected seawater. Calcite development appeared to increase slightly with reservoir heterogeneity, because CO$_2$ production increased as SRB oxidized more acetate. FeRB did not have a significant impact on calcite deposition, as it oxidized less acetate compared to SRB. Based on this, reservoirs in which SRBs are more active are expected to experience an increased amount of scaling problems due to seawater injection. In contrast, dolomite
in heterogeneous fields appeared to dissolve as more pore volumes are injected (results not shown), which was consistent with the results discussed for the 1D homogenous simulations. The precipitations of FeS$_{(am)}$ (Figure 5-14) and S$_{(s)}$ (Figure 5-15) were spatially consistent, as those minerals were mostly deposited due to the abiotic reduction of iron minerals by H$_2$S. Thus, the development of those minerals appeared more dominant in low permeability zones where iron concentrations were higher, and where they mixed with biogenic H$_2$S. However, the volume fractions of iron sulfides and metal sulfur were relatively smaller than those of calcite. Some oil fields may also experience other precipitates that include a wide range of different iron sulfides other than FeS$_{(am)}$, namely siderite (FeCO$_3$) and dolomite with varying Mg-Fe ratios.
Figure 5.11: The growth SRB after 3.5 PVI for heterogeneous fields with different Dykstra-Parsons coefficients ($V_{DP}$) and correlation lengths ($\lambda$) that are on the order of the well-spacing (WS). SRB initial volume fractions is $1.0E^-6 \, m^3/m^3$ at initial conditions.
Figure 5-12: The growth of FeRB after 3.5 PVI for heterogeneous fields with different Dykstra-Parsons coefficients ($V_{DP}$) and correlation lengths ($\lambda$) that are on the order of the well-spacing (WS). FeRB initial volume fractions is 1.000E-6 m$^3$/m$^3$ at initial conditions.
Figure 5-13: The development of calcite after 3.5 PVI for heterogeneous fields with different Dykstra-Parsons coefficients ($V_{DP}$) and correlation lengths ($\lambda$) that are on the order of the well-spacing (WS).
Figure 5-14: The development of FeS$_{am}$ after 3.5 PVI for heterogeneous fields with different Dykstra-Parsons coefficients ($V_{DP}$) and correlation lengths ($\lambda$) that are on the order of the well-spacing (WS).
Figure 5-15: The development of $S_{(s)}$ after 3.5 PVI for heterogeneous fields with different Dykstra-Parsons coefficients ($V_{dp}$) and correlation lengths ($\lambda$) that are on the order of the well-spacing (WS).
Our results show that correlation lengths do not have significant effects on H$_2$S breakthrough time for a single 2D layer, where the range and average of H$_2$S breakthrough time are identical as correlation length increases. However, the correlation length could have more significant impact in the case of intermittent layers, at the correlation length in the vertical direction is expected to be at higher values. Figure 5-16 illustrates that H$_2$S breakthrough time decreases with V$_{DP}$, reconfirming that correlation length does not have a significant impact on H$_2$S breakthrough, as the simulation results examining different correlation lengths concluded an identical range and average time for H$_2$S breakthrough.

These results were also consistent with the bivariate correlation between permeability and ferric iron distributions. The increase in V$_{DP}$ resulted in a wider spread in permeability around its mean that followed a lognormal distribution, as illustrated in Figure 5-17. This wider spread was also translated to the normal distribution of ferric iron, where the presence of low FeOOH volume fractions corresponded to the spread of the permeability distribution toward higher values. This resulted in the generation of more permeable bodies with low ferric iron concentrations; these will be inhabited by SRB and generate more active sources for biogenic H$_2$S, thus resulting in an earlier H$_2$S breakthrough as seen in our simulation results. The change in correlation length did not have a significant impact on the probability density functions (PDFs) of permeability and ferric iron, as it mainly improves the clustering of permeable bodies.

In addition, the increase in average permeability would shift the PDF of ferric iron toward the left, resulting in lower ferric iron concentrations and earlier H$_2$S breakthrough. Also, the increase in permeability would improve hydraulic conductivity across oil reservoirs, and thus would also influence biogenic H$_2$S breakthrough. Reservoirs with higher permeability are expected to have a higher average storage capacity; therefore, the comparison between their simulation results should be scaled in terms of their corresponding pore volumes. Also, the increase in average ferric iron concentration ($\mu_{Fe}$) would impose further delay on H$_2$S breakthrough. This is illustrated
in Figure 5-18, where the time for H$_2$S breakthrough increased with $\mu_{Fe}$ and decreased with $V_{DP}$. Similarly to the correlation length, the negative correlation coefficient ($\rho$) imposes an insignificant impact on H$_2$S breakthrough, as these two parameters do not have a significant impact on ferric iron distribution. As the correlation coefficient approaches zero, permeability reduction and ferric iron concentration become less correlated, imposing a slightly further delay on H$_2$S breakthrough. The standard deviation of ferric iron ($\sigma_{Fe}$) has a similar impact to that of $V_{DP}$, where higher $\sigma_{Fe}$ results in a wider spread across the mean. This reflects the presence of a larger number of permeable bodies that have a higher potential to become sources of biogenic H$_2$S generation.
Figure 5-16: H$_2$S breakthrough verses Dykstra-Parsons coefficients ($V_{DP}$) in 2D heterogeneous simulations. The scatter data represent the simulation results of 25 random realizations per a correlation length ($\lambda$), which is on the order of the well-spacing (WS). H$_2$S cutoff for breakthrough is 10 mg/L.
Figure 5-17: The probability density functions (PDF’s) of permeability and ferric iron for 2D heterogeneous simulations at different Dykstra-Parsons coefficients ($V_{DP}$).
Figure 5-18: H$_2$S breakthrough verses average ferric iron concentration ($\mu_{Fe}$) in 2D heterogeneous simulations, at different Dykstra-Parsons coefficients ($V_{DP}$). H$_2$S cutoff for breakthrough is 10 mg/L.
It is important to mention that although column experiments can be utilized as an analogue of field systems, column experiments cannot capture all features of these systems. Microbial column experiments are usually conducted at isothermal conditions with low temperatures between 20 to 45°C. Few column experiments, like the one conducted by Chen et al. in 1993 to evaluate reservoir souring in Kuparuk field in Alaska, has maintained higher temperatures within 60°C during their experiment that are similar to reservoir conditions. In addition, although column experiments maintain Darcy flux values that are within those observed in the fields, the injection pressures in column experiments are lower than those in the fields. However, several studies showed that increasing pressure to reservoir conditions has minimum impact on SRBs activity (Rosnes et al. 1991, Magot et al. 2000, Kallmeyer et al. 2004, Barton et al. 2007). Studies show that microbial growth decreases as temperature increase beyond the optimum growth temperatures, at which it could be completely inhibited at high temperatures that exceed 90°C. However, some field studies highlight the challenge of culturing thermophilic bacteria from injection wells, as they are dominated by mesophilic bacteria where reservoir temperatures are low due to cooling (Girgoryan et al. 2008). Thermophilic bacteria will be more dominant deep into oil reservoirs where temperatures are relatively higher, and thus they become harder to sample and analyze. In addition, some deep oil fields have reported the presence of hyperthermophilic SRBs that could thrive at temperatures higher than 100°C (Stetter et al. 1987 and 1993). In the case of FeRBs, several strains had been sampled from oil reservoirs with temperatures between 50 and 84°C (Nazina et al. 1995, Greene et al. 1997, Slobodkin et al. 1999). However, studies show that there are some FeRBs that can grow at sediments with high temperatures up to 121°C (Kashefi et al. 2003). Other factors that could restrict microbial growth include changes in pH level lower or higher than optimum growth conditions, the increase in formation salinity, and the increase in the concentration of toxic species that include H₂S (Badziong et al. 1978, Reis et al. 1992, Elliott et al. 1998, Barton et al. 2007). All
those factors could affect the microbial growth rates differently from what is experience in column experiments, and thus impact microbial reductions of sulfate and iron in oil reservoirs.

Furthermore, comparison between experimental results and field simulations show that ferric iron bio-availability is much lower at field conditions than experiment (Li et al. 2009). In natural field systems, the main source of ferric iron bio-availability would iron-rich clays (Li et al. 2010), and these minerals are mostly associated with permeability reduction in sandstone reservoirs (Worden et al. 2003). Experiments show that about 20% of ferric iron within clay structures would be available for bio-reduction as FeOOH (Kostka et al. 2002), where most of the bio-reduction would occur at the edge of clay minerals (Liu et al. 2012). In the case of carbonate reservoirs, several studies report that these formations have negligible iron contents and H$_2$S adsorption capacities (Burger et al. 2005, Evans et al. 2006, Johnson et al. 2017), thus it would be expected that microbial reduction of ferric iron has less significant in carbonate reservoirs than in sandstones. Furthermore, the breakthrough of hydrogen sulfide in carbonate reservoirs would be more impacted by the hydraulic conductivity of the fractures where microbes are more active, compared to sandstone reservoirs that are dominated by channeling and have more complicated mineralogy.

Most column experiments are conducted such as VFAs are injected throughout the column, based on the dominant VFAs available in an oil reservoir. However, the process of generating VFAs in oil reservoirs is more complicated. Studies show that SRBs depend on fermentative bacteria to degrade oil components into VFAs that SRBs utilize in their metabolism (Herbert et al. 1985, Wolicka et al. 2010). Few column experiments, such those conducted by Myhr et al. (2002) and Callbeck et al. (2013), utilized residual oil for SRB growth while investigating nitrate treatment. The results of Callbeck et al. experiment show that SRB growth resulted in the loss of about 34% of C19-C20 alkanes, and that SRBs took long time to reach maximum reduction rates without having fermentative bacteria in the column. The results also show that the metabolism of SRBs result in high acetate concentrations, at which some bacteria had utilized to reduce more sulfate
and increase H$_2$S concentration, similar to our analysis of Chen et al. (1994) experiment. Other studies illustrate that SRBs depend on fermentative bacteria to oxidize aromatic components into VFAs, such as toluene (Tanji et al. 2014). In addition, SRB growth can be sensitive toward oil composition, at which their growth is could more efficient with heavier oils with less water-soluble toxic compounds and higher water-cuts (Lillebø et al. 2010). In the case of FeRBs, these microbes could directly oxidize aromatic hydrocarbons in their metabolism without fermentative bacteria (Lovley et al. 1994, Schmitt et al. 1996), which could be similar to findings of Callbeck et al. (2013) that NRBs were not dependent on fermentative bacteria to degrade the residual oil and out compete SRBs. Thus, considering the complexity of oil compositions while evaluating reservoir souring requires better understanding of the biodegradation process of oil components, how they impact microbial metabolism and bio-competitions. Finally, biogenic H$_2$S and CO$_2$ gases can be miscible in the oil phase, depending on reservoir temperature and pressure. While some volumes of these gases result in the precipitation of biogenic minerals, some might impact the recovery of oil reservoirs.
Chapter 6
Conclusions and Future Research

6.1 Conclusions

We examined for the first time the potential impact of dissimilatory iron reduction on microbial reservoir souring in oil fields under seawater injection. The main objectives of this research were to examine the delay in hydrogen sulfide breakthrough in the presence of ferric iron minerals, and to evaluate the influence of heterogeneity on microbial processes. The results of microbial experiments conducted in upflow porous reactors were used to obtain biochemical reactions and growth parameters. Homogeneous simulations of seawater injection were utilized to evaluate the impact of increasing ferric iron concentration on the souring process, while heterogeneous simulations were run at different correlation lengths and Dykstra-Parson coefficients. In the heterogeneous media, the distribution of ferric iron was correlated with the spatial reduction in permeability.

Our results highlight the potential for dissimilatory iron reduction within biofilm reactors in the presence of small volume fractions of ferric iron, in which microbes would favor its reduction over sulfate to produce energy. This process would inhibit microbial souring and delay hydrogen sulfide breakthrough by several pore volumes of injection. In homogeneous media, increasing the volume fraction of ferric iron sustains dissimilatory reduction for a longer time, and thus inhibits the microbial process and delays further hydrogen sulfide breakthrough. Our results show that sulfate-reducing bacteria will grow to a larger extent near the injection source, at which both substrates and sulfate are available. The growth of iron-reducing bacteria will be higher in the formations containing higher volume fractions of bio-available ferric iron. In heterogeneous fields, formations with higher Dykstra-Parson coefficients will experience earlier breakthrough of
hydrogen-sulfide. The presence of highly permeable zones in those fields, where iron-content is relative to the inhibition of SRB growth, leads to the generation of rapid H₂S sources. Our results illustrate that increasing correlation lengths does not impact hydrogen sulfide breakthrough; however, it does have a slight impact on the maximum concentration of hydrogen sulfide as steady state conditions are approached.

In addition, the results of waterflooding simulations show that calcite would be the dominant precipitate due to the production of carbonate as microbes oxidize acetate, while dolomite dissolves due to the higher solubility of magnesium in water. Additional precipitates include metal sulfur, iron-sulfide and biomass. The generation of these precipitates could impose significant formation damage; thus, permeability reduction may harm the injectivity-productivity of wells in the field.

Our research highlights the importance of considering the mineral compositions of oil reservoirs while investigating the potential of microbial reservoir souring. Conducting sensitivity analyses of ferric iron bio-availability and its microbial reduction help to evaluate H₂S breakthrough more efficiently throughout these heterogenous reservoirs. These analyses will help to improve financial decisions that impact the operations and developments of oil fields; thus, they will help to reduce financial losses and sustain profits.

### 6.2 Future Research

One aspect that requires further assessment in evaluating microbial reservoir souring is that of potential substrate sources in oil fields. This includes an understanding of the microbial reactions and growth of oil-degrading bacteria, which SRB would depend on to generate their substrates. Callbeck et al. (2013) observed that SRB growth would be significantly slower without the presence of oil-degrading microbes in a column experiment; thus, the direct utilization of residual oil by
SRB may not be critical. However, Holba et al. (2002) suggested that ferric-iron was a potential TEA for microbes in an Alaskan field to degrade oil and generate tar, where no other TEAs were potentially available. This assessment may vary between oil fields depending on the fractions and types of heavy components and water-cuts (Lillebø et al. 2010); thus, the development of more common grounds would be sufficient.

Considering more complex residual oil compositions during the evaluation of reservoir souring must cover the main aspects concerning reactive transport and multiphase simulations. Commonly most studies consider is the partitioning of biogenic H₂S between oil and water. However, one area that these simulation studies have not considered is the bio-availability of iron oxides in the presence of oil components (Lovley et al. 1994, Schmitt et al. 1996). In addition, accounting for potential ion-exchange reactions could improve the analysis of microbial activities. The presence of iron-bearing minerals is largely associated with oil-wet reservoirs (Willhite 1986, Worden et al. 2000 and 2003), where those minerals could form preferable surface sites on which microbes could attach due to their negative surface charge (Dong et al. 2014). Differences in zeta potential in oil reservoirs could affect microbial transport and attachment, and may alter the formation’s wettability and the bio-availability of charged components.

Understanding the distribution of ferric iron (III) for microbial reduction could improve the planning of treatment options through bio-competition, which may include nitrate and perchlorate injection. The implementation of these plans could be coordinated with potential zones of microbial processes, identified based on Gibbs’ energies and TEA accessibility, and by establishing accurate correlations with reservoir heterogeneity. A simple configuration of the existence of these metabolic microbial zones is illustrated in Figure 6-1 for environmental applications. Oil reservoirs may experience such segmentation based on injected concentrations, residual oil saturations and formation mineralogy. The spatial extent of microbial iron reduction could have a significant interaction not only with SRB, but with other microbes, the enhancement
of which aims to outcompete SRB. For example, Obuekwe et al. (1981) found that nitrate enhances ferric iron reduction by FeRB that were retrieved from an oil field. Their results showed that high nitrite concentrations can oxidize ferrous iron back to ferric iron; thus, it could mischaracterize nitrate impact as if it inhibits FeRB. Such process could increase the bio-availability of ferric iron in oil reservoirs and possibly impact SRB growth.

Figure 6-1: Conceptual distribution of metabolic microbial processes in deep fresh aquifers (top) and contaminated shallow aquifers (bottom) (Lovley et al. 1994).
Appendix: Mathematics of Reservoir Souring Models

A.1 Mixing-Zone Model

This analytical 1D model was developed by Ligthelm et al. (1991) to evaluate reservoir souring between an injector-producer pair. H₂S generation is limited to the mixing zone between injected seawater and formation, where the width of the H₂S source (Δx, m) can be estimated based on dispersion \( \left(D, \frac{m^2}{s}\right) \) as (Eq. A-1):

\[
\Delta x = 2\sqrt{D \tau_b}.
\]

Eq. A-1

\( \tau_b \) is a time constant of bacterial reaction, which reflects the time needed for bacteria to convert sulfate and VFAs into H₂S. It is therefore inversely proportional to the total number of SRB in the system. The cumulative H₂S production \( (P, \frac{kmole}{m^2}) \) at time \( t \) seconds is estimated in the model as (Eq. A-2):

\[
P_{H_2S} = C\sqrt{D} \cdot t.
\]

Eq. A-2

C is a constant equal to 0.03 \( \frac{kmole}{m^3} \) for North Sea oil fields, which depends on the compositions of formation water and seawater. The production of H₂S in the water phase \( \left(\frac{R_W}{kmole}{m^3.s}\right) \) can be calculated by taking the derivate of Equation A-2 with respect to time (Eq. A-3):

\[
R_W = \left(\frac{C\sqrt{D}}{2\sqrt{t}}\right) \left(\frac{1}{\Delta x}\right).
\]

Eq. A-3

and by substituting Equation 6-1 into 6-3 (Eq. A-3):
\[ R_H^w = \left( \frac{C}{4\sqrt{t}} \right) \left( \frac{1}{\sqrt{\tau_2}} \right) \]  

Eq. A-3

The model considers three phases \((j)\) of water \((j = w)\), residual oil \((j = o)\) and solid \((j = s)\); thus, the molar balance equation in the model can be expressed as (Eq. A-4):

\[
\sum_{j=1}^{3} \left[ S_j^w K_{H_2S}^{jw} \rho_j \frac{\delta X_{H_2S}^w}{\delta t} \right] + \sum_{j=1}^{3} \left[ u_j K_{H_2S}^{jw} \rho_j \frac{\delta X_{H_2S}^w}{\delta x} \right] = \sum_{j=1}^{3} \left[ D_j K_{H_2S}^{jw} \rho_j S_j^w \frac{\delta^2 X_{H_2S}^w}{\delta x^2} \right] + \frac{H_2S}{\frac{\phi}{S_w} R_H^w} .
\]

Eq. A-4

\( S_j^w \) is the bulk volume fraction, \( \rho_j \) is the phase density \( \left( \frac{Kg}{m^3} \right) \), \( M_j \) is the phase molecular weight \( \left( \frac{Kg}{mol} \right) \), \( K_{H_2S}^{jw} \) is the molar portioning coefficient of \( H_2S \) between phase \( j \) and water, \( X_{H_2S}^w \) is the molar fraction of \( H_2S \) in water, \( u_j \) is the Darcy velocity \( \left( \frac{m}{s} \right) \), \( D_j \) is the dispersion coefficient, \( \phi \) is the porosity and \( S_w \) is the pore volume fraction of water phase. The partitioning of \( H_2S \) between phases would result in a slower velocity compared to that of the water phase \( \left( \frac{v_x}{m/s} \right) \), where a retardation factor \( (A_{ow} \frac{m}{s}) \) can be expressed as (Eq. A-5):

\[
A_{ow} = \frac{v_x}{\sum_{j=1}^{3} \left[ u_j K_{H_2S}^{jw} \rho_j S_j^w \right]} = 1 + \left\{ K_{H_2S}^{ow} \frac{\rho_0 M_w S_0}{\rho_w M_s S_w} + K_{H_2S}^{sw} \frac{\rho_s M_w (1 - \phi)}{\rho_w M_s S_w} \right\}.
\]

Eq. A-5

Substituting Equation 6-5 into 6-4 yields the following expression of \( H_2S \) molar balance equation (Eqs. A-6, A-7, A-8, A-9):

\[
\frac{\delta X_{H_2S}^w}{\delta t} = \alpha \frac{\delta^2 X_{H_2S}^w}{\delta x^2} + \frac{M_w}{v_x \rho_w} R_H^w .
\]

Eq. A-6

\[
x' = x - \frac{v_x}{A} t
\]

Eq. A-7

\[
t' = \frac{v_x}{A} t
\]

Eq. A-8
\[ \alpha = \frac{D_w}{v_x} \]  

Eq. A-9

This model was developed by Sunde et al. (1993). The model assumes \( \text{H}_2\text{S} \) is generated near injection wells as the required nutrients would be more readily available for SRB growth and biofilm development. Similar to the mixing-zone, this model simulates reservoir souring between

Figure A-1: Conceptual illustration of the 1D mixing zone analytical model (Ligthelm et al. 1991).

A.2 Biofilm Model
an injector and a producer in 1D media separated by a distance $L$. The model assumes two phases of solid and water, in addition to six components ($i$) to account for the nutrient limitations for SRB growth. These six components are: $H_2S$, SRB, sulfate, nitrogen, organic carbon and phosphorus. The conservations of the masses of these components in the aqueous phase can be expressed as (Eq. A-10):
\[
\frac{\partial C_i^w}{\partial t_D} + \frac{\partial C_i^w}{\partial x_D} = \varepsilon \frac{\partial^2 C_i^w}{\partial x_D^2} + \frac{\partial P_i^w}{\partial t_D} + r_i^w.
\] Eq. A-10

$C_i^w$ is the concentration of component $i$ in water, $\varepsilon$ is a constant diffusion coefficient, and the dimensionless distance ($x_D$) and time ($t_D$) are calculated as (Eqs. A-11, A-12):
\[
x_D = \frac{x}{L} \quad \text{Eq. A-11}
\]
\[
t_D = \frac{vt}{\varepsilon L}. \quad \text{Eq. A-12}
\]

Because SRB is assumed to be immobilized, $\left(\frac{\partial C_{SRB}^w}{\partial x_D}\right)$ converges to zero. Furthermore, $r_i^w$ is the adsorption of the component $j$ into the water phase; this relates to the one of the solid phases as (Eq. A-13):
\[
\sum_{w,s} r_i^{w,s} = 0. \quad \text{Eq. A-13}
\]

The adsorption of $H_2S$ into the solid phase is estimated based on affinity of the adsorption ($R_{ads}$) and $H_2S$ maximum capacity in the solid phase ($C_{max}^S$) (Eq. A-14):
\[
r_{H_2S}^s = R_{ads} \frac{C_{max}^s - C_{H_2S}^s}{C_{max}^s} C_{H_2S}^w. \quad \text{Eq. A-14}
\]

In addition, $\left(\frac{\partial P_i^w}{\partial t_D}\right)$ is the accumulation of component $i$, calculated using the Michaelis-Menten rate law as discussed in the literature review (chapter 2).
A.3 Thermal Viability Shell (VPS) Model

This model was developed by Eden et al. (1993) to account for the impact of temperature gradient on microbial growth, when it is mainly imposed by injecting cold seawater into hot oil reservoirs. The functions that describe this relation for North Sea oil fields is expressed as (Eq. A-15):

$$\beta = 0.613P_r - 10.76T_o - 0.07048P_r T_o + 1.476T_o^2 + 0.001015P_r T_o^2 - 0.0249T_o^3.$$  \hspace{2cm} \text{Eq. A-15}

$P_r$ is the reservoir pressure in atmosphere units and $T_o$ is calculated based on reservoir temperature $T$ in °C (Eq. A-16):

$$\frac{T_o - 20}{50 - 20} = \frac{T - T_o}{T_{\text{max}} - T_{\text{min}}},$$  \hspace{2cm} \text{Eq. A-16}

which sets a boundary for the viability region of microbial activity between a lower temperature ($T_{\text{min}}$) and an upper temperature ($T_{\text{max}}$). The purpose of this function is to calculate the slope of the middle line ($\beta$) that trilinearly approximates sulfate consumption based on statistical techniques (Figure 6-2).
A.4 History-Match Souring Algorithm

This model was developed by Burger et al. (2005-2009) to history-match and forecast biogenic H₂S production in fractured chalk formations. The algorithm calculates the moles of biogenic H₂S generated from seawater injection (SW) and produced-water re-injection (PWRI), as follows (Eqs. A-17, A-18):

\[
\text{moles } H_2S|_{SW} = K_1 \left( 1 - e^{-\frac{K_2}{PVT}} \right) C_{SO_4^{2-}} V_{fracture} K_3
\]

Eq. A-17
moles \( H_2S_{\text{PWR1}} \) = \( K_1 \left( 1 - e^{-K_2 PVI} \right) C_{SO_4^{2-}} + \frac{C_{\text{DOC}}}{K_4} V_{\text{fracture}} K_3 \), \hspace{1cm} \text{Eq. A-18}

where \( K_1 \) is the maximum reducible sulfate fraction, \( K_2 \) is the effective rate of nutrient supply, \( K_3 \) describes the effectiveness of sulfate reduction as a function of temperature, \( K_4 \) defines the moles of dissolved organic content (DOC) in produced water consumed per one mole of reduced sulfate, and \( V_{\text{fracture}} \) is the volume of the fracture. The coefficients \( K_3 \) and \( K_4 \) are determined from experiments, while \( K_1 \) and \( K_2 \) are determined from history-matching.

A.5 UTCHEM

Several reservoir-souring studies have been conducted using UTCHEM (Delshad et al. 2009, Farhadinia et al. 2010, Haghshenas et al. 2012, Hosseininoosheri et al. 2017). This 3D model uses governing equations for overall mass and energy balances, and utilizes Monod kinetics to describe microbial growth and metabolism. In regard to reservoir souring, the delay in \( H_2S \) breakthrough due to phase-portioning is estimated as (Eq. A-19):

\[ A_{\text{ow}} = 1 + \frac{C_{H_2S}^o}{C_{H_2S}} \] \hspace{1cm} \text{Eq. A-19}

while \( H_2S \) retardation due to phase-adsorption is calculated as (Eq. A-20):

\[ A_{\text{ads}} = 1 + \frac{\rho_s R_{\text{ads}}(1 - \varphi)}{\varphi \rho_j C_j} \] \hspace{1cm} \text{Eq. A-20}

In a recent study, the model attempts to account for optimum abiotic conditions for microbes at their maximum growth rate. These conditions include reservoir salinity (\( C_{\text{sat}} \)), temperature and pH (Eqs. A-21, A-22, A-23, A-24):

\[ \mu_{\text{max}} = \mu_{\text{opt}} \cdot \gamma(T) \cdot \gamma(pH) \cdot \gamma(C_{\text{sat}}) \] \hspace{1cm} \text{Eq. A-21}
\[\gamma(T) = \frac{(T-T_{\text{max}})(T-T_{\text{min}})^2}{(T_{\text{opt}}-T_{\text{min}})[(T_{\text{opt}}-T_{\text{min}})(T-T_{\text{opt}})-(T_{\text{opt}}-T_{\text{max}})(T_{\text{opt}}+T_{\text{min}}+2T)]}\]

\[\gamma(C_{\text{sal}}) = \frac{(C_{\text{sal}}-2C_{\text{sal}}^\text{opt} + C_{\text{sal}}^\text{max})(C_{\text{sal}}^\text{max} - C_{\text{sal}})}{(C_{\text{sal}}^\text{max} - C_{\text{sal}}^\text{opt})^2}\]

\[\gamma(pH) = \frac{(pH-pH_{\text{min}})(2pH_{\text{opt}} - pH_{\text{min}} - pH)}{(pH_{\text{opt}} - pH_{\text{min}})^2}\]
References


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