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Control of Microbial Sulfide Production with Nitrate and Biocide in Oilfield-Simulating Bioreators

Xue, Yuan (Fiona)

UNIVERSITY OF CALGARY

Control of Microbial Sulfide Production with Nitrate and Biocide in Oilfield-Simulating Bioreactors

by

Yuan (Fiona) Xue

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Abstract

Souring, the production of sulfide by sulfate-reducing bacteria in oil fields, can be remediated by nitrate injection. However, continuous amendment of sulfate-containing injection water with nitrate leads to microbial zonation. The combination of nitrate and biocide that may break this zonation and control souring more effectively was investigated here using bioreactors injected with excess volatile fatty acids and 2 mM sulfate or 2 mM sulfate and 2 mM nitrate. Biocide was pulsed with long duration (5 days) at low concentrations or short duration (1 h) at high concentrations. The success of these strategies was determined by the time needed for sulfide recovery. The results indicated that pulsed biocide can be synergistic with continuous nitrate. However, it depends on the type of biocide, its concentration, and the length of the pulse. Hence, pulsed biocide can improve souring control with continuous nitrate injections, if the appropriate product and injection strategy are chosen.

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List of Symbols, Abbreviations and Nomenclature

Symbol	Definition
AMP	Adenosine monophosphate
AP	Postulated nitrate/nitrite antiporter
API	American Petroleum Institute
APS	Adenosine phosphosulfate
ASTM	American Society for Testing and Materials
ATP	Adenosine triphosphate
BAC	Benzalkonium chloride
BTEX	Benzene, toluene, ethylbenzene, and xylene
CH_4	Methane
CH_3COOH	Acetic acid
CMIC	Chemical microbially influenced corrosion
CO_2	Carbon dioxide
DNRA	Dissimilatory nitrate reduction to ammonium
EMIC	Electrical microbially influenced corrosion
EOR	Enhance oil recovery
FeS	Iron sulfide
FICI	Fractional inhibitory concentration index
ΔG^0	Gibbs free energy at standard state
Glut	Glutaraldehyde
H_2	Hydrogen
H_2O	Water
HPLC	High-performance liquid chromatograph
hNRB	Heterotrophic nitrate-reducing bacteria
H_2S	Hydrogen sulfide
MEOR	Microbially enhanced oil recovery
MHGC	Medicine Hat Glauconitic C
MIC	Microbially influenced corrosion
	Minimum inhibition concentration
N_2	Nitrogen
NAP	Periplasmic nitrate reductase
NAR	Respiratory nitrate reductase
NIR	Nitrite reductase
NIWR	Near injection wellbore region
NO	Nitric oxide
N_2O	Nitrous oxide
NOR	Nitric oxide reductase
N_2OR	Nitrous oxide reductase
NRB	Nitrate-reducing bacteria
NR-SOB	Nitrate-reducing sulfide oxidizing bacteria
OOIP	Original oil in place
PWRI	Produce water reinjection
RT	Sulfide recovery time
SO_2	Sulfur dioxide

SRB	Sulfate-reducing bacteria
SRA	Sulfate-reducing archaea
SRP	Sulfate-reducing prokaryotes
soNRB	Sulfide-oxidizing nitrate-reducing bacteria
TSR	Thermochemical sulfate reduction
TT	Biocide treatment time
VFA	Volatile fatty acids

Chapter 1: **Introduction**

1.1 Stages to extract oil

1.1.1 Petroleum composition and physicochemical properties

Petroleum, also known as crude oil, is a non-renewable fossil fuel formed from dead organisms buried underground in sedimentary rocks subjected to millions of years of geological transformations (Speight 2014; Grigoryan and Voordouw 2008). Petroleum is typically a mixture of hydrocarbons (up to 97% w/w) with the numbers of carbon atoms ranging from 1 to over 100 (Speight 2014; Planckaert 2005). The major classes of hydrocarbons are cycloalkanes (= naphthenes = cycloparaffins), alkanes (=paraffins) and aromatics (= arenes) (Planckaert 2005; Speight 1999). The minor classes of hydrocarbons include alkenes (=olefins), non-hydrocarbons such as N, S, O organic compounds, and metals like nickel and vanadium (Wolicka and Borkowski 2012; Speight 1999). The composition of chemical compounds in petroleum fluids varies depending on the geological history of oil reservoirs, ranging from extremely dry gas up to bitumen, in spite of the narrow ranges in the elemental composition (Planckaert 2005). Correspondingly, the thermochemical background of reservoirs can be reflected by the composition of petroleum fluids. A widely-used technique to determine hydrocarbon composition is gas chromatography.

The varying properties of crude oils include API gravity, pour point, sulfur content and other bulk properties such as C/H ratio, flash point, and nitrogen content (Chang et al. 2012). API (American Petroleum Institute) gravity was arbitrarily defined as the oil density compared to water and is used by producers to qualify oil products. It is calculated by the equation ^{o}API= [141.5/specific gravity (at 60 ^{o}F)]-131.5 (Riazi 2007). Based on the API gravity crude oils are classified into light (>35 ^{o}API), medium (26-35 ^{o}API), heavy (10-26 ^{o}API) and extra heavy oil

(<10 ^{o}API) (Sandrea and Sandrea 2007). The data of world oil reserves in 2006 by API gravity showed that 22% is light oil, 44% is medium oil, 11% is heavy oil and 23% is extra heavy oil (Sandrea and Sandrea 2007). The pour point is the lowest temperature at which oil will still flow before solidifying. The sulfur content in crude oil can range from less than 0.1% to greater than 5% by weight percentage. If sulfur content in crude oil is less than 1 %, it is considered a sweet oil, while if it is more than 1%, it is a sour oil (Chang et al. 2012), which is low quality oil, requiring greater capital investment in the refining process to remove them.

1.1.2 Crude oil recovery

There are three basic phases in the recovery of oil. Primary oil recovery is driven by the initial high pressure in oil reservoirs. Once production wells are open, oil will be expelled out by the pressure difference between the underground and the surface. However, only a fraction (e.g. 20%) of original oil in place (OOIP) can be obtained by this means (Sandrea and Sandrea 2007). As natural pressure declines, it becomes too low to sweep out oil. In secondary oil recovery water is usually injected to maintain pressure. The injected water displaces and pushes oil towards production wells, which eventually produces a mixture of water (produced water) and oil. This process, also called water flooding, can recover up to 45-50% OOIP (Sandrea and Sandrea 2007). Because of the shortage of source water, produced water is usually re-injected into the reservoir after it is separated from oil. This process is also known as produced water reinjection (PWRI). Due to water loss and the displacement of oil in the reservoir, additional make-up water is needed from local water sources such as rivers, sewage treatment plants or deep formation waters (Voordouw 2011). The whole process is depicted in Figure 1.1. The leftover residual oil can be more than 50% (Sunde et al. 2004). More sophisticated techniques and methods are required to produce the remaining oil in the tertiary recovery stage. Enhanced

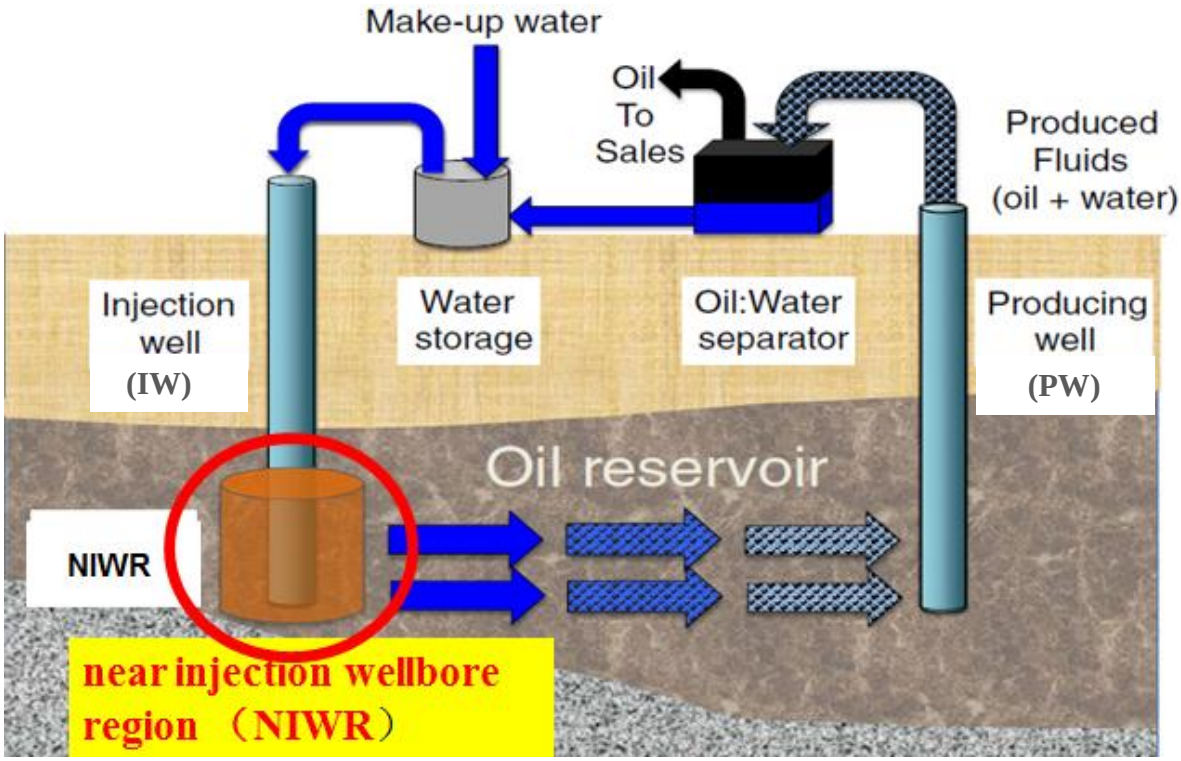


Figure 1.1 Secondary oil recovery by produced water re-injection (PWRI). Water is injected into the reservoir through the injection well (IW), to increase the pressure and sweep the oil towards the production well (PW). The produced fluids are a mixture of oil and water. Oil will be separated out and go to the refinery, while water is re-injected in case of water limitations. Make-up water is also needed to replace the volume of produced oil. Make-up water is supplemented at the water plant (or water storage) from local water resources. The brown zone is the near injection wellbore region (NIWR), where most of the sulfate-reducing bacteria and other microorganisms are growing because of readily obtained nutrients. The reduction of sulfate to sulfide mostly occurs here. If the reservoir is high temperature, this is also the area where a cool environment is established. The produced sulfide will be transported throughout the rest of reservoir to the production well (Augustinovic et al. 2012). The process is described in detail in 1.3.2 (adapted from Gieg et al. 2011).

oil recovery (EOR) methods include thermal, chemical, miscible and microbial methods (Planckaert 2005). An additional 7-15% of oil can be squeezed out by these EOR techniques (Sandrea and Sandrea 2007).

In order to accomplish the long term goal of recovering 70% of conventional oil and 30% of non-conventional heavy oils (Sandrea and Sandrea 2007), EOR methods must be given extra attention. Although thermal methods for the production of heavy and extra-heavy oil are most widely-used in the industry, limitations exist due to the huge investment of chemicals and energy (Bachmann et al. 2014). Microbially enhanced oil recovery (MEOR) seems promising due to its environmentally friendly nature and economic efficiency, as described elsewhere (Bachmann et al. 2014; Augustinovic et al. 2012; Rassenfoss 2011; Turkiewicz 2011; Youssef et al. 2009; Planckaert 2005).

1.2 Microorganisms living in oil reservoirs

Since 1926, when Edson S. Bastin and his colleagues first found that sulfate-reducing bacteria widely inhabited oil field produced waters, many studies of deep subsurface microbiology started emerging, even though the origin of reservoir microbes is still an open question (Magot 2005; Magot et al. 2000). Petroleum microbiology, the study of the microorganisms in oil extraction systems that may play a role in the formation, transport and recovery processes, thus appeared on the historic stage and gained people's attention. The activities of microorganisms can be beneficial or detrimental to industrial operations (Augustinovic et al. 2012; Lee et al. 1996). In order to manipulate those microbes as producers wish, it is vital to find out first what microorganisms are living in oil reservoirs. Substantially different from the surface, reservoir conditions are more severe and regarded as extreme (Wolicka and Borkowski 2012). They are anaerobic, having elevated level of salinity (some can

reach up to 10%) and most have high temperature (>80 ºC) and high pressure (Bachmann et al. 2014). All of these characteristics make it more interesting to study these extreme environment survivors. The major microbes occurring in oil reservoirs can be described as follows: syntrophs, sulfate-reducing bacteria, nitrate-reducing bacteria, and methanogens (Magot 2005).

1.2.1 Syntrophs

Syntrophic bacteria live thermodynamically dependent on other partners (McInerney et al. 2009). In anaerobic oil reservoirs, syntrophs are the pioneers of *in situ* oil biodegradation by breaking down high molecular weight hydrocarbons into small molecules such as acetate, formate, CO_2 and H_2 (Grigoryan and Voordouw 2008). However, these reactions are energetically unfavourable and only occur in the presence of other microbes (such as methanogens) that are able to consume the end products immediately to keep them at very low levels and drive the reaction forward (Sieber et al. 2012; McInerney et al. 2009). The produced energy is very low, less than 20 kJ/mol of methane, just enough for minimum ATP synthesis and basic maintenance of the community. The low energy yield and the need to share among more than one participant (Sieber et al. 2012; Gieg et al. 2011; Zengler et al. 1999) makes syntrophy an extreme lifestyle (McInerney et al. 2009).

The accompanying microbes are usually methanogens in oil reservoirs that lack higher redox potential electron acceptors such as sulfate, nitrate or Fe (III). As the terminal step of anaerobic metabolism, methanogens produce methane by using the end products of syntrophs. The whole process represents a mechanism through which high molecular weight organic matter is biodegraded to the simplest carbon compounds, which is a significant part of the whole carbon cycle and also explains the origin of heavy oil (Head et al. 2003). However, it is still unclear as to how syntrophs work together with their partners. Two of the theories are interspecies

metabolite transfer and direct electron transfer via conductive pili or nanowires (Sieber et al. 2012).

1.2.2 Sulfate-reducing bacteria

Sulfate-reducing bacteria (SRB) use sulfate as their final electron accepter ($SO_4^{2-} + 9H^+ + 8e^- \rightarrow HS^- + 4H_2O$) instead of O_2 in dissimilatory sulfate reduction (Wolicka and Borkowski 2012; Barton and Fauque 2009). The product of sulfate reduction is usually sulfide, which is the culprit causing biological souring (Gieg et al. 2011) and pipe corrosion (Barton and Fauque 2009). The potential electron donors for SRB to reduce sulfate include small molecules produced by syntrophs such as H_2, acetate and a wide range of organic compounds including some crude oil hydrocarbons (Liamleam and Annachhatre 2007; Vance and Thrasher 2005). Therefore, people sometimes use SRB as indicators to search for oil reservoirs (Wolicka and Borkowski 2012). SRB utilize oil organics in a specific order: volatile fatty acids (VFA) are the easiest compounds to be used (Tanji et al. 2014; Hao et al. 1996) followed by n-alkanes, monocyclic aromatic compounds (BTEX), polycyclic aromatic compounds and N, S, O compounds. Organic matter is usually oxidized in two ways: partially to acetate or completely to CO_2 (Muyzer and Stams 2008; Hamilton 1983). The particular metabolic ability of SRB (e.g. sulfide production) causes substantial trouble in oil production but can also be useful in certain circumstances. For example, SRB can detoxify dissolved heavy metals by precipitating them as metal sulfide (Teclu et al. 2008).

Reduction of sulfate to sulfide by SRB involves three distinct steps (Barton and Fauque 2009; Brock et al. 1994):

1) Sulfate activation. Because of the inert nature of sulfate, one ATP is required to activate sulfate to APS (adenosine phosphosulfate), which is catalyzed by ATP sulfurylase (Figure 1.2

reaction A);

2) Reduction of APS to sulfite by APS reductase. Two electrons are transferred from the energy source (e.g. H_2) to the sulfate portion of APS (reaction B in Figure 1.2);

3) Reduction of sulfite to sulfide (Figure 1.2 reaction C). In this step, sulfite is reduced directly to sulfide by dissimilatory sulfite reductase (Dsr). Six electrons are transferred.

SRB can thrive in a very broad range of temperatures (4-85 ^oC), salinities (0-17%), pHs (4- 9.5) (Javaherdashti 2011) and pressures (Wolicka and Borkowski 2012). SRB are strictly anaerobic microorganisms, but can often tolerate O_2 (Barton and Fauque 2009). SRB are usually living as biofilms in oil reservoirs by absorbing to the rocks (sessile cells), while some suspend in the fluid (planktonic cells). Most SRB are Gram negative bacteria (Wolicka and Borkowski 2012; Hao et al. 1996).

Most sulfate-reducing bacteria phylogenetically belong to the *Deltaproteobacteria, Firmicutes,* and 2 phyla within the *Archaea*, the *Euryarchaeota* and *Crenarchaeota* (Gieg et al. 2011; Barton and Fauque 2009; Youssef et al. 2009). Sulfate reducing Archaea (SRA) are thermophiles that can grow at temperatures of over 80 ^oC. Most are members of the genus *Archaeoglobus*. SRB and SRA are collectively called sulfate-reducing prokaryotes (SRP) (Gieg et al. 2011). However, the term SRB will be used in this thesis, as all the work was done at low temperature where no SRA are expected.

1.2.3 Nitrate-reducing bacteria

Nitrate-reducing bacteria (NRB) use nitrate as electron accepter and reduce nitrate to nitrite ($NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O$) and then to either N_2 or to ammonium. Dissimilatory nitrate reduction can proceed in two ways: denitrification or dissimilatory nitrate reduction to ammonium (DNRA) (Shartau et al. 2010; Zumft 1997). In denitrification, the intermediate nitrite

from nitrate reduction will be reduced further in the periplasm to N_2 ($NO_2^- + 4H^+ + 3e^- \rightarrow 1/2\ N_2 + 2H_2O$) with nitric oxide (NO) and nitrous oxide (N_2O) as intermediates ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$). In DNRA, nitrite will be reduced directly to ammonia ($NO_2^- + 8H^+ + 6e^- \rightarrow NH_4^+ + 2H_2O$; $NO_3^- \rightarrow NO_2^- \rightarrow NH_4^+$). The location of reactions and of enzymes involved in nitrate reduction is indicated in Figure 1.3. In a low temperature oilfield injected nitrate has been estimated to be reduced 95% to N_2 and 5% to NH_4^+ (Voordouw 2011).

In water flooded oil reservoirs, nitrate is typically absent or present in low concentration with the exception of nitrate injected wells to control souring. Hence, nitrate reduction may be a rare metabolic process in oil reservoirs (Magot 2005). However, NRB are common because NRB are living as fermentative bacteria when nitrate is absent (Shartau et al. 2010). Once nitrate is available, they switch to nitrate reduction. Unlike SRB, NRB can be aerobic, facultatively anaerobic, microaerophilic or anaerobic (Ollivier and Cayol 2005).

NRB can be divided into heterotrophic nitrate-reducing bacteria (hNRB) and sulfide-oxidizing nitrate-reducing bacteria (soNRB). hNRB derive energy from oxidation of organic chemicals like oil hydrocarbons; while most soNRB are chemolithotrophs, using CO_2 as sole carbon source and inorganic chemicals like sulfide as energy source (Voordouw 2011; Gevertz et al. 2000).

1.2.4 Methanogens

Methanogens, a major group of *Archaea*, are also present in oil reservoirs where there is often a scarcity of electron acceptors such as sulfate, nitrate and Fe (III). Because of the small change in redox potential when electrons flow from H_2 to CO_2 and the corresponding low energy yield, methanogenesis is the last step in anaerobic metabolism. Working together with syntrophs in the oil reservoir, methanogens convert acetate, H_2 and CO_2 to methane via the following

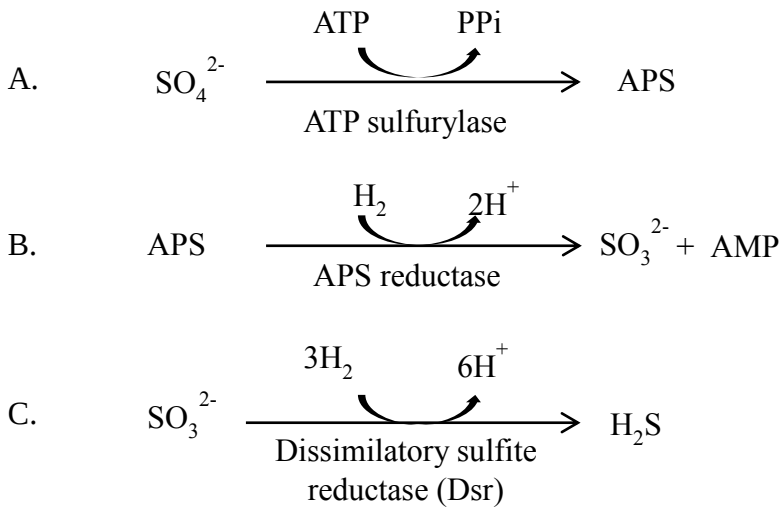


Figure 1.2 Dissimilatory sulfate reduction

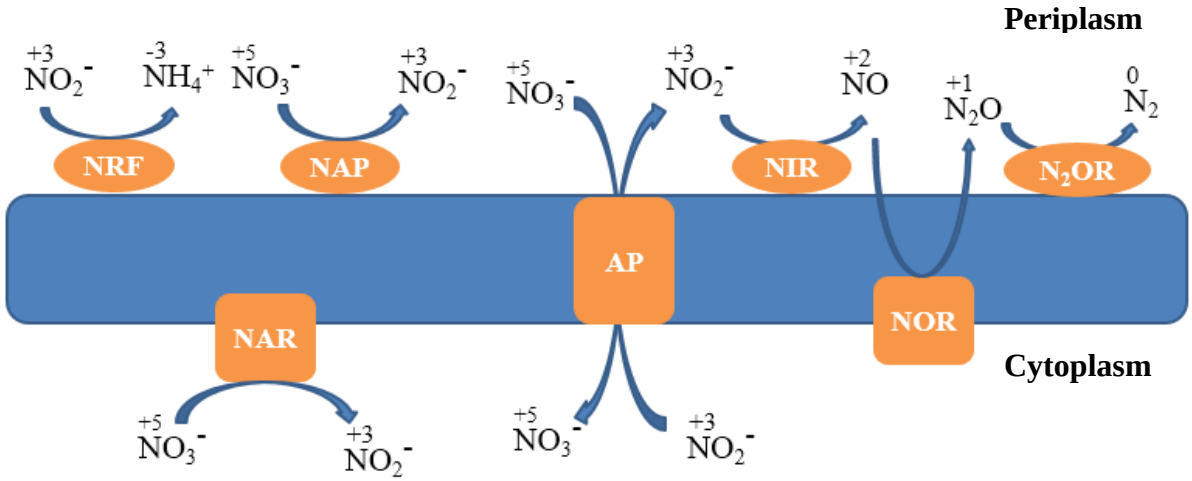


Figure 1.3 The sidedness of enzymes involved in dissimilatory nitrate reduction in the membrane. The number above each chemical is the oxidation state of N in each N-species. Nitrate is reduced to nitrite via respiratory nitrate reductase (NAR) under anaerobic condition. When oxygen is present, the periplasmic nitrate reductase (NAP) is active and able to reduce nitrate to nitrite in periplasmic membrane (Zumft 1997). Nitrite is reduced further to nitric oxide (NO) via nitrite reductase (NIR). NO reductase (NOR) is responsible for reducing NO to nitrous oxide (N_2O). N_2O to N_2 is the last step in denitrification performed by N_2O reductase (N_2OR) which is also the last enzyme discovered among all denitrification enzymes. Of these, NAR and NOR are both membrane-bound enzymes, while NIR, N_2OR and NAP are all periplasmic enzymes. Since nitrite reduction proceeds in the periplasm, the produced nitrite in the cytoplasm needs to be transported to the outside by AP, the postulated nitrate/nitrite antiporter. However, the mechanism to transport nitrate and nitrite is still unclear. NRF is nitrite reductase that converts nitrite to ammonium in the periplasm in DNRA (Kraft et al. 2011). The figure is partially adapted from (Zumft 1997).

reactions (Gieg et al. 2014):

1) $CH_3COOH = CO_2 + CH_4$ $\Delta G^{0/} = -31.0$ kJ/mol;

2) $4H_2 + CO_2 = CH_4 + 2H_2O$ $\Delta G^{0/} = -135.6$ kJ/mol.

Acetotrophic methanogens are responsible for (1), while hydrogenotrophic methanogens are involved in (2). Acetate and hydrogen are the most common substrates, while some methanogens can also use methylamines and methanol to produce methane (methylotrophic methanogens), such as *Methanohalophilus euhalobius*, *Methanosarcina mazei*, and *Methanosarcina siciliae* (Youssef et al. 2009). Not all methanogens can use acetate or H_2. For instance, *Methanosarcina siciliae* is unable to use acetate; *Methanosarcina mazei* can split acetate but is unable to use H_2 and CO_2 (Jeanthon et al. 2005).

Methanogens can thrive in a wide range of anaerobic environments. Most methanogens are mesophiles (Wolicka and Borkowski 2012; Jeanthon et al. 2005). Some reports have shown that as salinity increases, the activities of methanogens decrease (Borzenkov et al. 1997). Methanogens are also particularly sensitive to O_2 due to their requirement for a low redox potential (-330mV) (Wolicka and Borkowski 2012).

Which metabolic process occurs in an oil reservoir depends on the types of available electron acceptors. For instance, the presence of sulfate can induce SRB to conduct sulfate reduction; the addition of nitrate in injection water into a reservoir stimulates the growth of NRB; methanogens may be activated when only CO_2 is available to act as electron acceptor. Additionally, iron-reducing bacteria using Fe (III) as electron acceptor ($Fe_2O_3 + 6H^+ + 2e^- \rightarrow 2Fe^{2+} + 3H_2O$) have also been detected in produced water samples. Acetogens using H_2 and CO_2 to produce acetate were reported as well (Voordouw et al. 1996; Davydova-Charakhchyan et al. 1992). Among these electron acceptors, sulfate and CO_2 are usually most prevalent. Nitrate and

oxygen (aerobic condition) are scarce and Fe (III) will as a result also be limiting. The origin of microorganisms detected or isolated from oil reservoirs is still under debate because of the great difficulty to obtain representative samples without contamination (Magot 2005). How these microbes interact with each other is shown in Figure 1.4.

1.3 Souring in oil reservoirs

Souring, a common problem in oil fields, is the production of sulfide (S^{2-}, HS^- and H_2S depending upon pH) by SRB (Gieg et al. 2011; Hao et al. 1996). Souring can be found in both oil reservoirs and topside infrastructures, at low and high temperature, in both onshore and offshore operations (Gieg et al. 2011). SRB are usually living in the form of biofilms (Augustinovic et al. 2012) attached to the surface of reservoir rocks and pipeline walls (Sheng et al. 2007). This is a very crucial characteristic for SRB that equips them with higher resistance to unfavourable environments than planktonic bacteria. Besides SRB, sulfur and thiosulfate reducers (Magot et al. 2000) inhabiting oil reservoirs can also contribute to sulfide formation. SRA described in Section 1.2.2 are believed to play an important role on souring in high- temperature and high pressure oil reservoirs.

However, like other microorganisms from oil reservoirs, whether SRB are indigenous or introduced to oil reservoirs is also an open question (Struchtemeyer et al. 2011; Magot et al. 2000; Magot 2005). Instead of biotic causes, souring can be also attributed to abiotic causes (Khatib and Salanitro 1997), which include thermochemical sulfate reduction (TSR) (Machel 2001), thermal hydrolysis of S-containing organic compounds in heavy crude oil reservoirs (Zhang et al. 2005), dissolution of pyrite (Gieg et al. 2011) and liberation of H_2S gas from the aqueous phase as pressure declines (Seto and Beliveau 2000). However, most reports and studies credit souring mainly to microbiological causes in reservoirs subject to water flooding (Jones et

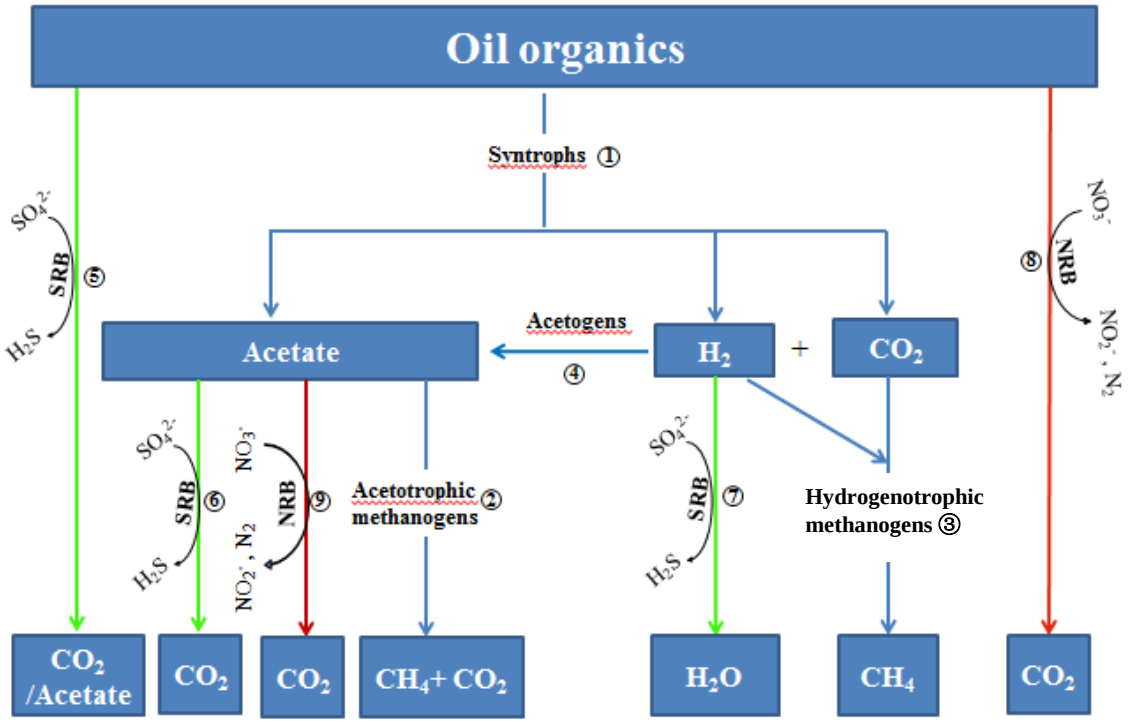


Figure 1.4 Carbon flow in microorganisms in the oil reservoir. [Blue lines] In natural oil reservoirs lacking electron acceptors, oil hydrocarbons are first attacked by syntrophs to form acetate, hydrogen and C_1 compounds such as CO_2 and formate (not shown in the figure). The produced acetate and hydrogen are removed immediately by acetotrophic methanogens and hydrogenotrophic methanogens, respectively. Methane is the terminal end product for both reactions. In the presence of acetogens, CO_2 and hydrogen can also be converted to acetate. The reactions are indicated by blue lines ①②③ and ④. [Green lines] In the presence of sulfate in the oil reservoir, SRB will oxidize oil organic matter completely to CO_2 or incompletely to acetate, while reducing sulfate to sulfide. SRB can also work together with syntrophs to oxidize the intermediates acetate and hydrogen to CO_2 and H_2O, respectively, which is represented by reactions ① ⑤⑥ and ⑦. [Red lines] when nitrate is injected into the oil reservoir, NRB are activated to conduct dissimilatory nitrate reduction to nitrite and then to N_2, while using oil organics as electron donors (reaction ⑧). Like SRB, NRB can be a partner of syntrophs as well by removing acetate (reactions ① and ⑨).

al. 2011; Vance and Thrasher 2005). Therefore, any souring mentioned here in this thesis refers to biological souring.

1.3.1 Detrimental effects of souring

Souring is a detrimental process that has a negative effect on oil industry operations (Gieg et al. 2011). Billions of dollars are spent every year to deal with souring problems (Chen et al. 1994). The detrimental effects of souring can be summarized into four aspects as follows:

1) The production of sulfide de-values the produced oil and increases the capital investment in refining. Sulfide produced by SRB increases the total sulfur content in oil, converting sweet oil to sour oil (sulfur content is more than 1%) and lowering the oil quality. In order to remove sulfur from oil, extra money will be paid in the refining process.

2) H_2S is a toxic and inflammable gas, presenting a health hazard for field workers and environmental safety. Its release into air must be under extremely strict controls by legislation. The toxicity effect of H_2S on humans has been studied extensively, and it is summarized in Appendix A. H_2S at 10 ppmv exhibits the notorious rotten egg smell; occupational exposure to 10 ppmv in Alberta is limited to less than 8 h/day, while exposure to 15 ppmv should be below 15 min/day; community evacuation must be done when H_2S reaches 20 ppmv in Alberta. Prolonged exposure of over 250 ppmv may lead to death via damage of respiratory and nerve systems; death may occur rapidly within minutes when H_2S is as high as 1000 ppmv (Guidotti 1994; Beauchamp et al. 1984). In the petroleum industry, actively souring oil reservoirs can produce large amounts of H_2S gas. For instance, the Skjold oilfield in the North Sea gave rise to maximum concentrations of 1000 ppmv of H_2S in the gas phase since the start-up of seawater injection with a maximum of 1.15 tons of total sulfide in all phases generated every day (Larsen 2002). Up to 40,000 ppmv of H_2S was reported in gas produced by the Huntington Beach field

in California (Khatib and Salanitro 1997). Some oilfields even had to shut down due to the high risk of sulfide poisoning (Macleod et al. 1994). The accidental or planed release of H_2S gas from petroleum industry operations into the environment can increase the level of SO_2, causing acid rain, damaging the ozone layer and impacting other ecological niches (Beauchamp et al. 1984). Therefore, the control of hydrogen sulfide gas in the oil and gas industry is of great importance for both safety and environmental concerns.

3) Sulfide precipitates (e.g. FeS) together with formation of SRB biomass may lead to plugging problems in injection systems. The notorious "schmoo" is actually formed by cementation of iron sulfide (Jones et al. 2010). A study by Roanea et al. (1991) using a flow rig simulating a high temperature, high pressure oil reservoir showed a 16% and 33% permeability reduction of a brine-saturated core following a 5 and 11 days incubation with an SRB inoculum, respectively.

4) Souring can lead to biocorrosion of metal infrastructures. SRB are widely accepted to be prime culprits in microbially influenced corrosion (MIC) (Enning and Garrelfs 2014) since SRB were initially proposed to cause corrosion in 1934. Even though the mechanism of MIC is very complicated involving more than one factor (Augustinovic et al. 2012; Sheng et al. 2007), the deposit of FeS on the surface of iron steel is undoubtedly the root cause and its formation must involve the presence of sulfide that is largely produced by SRB, no matter whether SRB acquire electrons from H_2 that is formed in cathodic depolarization (chemical microbially influenced corrosion, CMIC) (Barton and Fauque 2009; Sheng et al. 2007) or directly from iron steel e.g. the genus *Desulfobacterium* (electrical microbially influenced corrosion, EMIC) (Enning et al. 2012). Once FeS is formed, a more anaerobic environment will be created under the deposit which is quite favourable for SRB and methanogens to flourish leading to more

severe pitting problems. Moreover, the layer of FeS deposit is thought to facilitate electrochemical reactions and increase the corrosion rate by acting directly as a cathode (Javaherdashti 2011). Hence, biocorrosion is connected to souring via SRB activities and electrochemical reactions on the steel surface (Enning and Garrelfs 2014; Javaherdashti 2011).

1.3.2 Factors leading to souring

Souring mainly occurs due to water flooding during secondary oil recovery (Jones et al. 2011; Gieg et al. 2011; Vance and Thrasher 2005). Because natural oil reservoirs are very limited in electron acceptors such as sulfate (Magot, 2005), SRB are dormant or acting as fermenters with little sulfide produced. Under these conditions souring is not sufficient to cause problems. However, water flooding, especially with seawater which contains a high concentration of sulfate (up to 25-30 mM) brings sufficient electron acceptors to the reservoir. All seawater flooded fields examined are sour to some degree (Khatib and Salanitro 1997). Additionally, injected water also carries other nutrients that indigenous SRB need (Gieg et al. 2011), such as nitrogen sources, phosphate and even exogenous SRB (Struchtemeyer et al., 2011). SRB are then activated and start reducing sulfate to sulfide. Hence, the chemistry of the injection water is a major reason for souring causing production of H_2S following the onset of water injection (Larsen et al., 2004; Jenneman et al., 1999). In the Skjold oilfield, for example, souring was recorded just four months after the start of water flooding (Larsen 2002). During water flooding most SRB produce sulfide in the near injection wellbore region (NIWR, Figure 1.1) where formation and injection waters mix (Chen et al. 1994; Taylor et al., 1991; McKinley et al., 1988). The rest of the reservoir serves as a path to transport the produced sulfide towards the production well (Augustinovic et al. 2012). During transport, some sulfide will be removed

because of the formation of FeS deposit and the distribution to oil and gas phases (Voordouw et al. 2009).

Temperature is also an important factor to influence souring, because temperature impacts microbial growth. Temperature increases with depth at a rate of 3 ˚C per 100 m, the *in situ* temperature of deep oil reservoirs can be as high as 130-150 ˚C; (Wolicka and Borkowski 2012; Magot 2005; Magot et al. 2000), which is too hot for SRB to survive. The threshold temperature for SRB in oil reservoirs is considered 80-90 ˚C, and so far no sulfate reducers have been identified inhabiting oil reservoirs above these temperatures (Magot et al. 2000). Philippi (1977) claimed that no biodegradation was observed above 82 ˚C. Fisher (1987) found that the maximum fatty acid concentrations increased with the *in situ* temperature until 90 ˚C, suggesting that biodegradation activities in oilfield waters by microbes occurred up to 80-90 ˚C (Magot 2005). The recorded highest temperature for biological sulfate reduction is 93 ˚C (Roanea et al. 1991), although some hyperthermophilic SRB can be found growing at more than 100˚C (Stetter *et al*., 1993). Therefore, low temperature oil reservoirs are prone to be souring more than high temperature oil reservoirs. However, as water flooding is applied to sustain oil reservoir pressure, the mixing of cold injection water with hot formation waters in high temperature oil reservoirs creates a temperature gradient in the mixing zone, which becomes a suitable place for SRB growth (Figure 1.5). Therefore, even though protected by high temperature, hot oil reservoirs still have souring risks.

Besides the injection water chemistry and temperature, pressure and salinity are also important factors impacting SRB growth, but they are not the focus of this research.

1.4 Approaches to control souring

Since Bastin et al. first provided evidence for microbiological souring in 1926, different

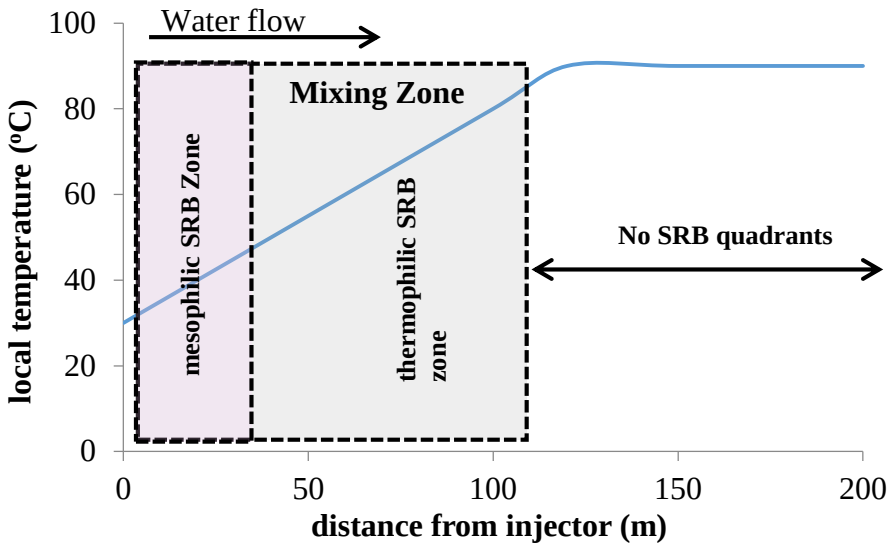


Figure 1.5 Temperature distribution in high-temperature oil reservoirs subject to water flooding. The light grey shaded box represents the mixing zone where a temperature gradient is created by continuous injection of cold water. The size of the mixing zone depends on the temperature of reservoir, the temperature of the injection water and the injection rate. The pink zone near the injector has a suitable temperature for mesophilic SRB to grow.

methods have been applied in the oil and gas industry to control or prevent souring (Table 1.1). There are usually three distinct approaches with respect to souring control (Jones et al. 2011):

1) Prevention of souring with the aid of mineral ores in natural reservoirs absorbing sulfide and with chemicals treatment at the start of water flooding;

2) Remediation of reservoir souring by nitrate or biocide treatment;

3) Post-souring control by using sulfide scavengers, such as amines or sodium hydroxide (Gieg et al. 2011).

The widely-used chemicals in preventative and remedial control approaches are biocides and nitrate. In the following section, their mechanisms, advantages, disadvantages and challenges to control biological souring will be further discussed.

1.4.1 Biocides

1.4.1.1 Advantages and disadvantages of using biocide to control SRB

Biocides are commonly used to control SRB in the oil industry. However, application of biocides is usually limited to above-ground infrastructures to control microbial corrosion and biofouling. Although biocides are rarely injected into oil reservoirs to control souring, they are designed to kill living organisms and thus possess great potential for souring control. As an advantage over other chemicals like nitrate, biocides directly kill bacteria and immediately remove the root cause of souring. However, increased resistance to biocide is always one of the big concerns. The transport of biocides in a reservoir is also an issue. Biocides are expensive preventing application of high concentration over long periods of time. As a result, biocide application can be erratic and haphazard (Augustinovic et al. 2012). Additionally, it is impossible for biocides to completely remove all bacteria within biofilms. Once biocide treatment is stopped, bacteria will eventually re-establish their activities. For example, 30 mg/L of THPS was

Table 1.1 Methods to control souring in the oil industry

Methods	Detailed methods	Descriptions	Limitations	References
Limiting SRB activity	Limit nutrients	Remove nutrients (sulfate, VFA) before water injection such as by reverse osmosis or membrane filtration	Expensive; SRB can still grow in topside facilities	Robinson; et al. 2010; Hubert 2010; Davis and McElhiney 2002
	Biocides	As seen in "Biocides" Section in this thesis	Development of resistance; expensive	Robinson et al., 2010
	Nitrate/nitrite	Inject nitrate and/or nitrite to inhibit SRB activity	Successful in high temperature oil reservoirs but challenging in low temperature oil fields; can cause corrosion	Voordouw et al., 2011; Vik et al. 2007; Larsen et al. 2004
	Combination	Combine nitrite with molybdate and/or biocide, or biocide with chelating agents	Cannot substantially change community structure, souring control was reversible	Gieg et al., 2011; Greene et al., 2006; Nemati et al., 2001
	Introduce antagonistic biomass	Inject microorganisms antagonistic to SRB like hNRB or soNRB	Proposed but not yet practiced *in situ*	Zuo 2007
	Aeration	Inject oxygenated water	Can cause corrosion	Kuijvenhoven et al. 2006
Remove sulfide	Chemical scavengers	Amines or sodium hydroxide; vapour recovery units to remove H_2S gas	Not applicable to higher concentration of sulfide	Larsen et al. 2004
	Nitrate/nitrite	Oxidize sulfide	Limited by nitrite reductase (Nrf) activity in SRB	Voordouw et al. 2002
Remove biomass	Remove biofilm	Mechanically remove biofilm from pipelines surface by using cleaning "pigs"	Not applicable to reservoirs	Larsen et al. 2004
Change pipeline	Novel materials	Use non-metallic pipes or protective coatings to prevent pipeline corrosion	Expensive	Gieg et al., 2011
Policy	Legislation	Regulate safety and odor control	N/A	N/A

continuously injected into a high pressure sour bioreactor with a highest concentration of 22 mg/L of produced H_2S. The dose of THPS was gradually reduced with no sulfide recovery. Only when injection was stopped sulfide recovery was seen at 130 days (Figure 1.6) (Jones et al. 2011).

The chemical structures and mechanisms of action of the biocides that are commonly used in the oil industry are given in Table 1.2 and Figure 1.7. Basically, they can be divided into two groups: chemically-reactive biocides that interact with biomass via chemical reactions, and physically-reactive biocides that interact with cell membranes via physical contact.

1.4.1.2 Synergy between two biocides

Combination of two biocides with expectation of synergy is commonly used in the oil industry in an attempt to slow down bacteria from developing resistance (Jones et al. 2010) and promote biocidal effect with cheaper price. When two biocides are used together, three results are expected: 1) synergy, the combined effect by two biocides is better than the sum of individual effects given by each biocide alone; 2) antagonism, the overall effect is decreased compared to the sum of individual effects; 3) indifference, the combined effect equals the sum of individual effects, or the addition of another agent dose not influence the effect of the agent. Synergy is most sought-after in the oil industry to combat microbial problems such as souring, MIC and fouling. It is already well- known that two biocides with different biocidal mechanisms tend to give a synergistic effect compared to the combination of two biocides with similar mode of action, for instance, Glut and BAC. Glut cross-links amino and sulfhydryl groups of proteins and nucleic acids and cause damage to bacteria. BAC as a cationic surfactant is believed to be able to alter interfacial tension on the surface of bacteria membrane and aid other biocides to penetrate better. This likely explains why Glut/BAC is most effective compared to other

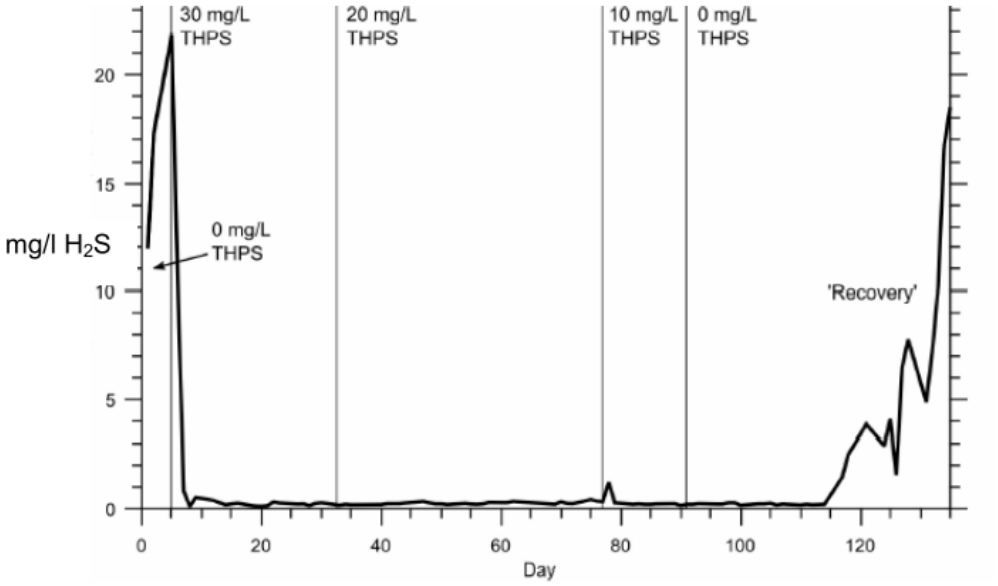


Figure 1.6 A high pressure column continuously injected with THPS to control H_2S production. The concentration was progressively decreased to 10 mg/L. H_2S was maintained at an undetectable level. When THPS was stopped, sulfide production slowly recovered until it reached the original level (adapted from Jones et al. 2011).

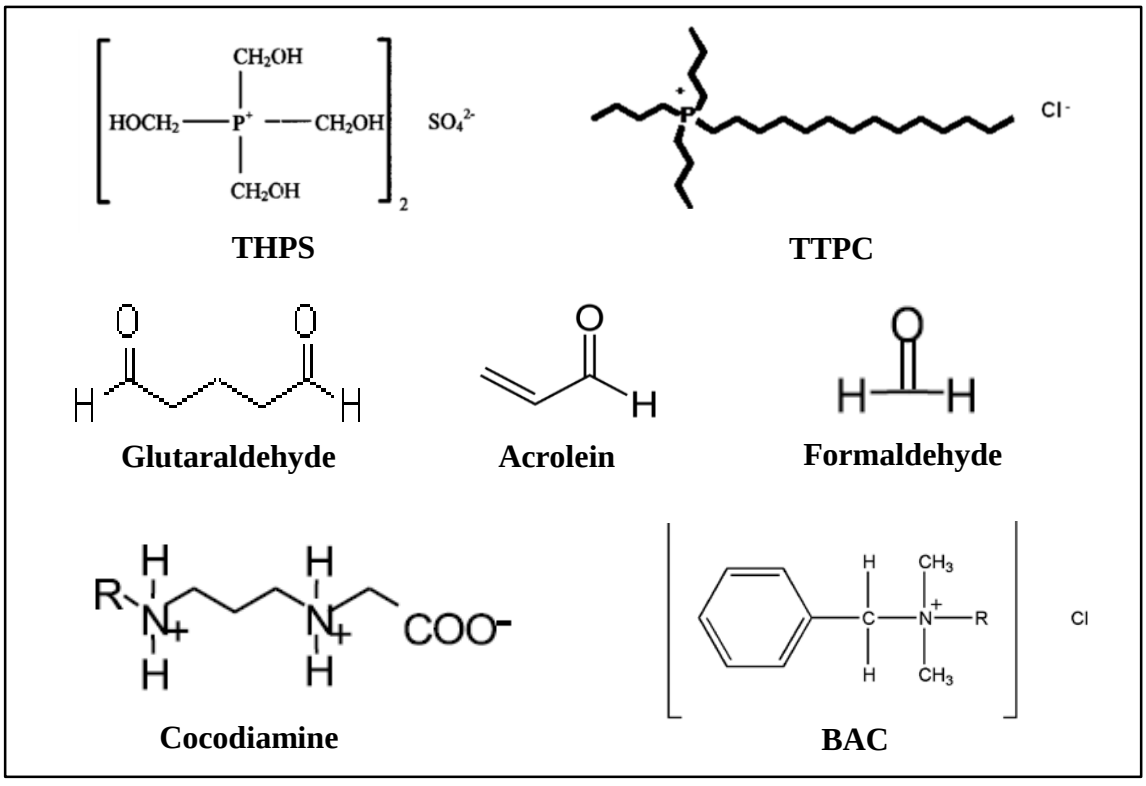


Figure 1.7 Chemical structures of biocides commonly used in the oil industry.

Table 1.2 Survey of biocides commonly used in the oil industry

(partially adapted from Greene et al. 2006)

Biocide	Mode of action		Description	Reference
Tetrakis hydroxymethyl phosphonium sulfate (THPS)	Chemically-reactive	Multiple modes of action: damages both outer and inner cell membrane; inhibits lactate dehydrogenase activity; inhibits cell respiration; inhibits sulfate reduction	Relatively new biocide in oil industry; biodegradable; dissolves FeS precipitates; fast-acting; effective against SRB; easily analysed.	Jones et al. 2012; Jones et al. 2011; Jones et al. 2010; Greene et al. 2006; Larsen 2002; Larsen et al. 2000; Downward et al. 1997; Macleod et al. 1994
Tributyl tetradecyl phosphonium chloride (TTPC)	Physically-reactive	Quaternary phosphonium cationic surfactant: solubilizes cell membranes	Fast-acting; broad spectrum; effective at low concentration; thermally stable; compatible with chlorine and H_2S.	Kramer et al. 2010; Kramer et al. 2008; Kramer 2006
Glutaraldehyde (Glut)	Chemically-reactive	Aldehyde groups cross-link with amino and sulfhydryl groups of proteins and nucleic acids	Most widely-used; broad spectrum; fast-acting; effective in a wide range of pHs and temperatures; deactivated by ammonia and amine-containing compounds; sulfide stable	Sunde et al. 2004; Thorstenson et al. 2002; Bødtker et al. 2008; Sunde et al. 2004; Ganzer et al. 2001; Gorman et al. 1980
Acrolein	Chemically-reactive	Damages proteins with aldehyde group	Scavenges sulfide; highly reactive; soluble in both water and oil; can dissolve FeS precipitates	Penkala et al., 2004
Formaldehyde	Chemically-reactive	Aldehyde groups react with amino groups of proteins and nucleic acids	Mechanism is similar to Glutaraldehyde	Kriel et al. 1993
Benzalkonium chloride (BAC)	Physically-reactive	Quaternary ammonium cationic surfactant: solubilizes cell membranes	Effective against biofilms; frequently used with Glut; high-foaming;	Kramer et al. 2010
Cocodiamine	Physically-reactive	Same as BAC	Same as BAC	Moore and Cripps 2012

individual biocides in laboratory studies (Kramer et al. 2010).

Many methods have been developed to detect synergy between two antimicrobial agents including diffusion test, spiral plating method, checkerboard method, time-kill, E test, etc. However, the checkerboard method and time-kill are most popular (Bonapace et al. 2000; White et al. 1996). In the checkerboard method, the combination of two antimicrobial agents A and B at various concentrations that are all below the minimum inhibition concentration (MIC) for each is tested against test bacteria. The fractional inhibitory concentration index (FICI) is used to evaluate the result, which is defined by: $FICI = [A]/MIC_A + [B]/MIC_B$ (Greene et al. 2006). A and B are synergistic if FICI ≤ 0.5, antagonistic if FICI ≥ 4, indifferent if FICI falls between 0.5 and 4 (Bonapace et al. 2000; White et al. 1996). In a time-kill test, test bacteria are incubated together with a single antimicrobial agent or with a combination of two agents for a specific contact time, the decrease of the bacterial count is measured. A 100-fold (2-log) decrease by the combined agents compared to the most effective agent is defined as synergy. Antagonism is defined as a 2-log increase by the combined agents compared to the most active agent, while indifference is defined as < 10-fold changes in bacterial counts (White et al. 1996). The synergy between two antimicrobial agents depends on the conditions used to study it, including the chosen test bacterium, the type of antimicrobial agents, and the medium.

1.4.2 Nitrate injection

Amendment of injection water with nitrate to control souring is a relatively new method starting from the 1990s (Grigoryan and Voordouw 2008). It is now gaining more and more attention because of the advantages over biocides (Voordouw et al., 2011; Myhr et al. 2002). Nitrate is relatively cheap, environmentally friendly and easy to use. It is water-soluble and does not bind to reservoir rocks, which enables it to penetrate deeper into oil reservoirs. It is also

compatible with other chemicals typically used in the production process (Larsen 2002). Both laboratory studies and pilot field applications have showed great success of using nitrate injection to control souring. Table 1.3 lists several successful examples of oil fields that have been subjected to nitrate injection to control souring.

1.4.2.1 Mechanisms of nitrate injection to control souring

Nitrate injection to control souring works by shifting the microbial community in oil fields away from sulfidogenesis (Bødtker et al. 2008; Thorstenson et al. 2002) and by inhibiting sulfide production. Several mechanisms behind this process are discussed in detail below and graphically indicated in Figure 1.8 (Gieg et al., 2011; Larsen et al., 2004).

Reactions ① and ② in Figure 1.8 show the competitive exclusion. Addition of nitrate stimulates the growth of heterotrophic nitrate reducing bacteria (hNRB) reducing nitrate to nitrite, then to nitrogen or ammonia by using organic hydrocarbons as electron donors. However, oil organics are also electron donors for SRB in oil reservoirs. Thus hNRB and SRB compete for the same nutrients. Since nitrate reduction has a higher reduction potential than sulfate reduction (Davidova et al. 2001), hNRB are able to outcompete SRB for the same electron donors and the growth of SRB is thus suppressed because of nutrient limitation (Giangiacomo and Dennis 1997). However, in actual oil reservoirs electron donors (oil hydrocarbons and organic acids) are usually in excess of electron acceptors, nitrate and sulfate (Davidova et al. 2001). Therefore, it is hard for competitive exclusion to be the main mechanism to control souring, unless the electron donor in the oil reservoir is limited. The Veslefrikk oil field in North Sea, for example, has limited electron donors to support SRB and NRB so that a low concentration of nitrate injection could shift sulfidogenesis completely to nitrate respiration; souring was thus greatly alleviated (Thorstenson et al. 2002).

Table 1.3 Examples of oilfields subjected to nitrate injection to control souring

Oil field	Location	Temperature (℃)	Production started	Water flooding started	Souring started	Nitrate started	Nitrate injection description	Results	Ref.
Coleville	Western Canada	29	-	1958	1958	1996	Continuous injection of potassium nitrate for 50 days	Significant sulfide reduction (40%-100%).	Jenneman et al. 1999
MHGC	Western Canada	30	-	2000	2006	2007	Field-wide nitrate injection was implemented either continuously or in alternating；weekly pulses of low- and high-nitrate concentrations	Pulsed injection of nitrate worked better than continuous injection	Voordouw et al, 2009
Skjold	North Sea	80	1982	1985	1985	2000	Souring resumed within 1–2 days of cessation of nitrate injection	Nitrate injection worked	Larsen 2002
Draugen	North Sea	71	1993	1994	-	-	Continuous injection of 50 mg/L of nitrate in above ground operation	Efficient to control of near-well reservoir souring, but increased corrosion rate	Vik et al, 2007
Halfdan	North Sea	80	1999	2001	-	2001	Nitrate injection to prevent souring	Nitrate injection worked	Larsen et al. 2004
Veslefrikk	North Sea	80	1989	1989	-	1999	Continuous injection of 30 ppm nitrate	20,000 fold decrease in SRB count, 50 fold decrease in SRB activity; corrosion rate reduced from 0.7 to 0.2 mm/year	Thorstenson et al. 2002
Gullfaks	North Sea	70	1986	1986	1990	1999	Continuous addition of 30-40 ppm $Ca(NO_3)_2$	1000-fold decrease in SRB count;10-20 fold decrease in SRB activity; Corrosion rate decreased by 50%	Sunde et al. 2004

Reaction ③ in Figure 1.8 shows sulfide removal by soNRB. Another group of bacteria enhanced by nitrate addition are the sulfide-oxidizing nitrate-reducing bacteria (soNRB). As indicated in Section 1.2.3, they can remove formed sulfide by oxidizing it to sulfate or sulfur while reducing nitrate to nitrite or completely to nitrogen (Greene et al. 2003; Voordouw et al. 2002). The types of products formed depend on the bacteria and initial nitrate/sulfide ratio (Grigoryan and Voordouw 2008). If nitrate is in excess of sulfide, sulfide will be oxidized to sulfate, while nitrate is reduced to nitrite. If sulfide is in excess, nitrate will be completely reduced to N_2, while sulfide is oxidized to sulfur (Gevertz et al. 2000). Since soNRB generate sulfate that is used by SRB, soNRB and SRB can grow symbiotically when electron donors are in excess (Coombe et al. 2004).

Reaction ④ shows intermediate inhibition. Nitrite, the metabolic intermediate of nitrate reduction is an analog of sulfite, having higher affinity to dissimilatory sulfite reductase (Dsr) that is a key enzyme in SRB to catalyze the reduction of sulfite to sulfide (Section 1.2.2) and thus preventing sulfate reduction (Greene et al., 2003; Myhr et al. 2002; Reinsel et al. 1996). Consequently, a good inhibition would be achieved if the reduction of nitrate by NRB stopped at nitrite and did not go all the way to N_2. Unfortunately, some SRB can detoxify nitrite via nitrite reductase (NRF, Figure 1.3), an enzyme able to metabolize nitrite further to ammonia (Haveman et al. 2004; Greene et al. 2003). Additionally, nitric oxide (NO) and nitrous oxide (N_2O), the other intermediates of nitrate reduction, are toxic to SRB and may inhibit SRB activity as well (Davidova et al. 2001).

Reaction ⑤ in Figure 1.8 shows that nitrite can also react with sulfide abiotically, forming sulfur, nitrogen or ammonia (Davidova et al. 2001; Sturman et al. 1999).

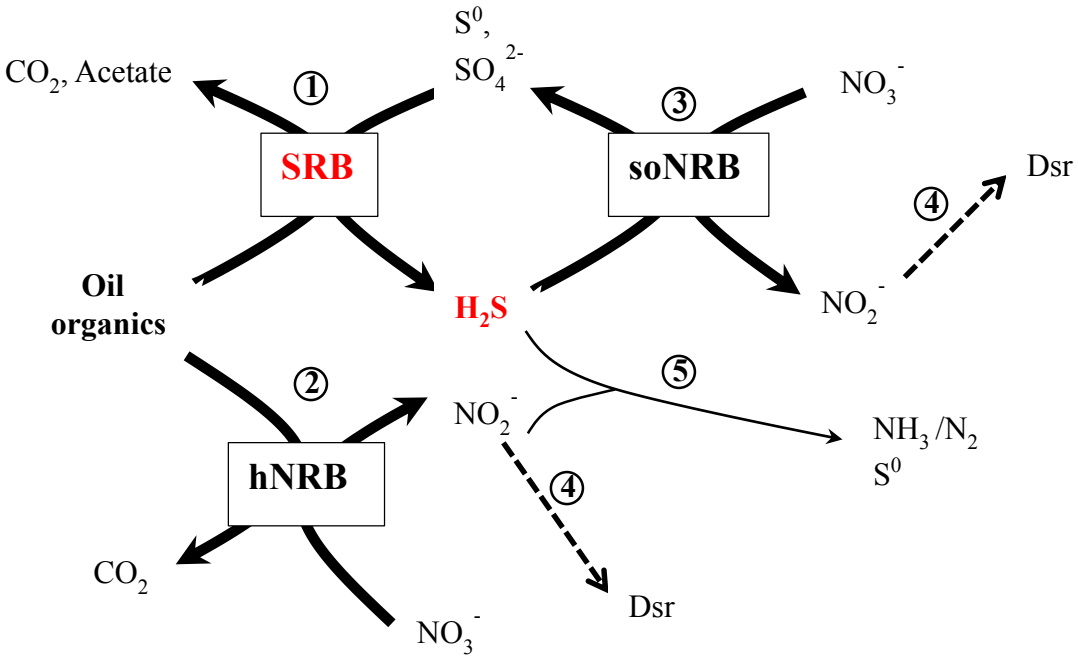


Figure 1.8 Schematic of mechanisms of nitrate addition to control souring. (① and ②) SRB and hNRB compete for the same electron donors, oil organics, and the activities of SRB are inhibited because of the preference of nitrate reduction. (③) soNRB can scavenge the produced sulfide by oxidizing it into sulfate or sulfur while reducing nitrate. (④) Nitrite, the intermediate of nitrate reduction can inhibit dissimilatory sulfite reductase (Dsr). (⑤)The produced sulfide can also chemically react with nitrite. The figure was adapted from (Gieg et al. 2011).

1.4.2.2 The application of nitrite and nitrate

Due to the abundance of electron donors in oil reservoirs, the inhibition of SRB by nitrite seems to be the most significant mechanism in controlling sulfide production based on the fact that nitrite can not only inhibit sulfate reduction, but can also eliminate existing sulfide. Whether nitrate or nitrite must be used to control souring is also an important question (Voordouw, 2003). If only SRB are present with little or no hNRB and soNRB in a reservoir, nitrite is preferred over nitrate to control souring (Davidova et al. 2001; McInerney et al. 1996), because nitrite can inhibit SRB and remove sulfide, whereas nitrate has no effect on SRB. If SRB, hNRB and soNRB are all present in a reservoir, nitrate is preferable due to its easier handling, cheaper cost and higher oxidation power. The overall reaction mediated by these three groups of bacteria is, in essence, the oxidation of oil organics coupled to the reduction of nitrate (Voordouw 2003). The needed dose of nitrate is therefore determined by the concentration of degradable oil organics, which is unfortunately hard to know.

The synergy between nitrite and other chemicals such as biocides and other metabolic inhibitors has been studied as an alternative to enhance sulfide containment. Greene et al. (2006) investigated synergistic inhibition of sulfide production by combining two chemical compounds. The results showed that nitrite is synergistic to all biocides tested except THPS. Nemati et al. (2001) also showed the synergistic effect between nitrite and molybdate, another metabolic inhibitor of sulfate reduction, to control H_2S production. The efficacy of these inhibitors depends on the composition of the microbial community and the metabolic state.

The combination of nitrate and biocides has not been studied yet except by Callbeck (2012), who showed in his research that nitrate was synergistic to BAC but not to other biocides (Glut, acrolein and bronopol). This was explained by their different modes of action. Glut, acrolein and bronopol are chemically-reactive biocides that kill bacteria by irreversible chemical

reaction (e.g. cross-linking), which inactivates the biocide. In contrast, biocides like BAC are physically-reactive biocides (cationic surfactant) that physically interact with bacteria biomass (e.g. adsorbing to biomass membranes via surfactant cations), in which biocides are still active after being released. However, no definitive results on whether these differences are important were obtained and this needs to be further investigated.

1.4.2.3 Problems associated with nitrate injection

In spite of successes of using nitrate to control or prevent souring, problems do exist. Many field studies and laboratory work on low-temperature oil reservoirs have shown that nitrate treatment indeed greatly decreased the concentration of sulfide in the first several weeks, but the sulfide then resumed to the level before treating with nitrate (Callbeck et al., 2011; Voordouw et al. 2009; Myhr et al. 2002). A typical example of this phenomenon has been observed in the Medicine Hat Glauconitic C (MHGC) oil field listed in Table1.3. The theory of microbial zonation was proposed since then.

The MHGC oilfield, located in the Western Canadian Sedimentary Basin near the city of Medicine Hat in South Eastern Alberta, is a shallow and low-temperature oil reservoir at a depth of 850 m below the surface (mbs) and an *in situ* temperature of around 30 ^{o}C. It produces heavy oil with API gravity of 16 degrees without the aid of water injection from the early 1980s to 2000, after which PWRI was initiated to promote oil production with supplemental make-up water coming from a local municipal sewage treatment plant containing 3-4 mM (300-400 ppm) sulfate, that was later diluted by mixing with produced water in a ratio of 1:3 to a final concentration of 0.8-1.2 mM before injection into the reservoir. Deep formation waters with some sulfide but no sulfate were used in a small section of the field. Increasing concentrations of sulfide were first detected in the gas phase as an indicator of souring in 2006, six years after the

initiation of water flooding (Voordouw et al. 2009). Field-wide injection of 2 mM nitrate was then implemented to control sulfide production. Sulfide initially decreased by 70% in the first 7 weeks. However, sulfide gradually increased until it reached the same level as prior to nitrate treatment (Figure 1.9). Pulsed injection of high concentration (760 mM) of nitrate for 1 hour/ week was used as an alternative at a specific injection well from July 2008 to March 2009. Nitrate breakthrough and no sulfide were observed in a neighbouring production well. However, this promising observation for a single well did not cause diminishing sulfide at other wells (Voordouw et al. 2009).

The phenomenon of initial decrease of sulfide in the first 7 weeks followed by rebound was explained by the microbial zonation model (Figure 1.10, I) and confirmed by community analysis in bioreactors conducted by Callbeck et al. (2011). In this model, the oil reservoir is divided into zones. Injection of water containing limiting sulfate into the reservoir results in SRB thriving in the NIWR and the production of sulfide. When nitrate is amended into sulfate-containing injection water to control souring, NRB instead of SRB take over the NIWR forming a nitrate reduction zone because of the higher redox potential of nitrate. The suppression of SRB activity by nitrite causes a decreased sulfide production at first. However, the suppression of SRB is transient and stops once SRB move further to a zone without nitrite inhibition. Because electron donors are in excess of electron acceptors in the MHGC oilfield, sulfide production will be continued in the new sulfate reduction zone, which can give a good explanation of sulfide recovery after week 7. The emergence of microbial spatial arrangement with nitrate reduction in the NIWR followed by a sulfate reduction zone contributes directly to the failure of using nitrate to control souring in low-temperature oil reservoirs. The pulsed injection of higher concentration of nitrate may control souring, possibly because the injected nitrate is able to penetrate to the

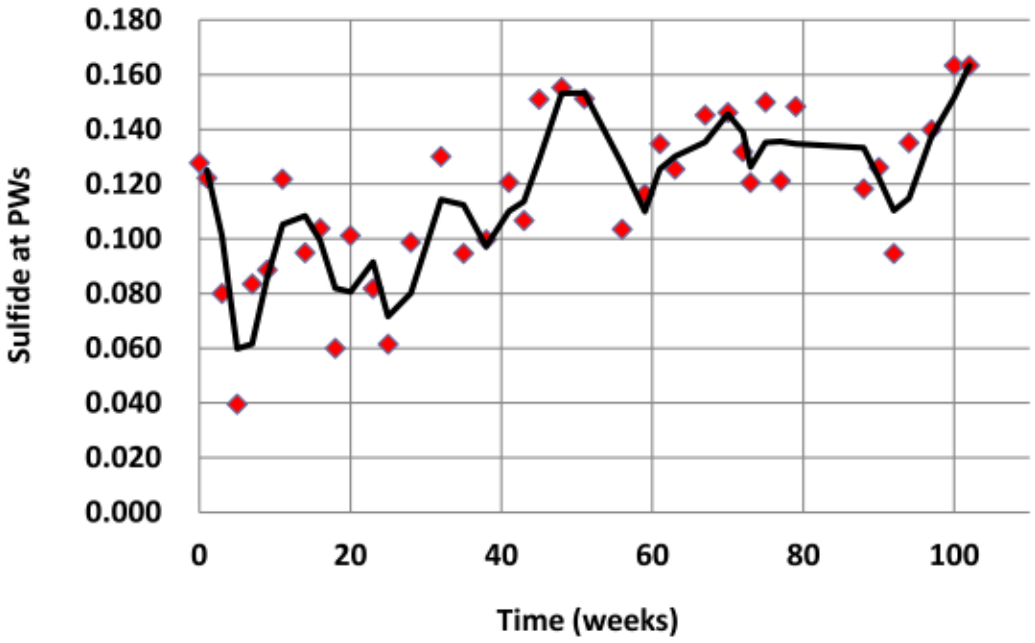


Figure 1.9 Average sulfide concentration from all monitored production wells in the MHGC oilfield (Voordouw et al. 2009).

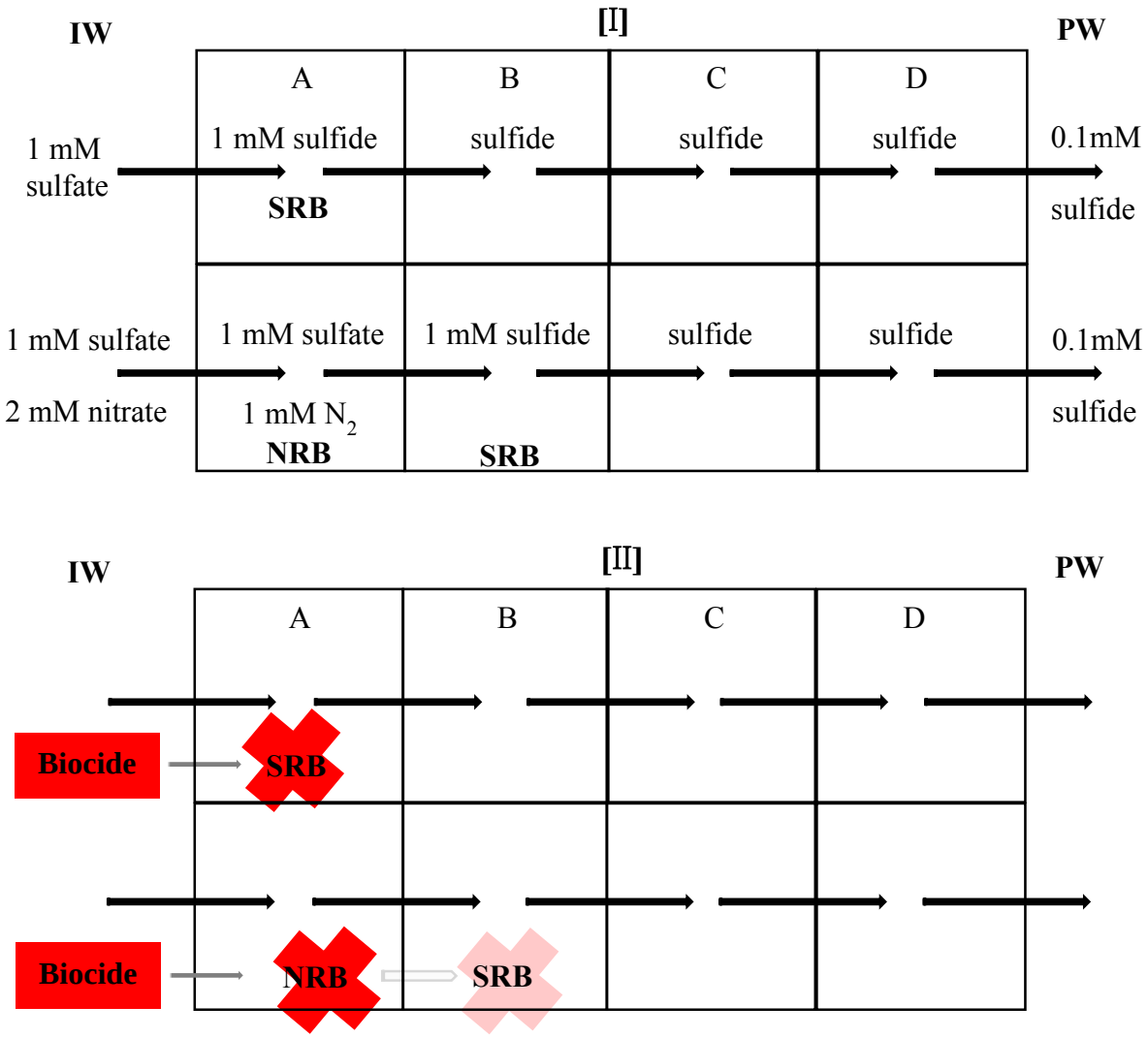


Figure 1.10 Microbial zonation model for low-temperature oil reservoirs (Voordouw et al. 2009). In this model, the reservoir is divided into blocks A, B, C and D. Oil is swept by injection water from A to D. A is the near injection wellbore region (NIWR). [I] (Top) Nitrate is absent, 1 mM sulfate is reduced to 1 mM sulfide at A, resulting in 0.1 mM sulfide in production well because of formation of sulfide precipitates and loss of sulfide to other phases (e.g. oil, and gas) during reservoir transport; (Bottom) Nitrate is present. When nitrate is first applied, there is inhibition of the SRB in A. However, this is only transient and stops once SRB activity is established in B. No souring control is achieved under these conditions. [II] When injected with biocide, (Top) biocides kill SRB immediately; (Bottom) biocides kill NRB first, and then may kill SRB.

deeper sulfate reduction zone (Voordouw et al. 2009).

Fortunately, high-temperature oil reservoirs (60 - 80 ^{o}C) may not have this problem. It is known that all viable SRB are limited to the NIWR where the high temperature is cooled down by continuous injection of colder water. It is easier to inhibit these SRB by treating with nitrate than in low-temperature oil reservoirs (Voordouw et al. 2009), because SRB cannot re-establish a sulfate reduction zone deeper in the reservoir where the temperature is too high to support SRB growth. Whether high temperature prevents microbial zonation depends on the size of the mixing zone with temperature gradients that was described in Section 1.3.2 (Figure 1.5). If the mixing zone extends far enough to allow SRB growth, then even high-temperature oil reservoirs can suffer nitrate injection failure because of microbial zonation.

In spite of the success of nitrate, its application is complex and success is variable. In addition to possible failure caused by microbial zonation at low temperature, nitrate applications may fail if the injection water contains high concentrations of organic acids such as acetate (Jones et al. 2011). Some studies have shown that the addition of nitrate may enhance corrosion and cause plugging due to biomass (Rempel et al. 2006; Voordouw et al. 2002). Furthermore, the presence of nitrate does not directly kill SRB like biocides (Coombe et al. 2004). SRB will be reactivated and cause souring once the conditions are favourable.

In addition to biocide and nitrate addition, there are some other approaches to eliminate souring. Because the sulfate concentration in the makeup water is the main cause of souring, some companies have installed filters or use reverse osmosis to remove sulfate from the injection water. Alternatively, the produced water can be treated with sulfide scavengers, like amines, sodium hydroxide and vapor recovery units to remove H_2S. Different approaches to control souring are summarized in Table 1.1.

1.5 Methods to study souring control

SRB are the main cause of biological souring. Studies of souring control typically involve culture of SRB, treatments with chemicals (e.g. nitrate, nitrite, biocides), or altering nutrients to inhibit SRB growth. In other words, methods to study souring control are based on methods to culture SRB. Three ways of culturing SRB are widely used in laboratory studies.

1.5.1 Batch cultures in serum bottles

Batch cultures in serum bottles are a basic tool to study anaerobes. Butyl rubber stopper and aluminum crimps are usually used to seal the bottles to create an airtight microcosm. Flushing with oxygen-free gas makes the bottles anaerobic. For example, to measure the minimum inhibitory concentration (MIC) of a biocide for SRB, a known starting concentration of SRB (either a pure culture or an SRB consortium enriched from oil field produced water) and biocide are added together. After a certain time of incubation, the number or activity of the remaining SRB is recorded and then plotted against the concentration of used biocides. Based on this graph, the MIC of the given biocide to SRB can be obtained.

Cells growing in serum bottle microcosms are planktonic cells, which are most sensitive to antimicrobial agents, because they have a larger contact surface to be targeted.

1.5.2 Robbins device or equivalent

It is generally thought that SRB grow in oil reservoirs in the form of biofilms. Cells in biofilms have different physical and chemical characteristics from planktonic cells (Gieg et al., 2011). For instance, cells in biofilms have more resistance to antimicrobial agents like biocides (Ganzer et al. 2001). Therefore, in order to simulate actual oil reservoirs, bioreactors with a solid matrix allowing the formation of sessile cells are used regularly to study souring control rather

than serum bottles that culture enrichments of planktonic cells (Callbeck et al., 2011; Hubert et al., 2003; Myhr et al. 2002; Reinsel et al., 1996).

The Robbins device or equivalent provides a surface by inserting coupons or studs into a sealed column or chamber (Figure 1.11) for SRB to attach to forming biofilms. Medium flow through the chamber creates a controlled hydrodynamic condition (McGinley and Van Der Kraan 2013). Because the biofilm is more resistant, the MIC measured with the Robbins device or equivalent is expected to be higher than the MIC obtained in serum bottle tests (Yin et al. 2012). In addition, samples at different ports can be collected to allow the study of SRB or souring in different spatial positions.

1.5.3 Sand-packed columns

Even though the Robbins device or equivalent allows the formation of biofilms, the quantity and thickness of biofilm is still substantially less than that in actual oil reservoirs where rocks or packed sands provide much larger surface areas than the flat surfaces in the Robbins device or equivalent. Therefore, sand-packed columns (Figure 1.12) mimic reservoirs more closely, the development of a larger quantity of SRB biofilm is thus expected (Grobe and Stewart 2000). Chen et al. (1994) compared the amount of biomass in liquid phase (planktonic cells) with the biomass attached to the solid sand (biofilm) from an up-flow packed-bed bioreactor system, finding approximately four orders of magnitude more biomass in the latter phase based on dry weight. Therefore, we expect that the MIC measured with sand-packed columns is much higher than that measured by the use of serum bottles or a Robbins device.

Many studies of souring control are based on sand-packed columns. Myhr et al. (2002) used a Perspex column (10 ×192 cm) simulating the Gullfaks oilfield in the North Sea to prove the feasibility of using lower concentration of nitrate to eliminate sulfide production. This was

Figure 1.11 Robbins device used to establish SRB biofilms in souring control studies. (Pictures are from Tyler Research Corporation: http://www.tylerresearch.com**)**

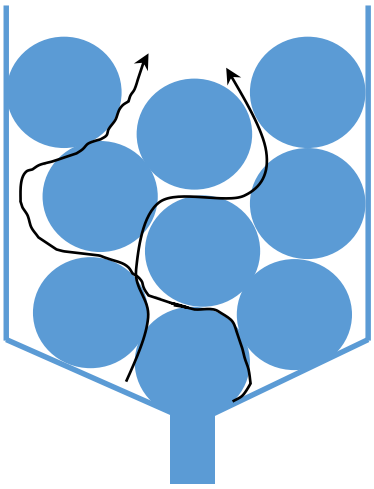


Figure 1.12 Schematic of sand distribution and space between sand grains in a sand-packed column.

then employed in the Gullfaks and Veslefrikk fields to replace glutaraldehyde treatment as a souring control strategy (Bødtker et al. 2008). Working with a sand-packed high pressure bioreactor column, Jones et al. (2011) claimed that continuous injection of a low concentration of third generation THPS (Jones et al. 2010) could be used as an alternative to control souring in an oil reservoir with lower permeability and a bigger mixing zone (Figure 1.5).

1.6 Techniques used to measure souring control

SRB activities can be monitored by the amount of products they generate or substrates they consume, or even by direct bacteria count. As molecular techniques advance, substantial culture-independent methods have been developed and employed to determine SRB activity and numbers in different samples at different time points. The choice of techniques depends on proximity, availability and allowable expenses.

1.6.1 Chemical analysis

SRB conduct sulfate reduction and organic matter oxidation, so that the rate of product (H_2S) generation and substrate consumption can definitely be used to represent the activities of SRB. Activity is usually expressed based on chemical concentration in mM/day or ppm/day or in the $\mu g/cm^2/d$ (Sunde et al. 2004).

1.6.2 MPN

Viable counts of SRB are commonly determined by the "most probable number" (MPN) method using a selective medium (e.g. lactate and sulfate) containing Fe^{2+} used as an indicator of positive growth of SRB when blackening occurs after 4 weeks incubation (Thorstenson et al. 2002). NRB and APB (acid producing bacteria) can also be enumerated by MPN, but using

different media and different indicators. For instance, mineral medium containing yeast extract and thiosulfate as electron donors was used by Davidova et al. (2001) for NRB counts with more than 10% nitrate consumption being scored as positive. In spite of feasibility of counting these bacteria, the enumeration of NRB is not as frequent as that of SRB and of APB in analysing oil field waters. The count of bacteria allows us to gain direct information. However, there is not always a correlation between bacterial count and activity. For example, samples taken from the Marion Lake field (Alberta, Canada) and an Oklahoma field had the same SRB count, 2.5×10^3 ml^{-1}, but the SRB activity from the latter was an order of magnitude higher than from the former (Davidova et al. 2001). In spite of this, the MPN method is useful for biocide studies on the size of microbial populations.

The MPN method enumerates bacteria based on culture and usually takes up to a month to obtain results. Besides the MPN method, other methods can be also used to count SRB such as the double staining fluorescent antibody technique (FA) (Voordouw 2011; Sunde et al. 2004; Myhr et al. 2002; Thorstenson et al. 2002).

1.6.3 Culture-independent methods

Culture-independent methods include 454 pyrosequencing to analyze microbial community compositions, Fluorescence In Situ Hybidization (FISH) counting of bacteria and qPCR of 16S rRNA genes or of functional genes (Augustinovic et al. 2012). Due to the culture-free characteristics, a shorter time is needed to get results and a broad-spectrum of bacteria can be detected. Its development has substantially improved the understanding of microbiology, including of petroleum microbiology.

Chapter 2: **Hypothesis and Objectives**

It is known that continuous amendment of sulfate-containing injection water with nitrate can lead to microbial zonation as in the MHGC oilfield, where nitrate reduction occurs in the near injection wellbore region followed by sulfate reduction. Once microbial zonation has been established, nitrate is no longer effective in removing sulfide. Disturbing zonation is therefore a key to achieve souring control in low-temperature oil reservoirs. Even though biocides are rarely injected into actual oil reservoirs to control souring, the possibility that a combination of biocide and nitrate can successfully break zonation and lead to more effective souring control needs to be investigated. The overall hypothesis of this work, therefore, is that combination of nitrate and biocide can lead to more effective souring control by breaking microbial zonation. Up-flow packed-bed bioreactors are going to be employed as the major tool to closely simulate souring in a zoned system.

To validate or refute this hypothesis, several specific objectives are defined as indicated below:

Objective 1: Mimic the MHGC oilfield by sand-packed columns and run them under appropriate conditions;

Objective 2: Sour columns and pulse with biocides to investigate the effect of pulsed biocides without nitrate on souring control;

Objective 3: Continuously inject sour columns with nitrate to establish microbial zonation and investigate the additional effect of pulsed biocides on souring control;

Objective 4: Determine whether synergy between pulsed biocides and continuous nitrate exists by comparing the results of (2) and (3).

In addition to the overall hypothesis that the combination of nitrate and biocide may be synergistic, we also hypothesize that the synergy applies to physically-reactive biocides but not to chemically-reactive biocides based on previous studies done by (Callbeck 2012).

When biocide is injected into a low-temperature reservoir under PWRI with continuous nitrate injection (nitrate and sulfate both present), the biocide will interact with NRB biomass first because of its upstream location (Figure 1.10 bottom). For chemically-reactive biocides, NRB may act like a shield protecting SRB by inactivating the biocide before it goes deeper into the sulfate reduction zone. Thus it takes a shorter time for SRB to re-establish activities after the cease of biocide treatment than the condition where only SRB inhabit the NIWR (no nitrate addition). For physically-reactive biocides, the NRB may act like a sponge by holding biocides via adsorption and releasing them later, which increases the biocide treatment time on SRB. Thus, a longer sulfide recovery time may be observed than in the absence of NRB. Hence a further refinement of the hypothesis is that physically-reactive biocides will work synergistically with nitrate to control souring, but this will not occur with chemically-reactive biocides.

Chapter 3: **Materials and Methods**

3.1 Samples collection

Produced water samples were collected from the MHGC oilfield that has been discussed in Section 1.4.2.3 with 1-L Nalgene plastic bottles sterilized before use. Bottles were filled to the brim to exclude air and were capped tightly. Samples were transported to the lab within 5 hours and stored in the anaerobic hood (Coy laboratory products, Inc) filled up with 90% N_2 and 10% CO_2 at room temperature.

3.2 Media and enrichment cultures

Modified Coleville synthetic brine (CSB) medium, CSBA (Callbeck et al., 2011; Hubert et al. 2003) containing 2 mM sulfate (CSBA-S) was used as the basic medium in this research. The detailed components are listed in Table 3.1. Sulfate was used at a concentration of 2 mM instead, higher than the 1 mM in the MHGC oilfield for ease of analysis. CSBA-SN medium was CSBA-S medium amended with 0.17 g/L (2 mM) of sodium nitrate. Reduction of 2 mM nitrate will give maximally 1 mM N_2.

VFA were used as electron donors, because they are often present in produced waters and are easier to work with than crude oil. The preference order for SRB to utilize VFA is propionate> butyrate> acetate (Callbeck et al. 2013). SRB from the MHGC oilfield do not use acetate (Callbeck 2012; Grigoryan et al. 2008). Therefore the calculation was based on propionate and butyrate only. NRB are also able to reduce nitrate completely to N_2 by using all constituents of VFA (Callbeck et al. 2011). The calculation for NRB was therefore, based on all components of VFA and complete reduction of nitrate to N_2. Consequently, 1.6 mM VFA (1.6 mM propionate and 1.6 mM butyrate) can reduce 2 mM sulfate (Table 3.4; equation 1 and 2),

Table 3.1 Components of CSBA-S medium used in this research

(adapted from Callbeck 2012)

components	amount per L
NaCl	7.0 g
KH_2PO_4	0.2 g
NH_4Cl	0.25 g
$CaCl_2 \cdot 2H_2O$	0.15 g
$MgCl_2 \cdot 6H_2O$	0.4 g
KCl	0.5 g
[1] Na_2SO_4	0.28 g
Resazurin	100 µL
Added after autoclaving	
$NaHCO_3$ (1 M)	30 mL
[2] Trace element stock	1 mL
[3] Selenate/tungstate stock	1 mL
[4] VFA (1 M)	3 mL
Adjust pH to 7.4-7.6	

[1] equals 2 mM;

[2]Trace element stock: refer to Table 3.2;

[3] Selenate/tungstate stock: refer to Table 3.3;

[4] VFA (1 M) contains 1 M each of acetate, propionate and butyrate.

Table 3.2 Trace element stock solution (adapted from Callbeck 2012)

components	amount per L
Na_2EDTA	5.20 g
$FeSO_4 \cdot 7H_2O$	2.10 g
H_3BO_3	30.0 mg
$MnCl_2 \cdot 4H_2O$	100 mg
$CoCl_2 \cdot 6H_2O$	190 mg
$NiCl_2 \cdot 6H_2O$	24.0 mg
$CuSO_4 \cdot 5H_2O$	3.00 mg
$ZnCl_2$	68.0 mg
$NaMoO_4 \cdot H_2O$	36.0 mg
2M HCl	adjust to pH 7.0

Table 3.3 Selenate/tungstate stock solution (adapted from Callbeck 2012)

components	amount per L
NaOH	400 mg
$Na_2Se_2O_3 \cdot 5H_2O$ (Na Selenite)	6.0 mg
$Na_2WO_4 \cdot H_2O$ (Na Tungstate)	8.0 mg

while 0.24 mM VFA (0.24 mM of each acetate, propionate and butyrate) was enough for 2 mM nitrate reduction (Table 3.4; equation 3, 4 and 5). In total, 1.84 mM VFA is required for 2 mM nitrate and 2 mM sulfate reduction. Since excess oil electron donors are present in MHGC oilfield (Callbeck et al. 2013; Callbeck et al. 2011), 3 mM VFA that was in excess of 2 mM nitrate and 2 mM sulfate was used as the final concentration in this research. All media were gassed with 90% of N_2 and 10% CO_2 (N_2-CO_2) to make them anaerobic before use. Different types and concentrations of biocides were added into medium based on different experimental objectives.

An SRB enrichment was made by adding 10% of produced water from the MHGC oil field into 100 ml of CSBA medium amended with 10 mM sulfate and 8 mM VFA in a 150 mL serum bottle sparged with N_2-CO_2 and sealed with a rubber stopper and an aluminum crimp (Callbeck et al. 2011). Incubation of this enrichment was at 30 ^oC in a shaker. The culture was transferred to a new medium bottle when the culture turned turbid and most of sulfate was reduced. After two transfers, the enrichment was ready for inoculation of bioreactor columns.

3.3 Bioreactor setup and establishment of SRB biofilms

Plastic or glass syringe columns without piston were used as bioreactor columns, packed tightly with sand (Sigma-Aldrich, 50-70 mesh particle size) with a thin layer of polymeric mesh (~ 3 mm) and glass wool (~1 mm) at the bottom and another layer of glass wool on the top (~ 1 mm) to contain the sand (Callbeck et al. 2011; Hubert et al. 2003). A rubber stopper with size of 5 was used to seal the column with a hole drilled to allow the effluent to flow out of column. Zip ties were used on the outside to enhance the seal. Two three-way Luer-Lock valves were placed close to the influent and effluent stream so that samples could be taken for chemical analysis. Materials used to set up this bioreactor column and a finished packed column are shown in Figure 3.1. The

Table 3.4 Chemical reactions of VFA and sulfate or nitrate

SO_4^{2-}	Propionate	$4C_3H_5O_2^- + 3SO_4^{2-} \rightarrow 4C_2H_3O_2^- + 3S^{2-} + 4\ CO_2 + 4H_2O$	(1)
	Butyrate	$2C_4H_7O_2^- + SO_4^{2-} \rightarrow 4C_2H_3O_2^- + S^{2-} + 2H^+$	(2)
NO_3^-	Acetate	$5C_2H_3O_2^- + 8NO_3^- \rightarrow 10CO_2 + 4N_2 + H_2O + 13OH^-$	(3)
	Propionate	$5C_3H_5O_2^- + 14NO_3^- \rightarrow 15CO_2 + 7N_2 + 19OH^- + 3H_2O$	(4)
	Butyrate	$5C_4H_7O_2^- + 20NO_3^- \rightarrow 20CO_2 + 10N_2 + 5H_2O + 25OH^-$	(5)

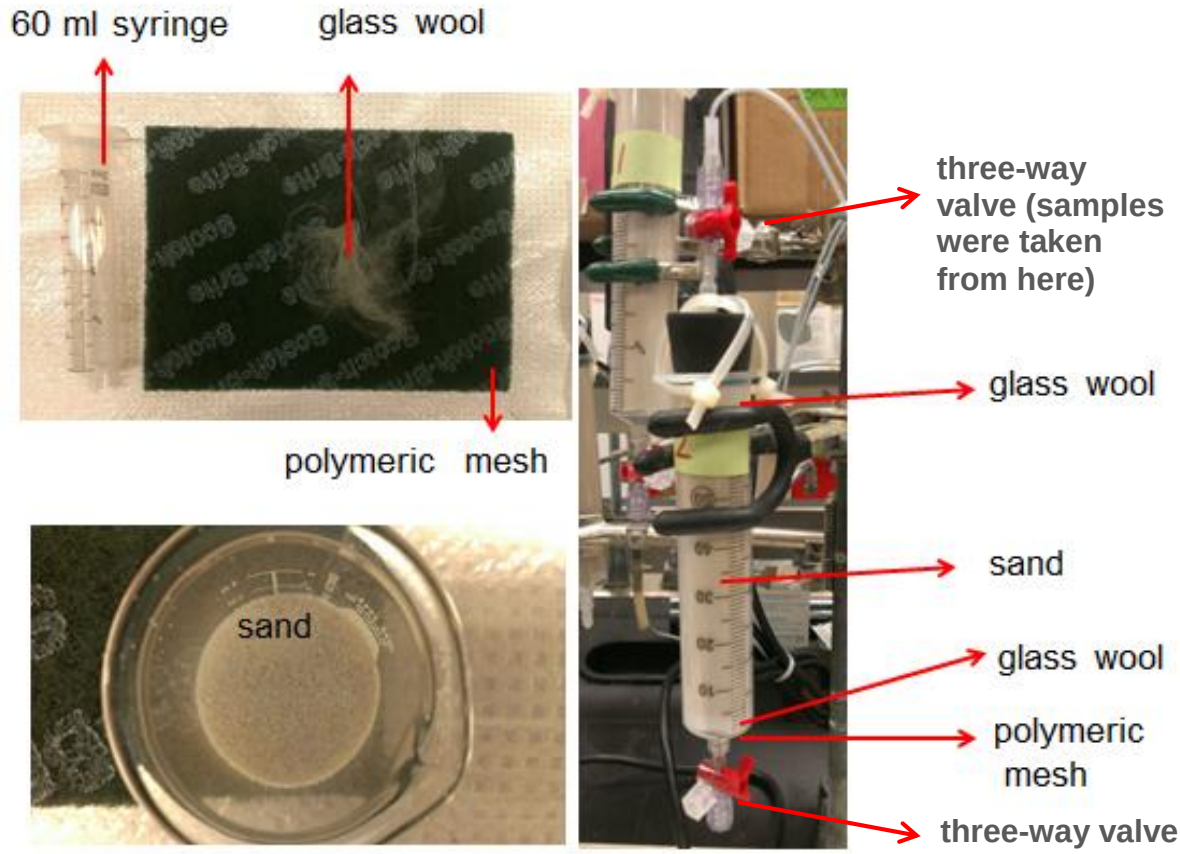


Figure 3.1 Bioreactor setup materials and dry bioreactor column. The inoculum was injected into the column through the bottom three-way valve, while samples were taken from the effluent three-way valve.

packed dry column was autoclaved. An effluent container was used to collect the effluent flowing out through the perforated rubber stopper. A peristaltic multichannel pump (Gilson Inc., Minipuls-3, 8-channel head) was used to deliver medium or water to the column. The whole system is indicated in Figure 3.2. The pore volume (PV) of the packed column, the fraction of the void volume over the total volume of the column, was determined by the weight difference between the column injected with sterilized water and the dry column using the following formula:

$$PV = \frac{m_{\text{wet column}} - m_{\text{dry column}}}{\rho_{H2O}}$$

where ρH_2O is the density of water (1 g/ cm^3) at room temperature, m is the weight of the column (g).

After the column was flooded with CSBA-S medium amended with 10 mM sulfate and 8 mM VFA, half of a pore volume of SRB enrichment (Section 3.2) was inoculated into the bioreactor column through the inlet three-way valve. The column was then incubated in the anaerobic hood to establish SRB activity without injection of medium for a couple of weeks until most of sulfate was reduced to sulfide. Continuous injection of CSBA-S medium at a low flow rate (e.g. 1/4 PV/day, the typical flow rate is 1 PV/day for the operation of oilfield-simulating bioreactors) was then performed outside the hood (Hubert et al. 2003). The flow rate was then progressively increased to allow SRB to attach to the sand matrix and establish biofilms. Otherwise, the planktonic SRB cells would be flushed out of the column. After another couple of weeks the flow rate was raised to the desired level (e.g. 3 PV/day or 6 PV/day) and the bioreactor was kept at this desired flow rate until the bioreactor consistently produced 2 mM sulfide. The bioreactor was then injected with biocides and/or nitrate to collect sulfide recovery times.

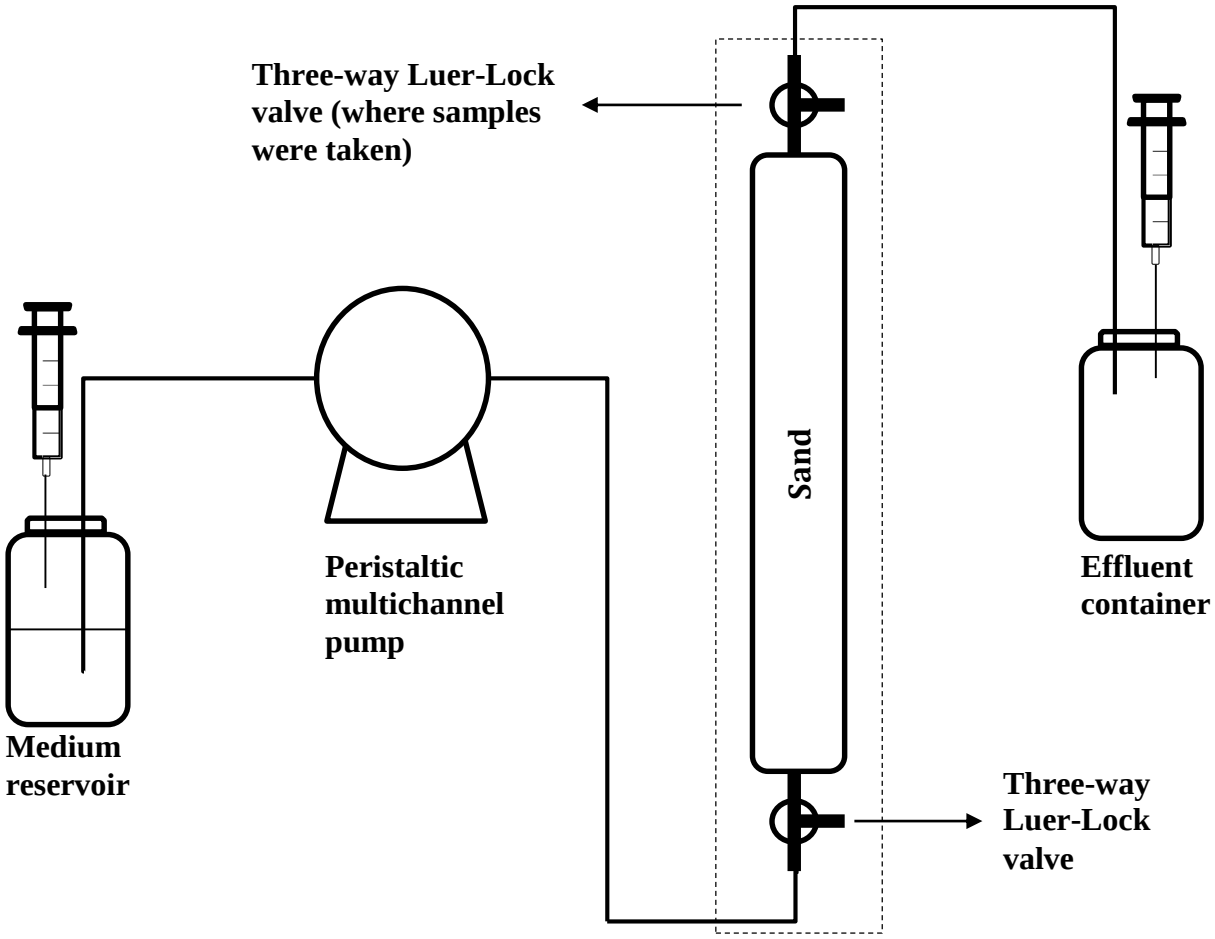


Figure 3.2 Schematic of up-flow sand-packed bioreactor system modeling MHGC oilfield subjected to water flooding. The tubing used to construct the flow path was Gilson PVC connecting tubing with the size of 0.76 mm OD. The tubing going through the pump was Gilson PVC MP pump tubing with the same size as connecting tubing. A 60 ml syringe containing 90% N_2 and 10% CO_2 was used in the medium reservoir bottle and an empty syringe was used in the effluent container respectively to balance pressure and maintain an anaerobic environment. The column in the dashed box was packed up with the materials in Figure 3.1.

3.4 Chemical analysis

A volume of ~ 0.5 mL samples were taken from the effluent three-way valve to assay sulfate, sulfide, nitrate and nitrite.

Since the pH of effluent samples was around 8.4 where aqueous sulfide (HS^- and S^{2-}) were more than 90% of the total sulfide species (HS^- and S^{2-} and H_2S) (Figure 3.3), the total sulfide concentration was determined by the aqueous sulfide concentration. The concentration of aqueous sulfide was measured using the methylene blue method (Truper and Schlegel 1964). For this method, an aliquot of 17 µL sample was added into 200 µL zinc acetate solution (1 mL of 20% acetic acid added to 24 g $Zn(CH_3COO)_2 \cdot 2H_2O$ in a volume to 1000 mL water) immediately after a 0.5 mL of sample was taken from the effluent. To this solution, 600 µL of Milli-Q water, 200 µL of diamine reagent (2 g N, N-dimethyl-*p*-phenylenediamine dissolved into 600 mL distilled water pre mixed with 200 ml H_2SO_4 concentrate, adjusted to 1000 mL) and 10 µL of iron aluminum solution (2 mL H_2SO_4 concentrate added to 10g $NH_4Fe(SO_4)_2 \cdot 12H_2O$, adjusted to 100 mL) were added in and mixed by vortexing. After 10 minutes the absorbance was measured by a spectrophotometer (Thermo Scientific GENESYS 20) at 670 nm wave length against the blank made using 17 µL of water. The result was calculated from a standard curve that was made from a series of sulfide standard solutions. The standards were diluted from 1 M sulfide stock solution (240.18 g Na_2S in 1 L anaerobic deionized water) by using anaerobic deionized water.

The remained samples were stored in a -20 ºC freezer after centrifugation (13,000 rpm, 5 min) until high-performance liquid chromatography (HPLC) was available to measure the concentration of sulfate, nitrate and nitrite. Sulfate was analyzed by the HPLC using a conductivity detector (Waters 423) and an IC-PAK anion column (4 x 150 mm, Waters) with acetronitritle buffer at a flow rate of 2 mL/min. Nitrite and nitrate were detected by the HPLC using an UV detector (UV/VIS-2489, Waters) with the absorbance at 220 nm. The column used was IC-PAK

Anion HC (150×4.6 mm, Waters). Acetonitrile buffer was used and run at a flow rate of 2.0 mL/min. The results were also calculated from standard curves that were made from a series of standard solutions. The standards were diluted from 1 M sulfate, nitrate and nitrite mixture stock solutions.

3.5 Biocides

Five biocides were used in this research: glutaraldehyde (Glut), benzalkonium chloride (ADBAC or BAC), blend of glutaraldehyde and benzalkonium chloride (Glut/BAC), THPS and cocodiamine. Glut/BAC, THPS and cocodiamine were provided by the collaborating companies. Glutaraldehyde and BAC were purchased from Sigma-Aldrich and ICN, respectively. Glut, THPS and Glut/BAC were stored at room temperature sealed with special tapes. Cocodiamine was placed in the cold room in a plastic bottle. BAC was stored at room temperature. The detailed information of these biocides is listed in Table 3.5. All biocides were added into medium directly from these products except for BAC when it was pulsed for 5 days at low concentrations. In this case, a stock solution of 50 mM of BAC was made by dissolving 3.6 g BAC into 200 mL Milli-Q water, from which BAC was amended into medium to the desired concentration. The detailed amount of each biocide that was added into medium is listed in Tables 4.2 and 5.2.

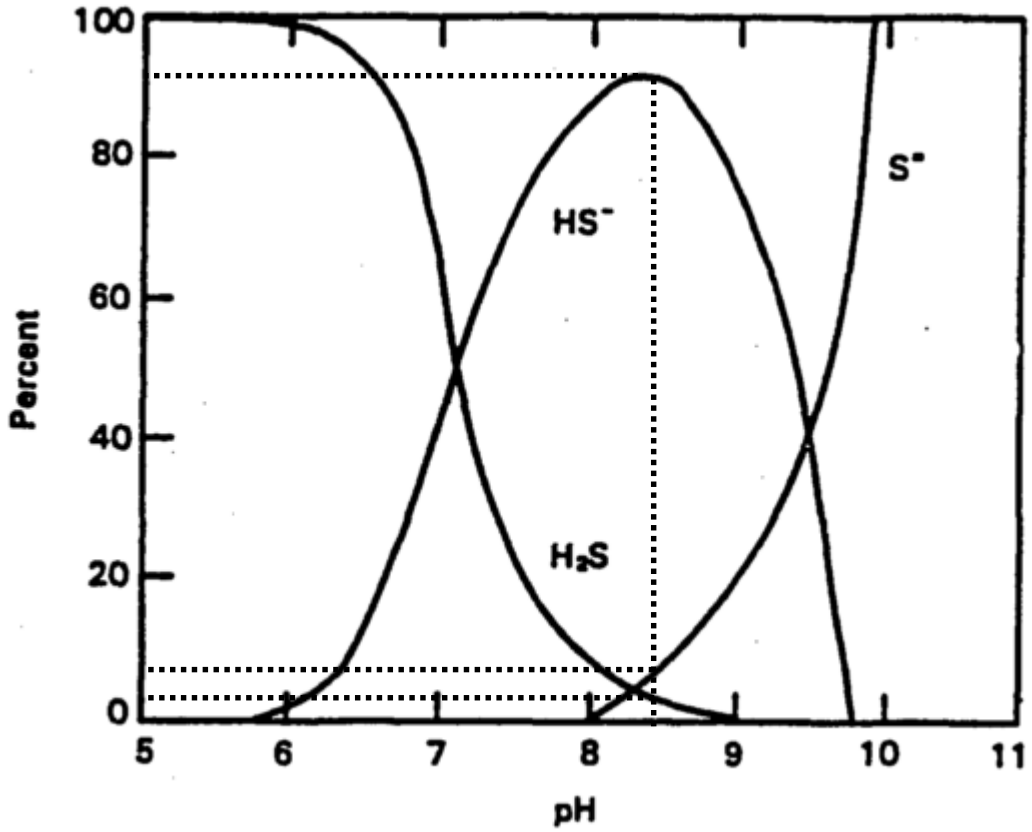


Figure 3.3 Sulfide species distribution in different pH systems (adapted from Hao et al. 1996).

Table 3.5 Information of biocides used in this research

Company	Product #	Batch #	Active ingredient	Active ingredient %	Other compounds
Sigma-Aldrich	G6403	069K1528	Glutaraldehyde	50	Methanol (0.5%)
ICN	6374E	190158.	BAC	99.8*	N/A
DOW	Aqucar GA 42	00266295	Glut + ADBAC	42.5 + 7.5	Ethanol, Methanol
DOW	Aqucar PS 75	300475	THPS	76.5	N/A
Baker Hughes	XC 424	N/A	Cocodiamine	30	N/A

*All products are liquid except BAC. BAC is solid powder;
"N/A" means not applicable, we did not have access to the information, because it is confidential to the company.

Chapter 4: **Biocides Pulsed for 5 Days to Control Souring**

4.1 Introduction

Combination of two chemicals including two types of biocides (Section 1.4.1.2), a biocide and a metabolic inhibitor, or two types of metabolic inhibitors (Section 1.4.2.2) has been studied to mitigate souring. However, souring control by a combination of nitrate and biocide has only been studied by Callbeck (2012) who used pulsed biocide together with continuous or pulsed nitrate to control sulfide production. Interestingly, he found that nitrate was synergistic with BAC but not with Glut, acrolein or bronopol. This suggested that the nature of biocides (e.g. chemically-reactive biocides or physically-reactive biocides) may contribute to the synergy with continuous nitrate addition. However, in this research the SRB enrichment was re-injected into the column after each pulse of BAC to recover sulfate reduction, which made it not comparable to other biocide treatments. The pulse of BAC was implemented before sulfide had recovered to 2 mM, and BAC contact times were not constant for different pulses. More research on combining nitrate and biocide is therefore needed.

Biocide pulses of 5 days were given in this chapter to correspond with Callbeck's experiment. These were pulses of long duration and low concentration (L-L). Biocides are usually dosed in a pattern defined by concentration, duration (or contact time) and frequency (Jones et al. 2010; Maxwell 2005), for example, 200 ppm for 7 hours every week. In this chapter only sulfide recovery times were recorded following biocide treatment of long duration, low concentration (L-L). The treatment of short duration at high concentration (S-H) will be presented in Chapter 5.

4.2 Materials and methods

4.2.1 Medium, SRB enrichment, bioreactor setup, establishment of biofilm and chemical analysis

Bioreactors were set up following the method indicated in Section 3.3. Plastic syringes (60 mL) without piston were used as bioreactor columns. The medium, SRB enrichment and the chemical analyses used were as described in Sections 3.2 and 3.4.

4.2.2 Biocide and nitrate injection

CSBA-S medium containing 10 mM sulfate and 8 mM VFA was used initially to inject into columns, then the columns were inoculated with SRB enrichment. The columns were incubated for a couple of weeks without medium flowing through until most of sulfate had been reduced. CSBA-S medium containing 2 mM sulfate and 3 mM VFA was pumped into the columns progressively to develop SRB biofilms and souring. When columns consistently produced 2 mM sulfide, a certain concentration of biocide (Table 4.2), the products provided by the companies (Table 3.5), was amended into 500 mL medium and injected into the bioreactors for 5 days (treatment time TT=5 days). Biocide concentration was indicated as ppm of the active ingredient (e.g. 400 ppm Glut). Biocide treatment was stopped 5 days later by switching back to CSBA-S medium without biocide. SRB recovery time, determined by the rebound of sulfide concentration to 1 mM, was quantified in this research. The next pulse was initiated after the sulfide recovered to 2 mM and was maintained at that level for at least 4 days to ensure the complete recovery of SRB activity. A theoretical graph in Figure 4.1 is given to help illustrate this process.

Continuous injection of CSBA-SN medium containing 2 mM sulfate and 2 mM nitrate was used to establish microbial zonation by nitrate addition to control sulfide production. The

nitrate concentration in CSBA-SN medium could be increased to 4, 8 or 13 mM, to test the effect of different concentrations of nitrate on souring control. Once 2 mM sulfide was continuously produced in the effluent and maintained for at least four days, biocides were amended into 500 mL medium to start biocide treatment, which was stopped by switching back to CSBA-SN medium without biocide. The next pulse was initiated after sulfide had recovered to 2 mM for at least 4 days.

4.3 Results

4.3.1 Bioreactors parameters

Four bioreactors BV0, BV1, BV2 and BV3 (BV refers to bioreactors with VFA as electron donors) were set up and run in parallel. Bioreactor parameters are listed in Table 4.1. These parameters will apply regardless of the production of sulfide.

4.3.2 Effect of 5-day pulse of biocide on souring control in the absence of nitrate

BV0 injected with CSBA-S medium only containing 2 mM sulfate and 3 mM VFA without biocide and nitrate served as the control, whereas BV1, BV2 and BV3 injected with CSBA-S medium containing 2 mM sulfate and 3 mM VFA were pulsed with Glut, BAC and cocodiamine for 5 days, respectively. The effect of biocide treatment on effluent sulfide concentration is seen in Figure 4.2.

The control column BV0 continuously produced sulfide in the effluent from day 22 onwards (Figure 4.2, A). On average, the produced sulfide was (1.78 ± 0.11) mM, 89% of initial 2 mM sulfate reduced, which means 11% of sulfide was lost from the S-balance. It was possibly due to the formation of iron sulfide precipitate and sulfide gas released from the aqueous phase during transportation.

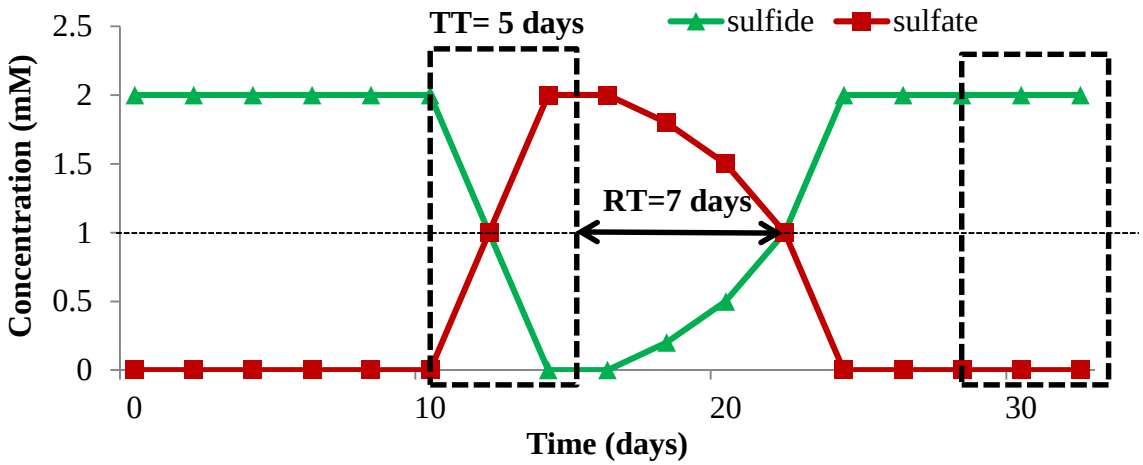


Figure 4.1 A theoretical graph to show the analysis of the biocide treatment. Red line with squares is the sulfate concentrations. Green line with triangles is the sulfide concentrations. Dashed boxes represent the biocide treatment time (TT), which is 5 days. Sulfide recovery time (RT) is 7 days.

Table 4.1 Typical parameters of 60 mL syringe bioreactors

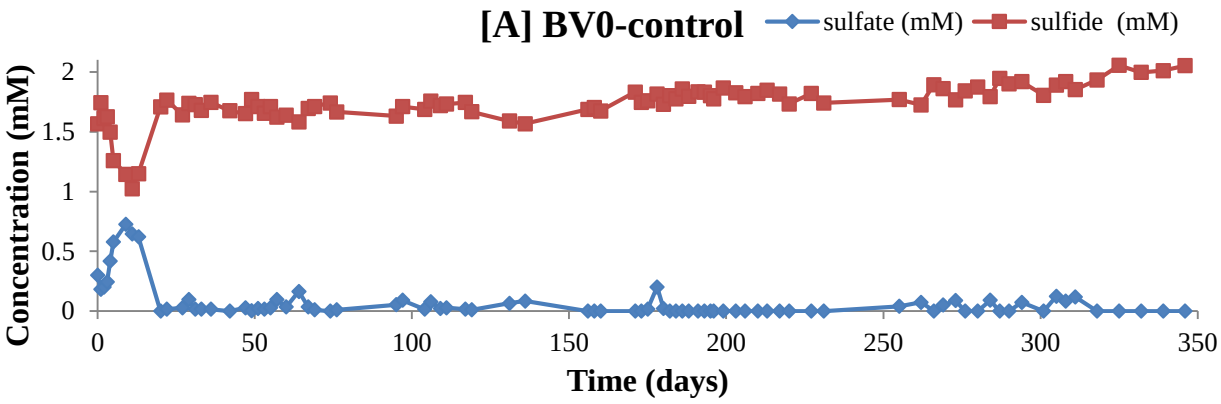
Column size L*D (cm)	Pore volume (PV, mL)	Total volume V (mL)	Porosity (%)	Flow rate (mL/h)	Retention time (h)	Velocity (cm/h)
12.2*2.7	25.7	69.8	36.8	3.7	6.9	1.8

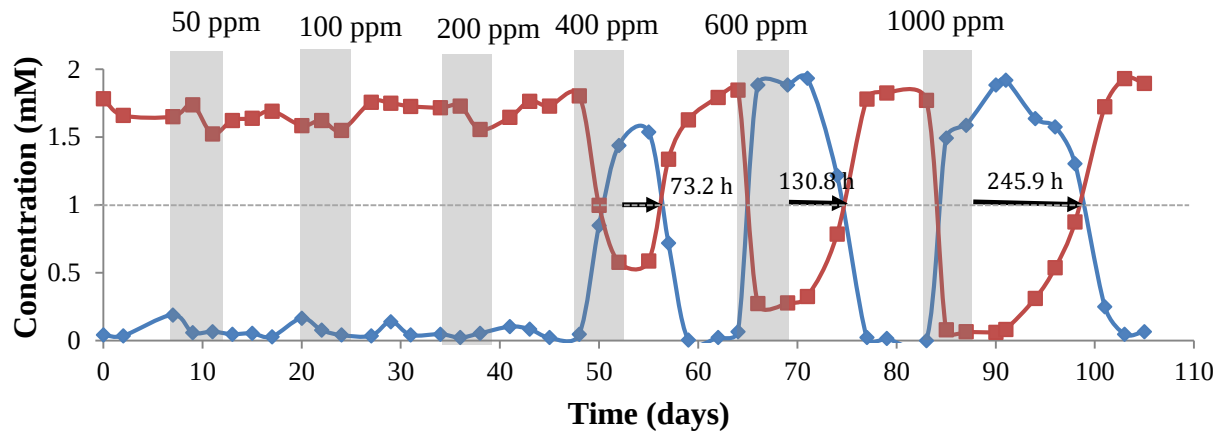
- L represents the length of the bioreactor columns;
- D represents the inside diameter of the columns;
- Total volume was calculated by $V = \pi(D/2)^2 * L$;
- Porosity is a fraction of the void volume over the total volume, calculated by $\frac{Pore\ volume}{Total\ volume} = \frac{25.7\ mL}{69.8\ mL} = 69.8\%$;
- Retention time is the time needed to elute one pore volume, calculated by $\frac{Pore\ volume}{flow\ rate} = \frac{25.7\ mL}{3.7\ mL/h} = 6.9$ h;
- Velocity is the rate of change of the fluid displacement, calculated by $\frac{Length\ of\ column}{retention\ time} = \frac{12.5\ cm}{6.9\ h} = 1.8$ cm/h.

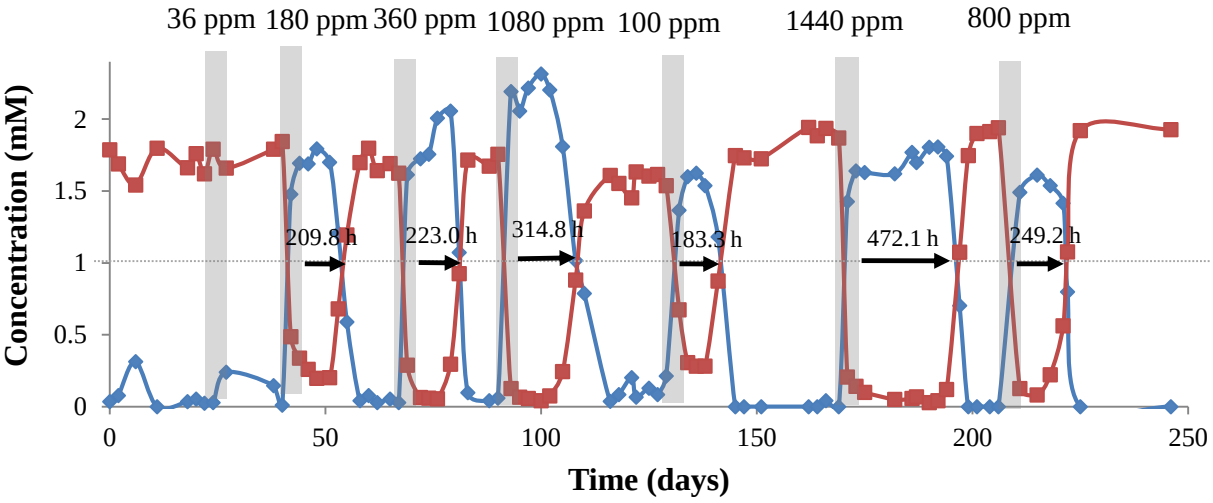
BV1 was pulsed with Glut for 5 days (Figure 4.2, B). The results showed that 50, 100 and 200 ppm Glut had no effect on sulfide production, while 400 ppm Glut started showing sulfide inhibition by lowering sulfide from 2 mM to 0.58 mM in 5 days. As Glut treatment stopped, sulfide concentration increased gradually to the initial 2 mM with an RT of 3.1 days (73.2 h). The second pulse of 600 ppm decreased sulfide further to 0.28 mM at the end of the 5-day treatment. Sulfide then recovered giving an RT of 5.4 days (130.8 h). The last pulse of 1000 ppm was initiated six days after the bioreactor had achieved a steady state at which it consistently generated 2 mM sulfide. Sulfide was reduced to nearly zero on the third day of treatment and maintained for another six days, then recovered with an RT of 10.2 days (245.9 h).

BV2 was pulsed with BAC for 5 days (Figure 4.2, C). The results showed that 36 ppm was not able to decrease sulfide in the effluent. When the concentration was increased 5-fold to 180 ppm, sulfide immediately decreased to 0.3 mM. After BAC was removed, sulfide remained at 0.20 mM for six days and then increased to 2 mM with an RT of 8.7 days. The third pulse of 360 ppm displayed a similar trend with the lowest sulfide concentration going near zero. The RT was 9.3 days. Following sulfide recovery a pulse of 1080 ppm showed a similar trend with a longer period of zero level sulfide and an RT of 13.1 days. Another three pulses of BAC at concentrations of 100, 1440 or 800 ppm gave an RT of 7.6, 19.7 and 10.4 days, respectively. The Longest RT (19.7 days) was observed for the highest pulse (1440 ppm).

Cocodiamine was injected into BV3 for 5 days (Figure 4.2, D). Surprisingly, a pulse of only 25 ppm decreased the sulfide concentration by 91%, resulting in an RT of 7 days. The RTs observed for pulses of 50, 100 or 150 ppm were 12.5, 9.5 and 11 days, respectively. Both at 100 ppm and at 150 ppm the sulfide concentration was zero for a few days. However, after the pulse of 150 ppm, the sulfide concentration did not recover to 1.8 mM, but stayed at 1.5 mM for nearly


[A] BV0-control
sulfate (mM)
sulfide (mM)
Concentration (mM)
Time (days)


[B] BV1- Glut
50 ppm
100 ppm
200 ppm
400 ppm
600 ppm
1000 ppm
73.2 h
130.8 h
245.9 h
Concentration (mM)
Time (days)


[C] BV2-BAC
36 ppm
180 ppm
360 ppm
1080 ppm
100 ppm
1440 ppm
800 ppm
209.8 h
223.0 h
314.8 h
183.3 h
472.1 h
249.2 h
Concentration (mM)
Time (days)

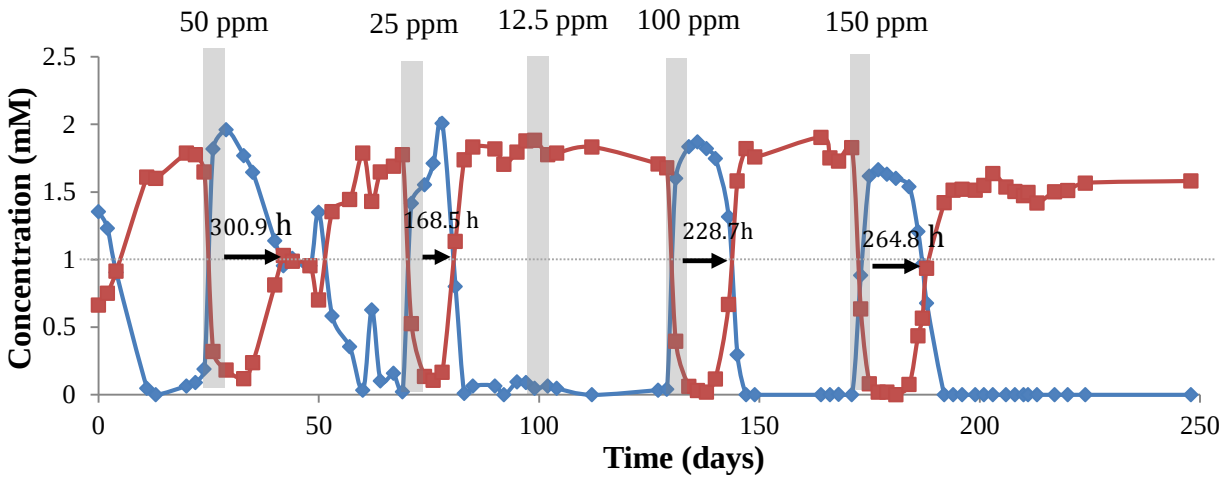


Figure 4.2 The effect of 5-day biocide treatment on sulfide production in bioreactors in the absence of nitrate. Shaded rectangles indicate the time periods for pulsing biocides (5 days). The biocide concentration is indicated above the boxes. Sulfide recovery times (RT) are indicated by black arrows and numerically presented with specific numbers above them. All the RTs are summarized in Table 4.2.

two months.

Sulfide recovery times and the corresponding biocide concentrations for BV1, BV2 and BV3 are summarized in Table 4.2. In Figure 4.4 A, sulfide recovery times are graphically plotted against biocide concentrations.

4.3.3 Effect of 5-day pulse of biocide and continuous nitrate injection on souring control

CSBA-SN medium containing 2 mM sulfate and 2 mM nitrate was continuously injected into bioreactors BV1, BV2 and BV3 after all biocides had been tested in the absence of nitrate (Section 4.3.2). Glut, BAC and cocodiamine were pulsed for 5 days to inhibit sulfide production. The results for each bioreactor are shown in Figure 4.3.

For BV1, increasing concentrations of nitrate (2, 4, 8 or 13.3 mM) were also injected into the column (Figure 4.3, A). Souring control was only observed with 13.3 mM nitrate. Under these conditions, all nitrate was completely reduced (no nitrite observed), but sulfate was only partially reduced to sulfide. Complete sulfate reduction resumed when the nitrate concentration was decreased to 2 mM. Subsequent addition of 200 ppm Glut had no inhibitory effect. The addition of 300 ppm for 5 days inhibited sulfide production with breakthrough of 0.81mM nitrate (no nitrite). The sulfide RT was 6.1 days. Adding 400 ppm Glut for 5 days gave souring control with breakthrough of 1.21 mM nitrate but no nitrite and a sulfide RT of 2.9 days. With 600 ppm of Glut, the RT was 10.2 days. Breakthrough of 2 mM nitrate was observed, but no nitrite. The longest RT of 24.5 days was observed for the highest Glut concentration, 1000 ppm. Nitrate and nitrite production was the same as the pulse of 600 ppm.

Injection of 2, 4 or 8 mM of nitrate into BV2 did not give notable souring control (Figure 4.3, B). A concentration of 2 mM nitrate was then continuously injected into the column. Complete inhibition of sulfate reduction with BAC was not observed until the pulsed

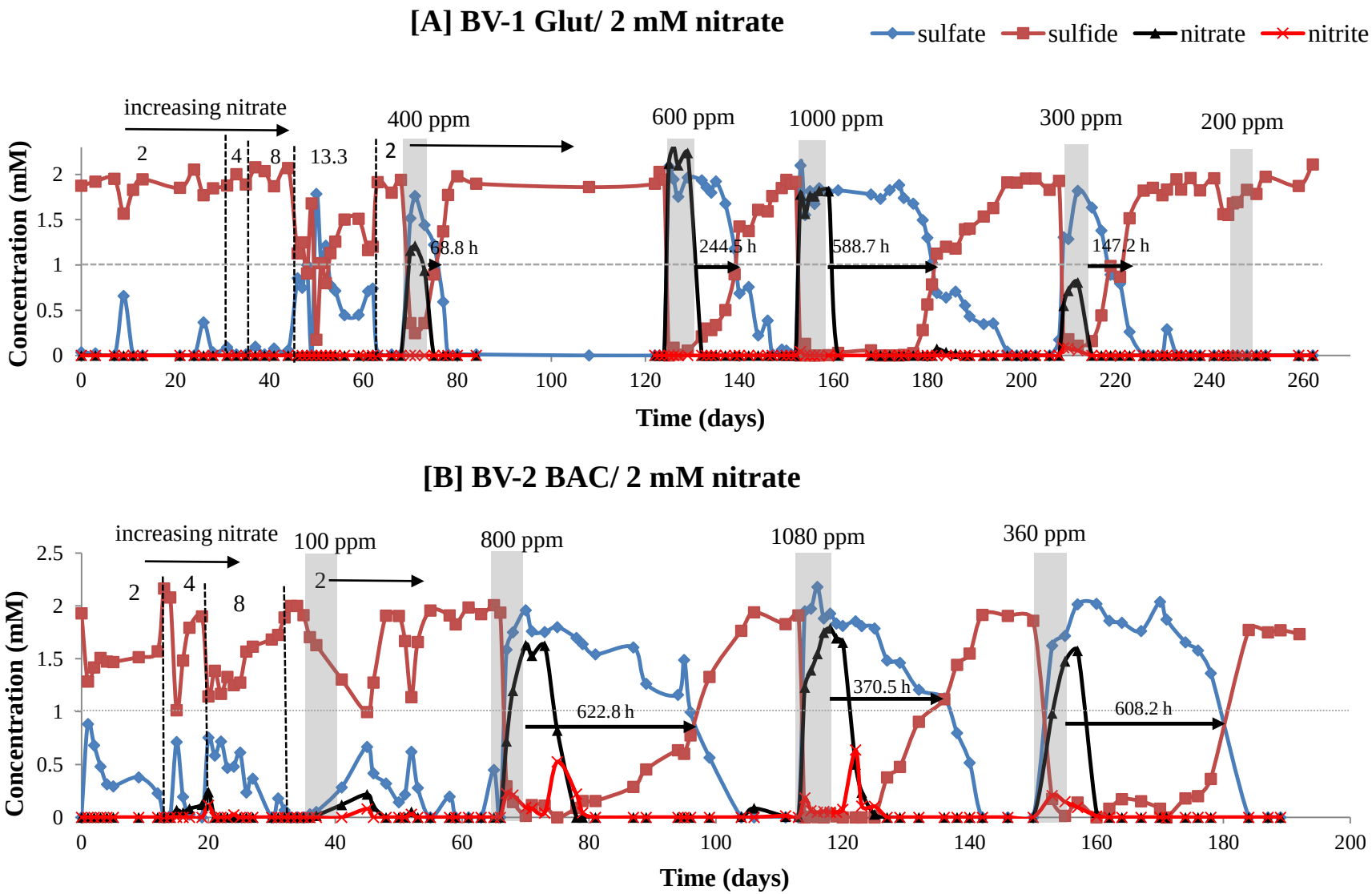
concentration was raised to 360 ppm. The RT was 25.3 days and breakthrough of 1.58 mM nitrate and 0.21 mM nitrite was seen. Adding 800 ppm BAC inhibited all sulfate reduction and gave breakthrough of 1.63 mM nitrate and 0.53 mM nitrite with an RT of 25.9 days. Nitrite was observed during BAC treatment at 0.23 mM and following BAC treatment at 0.53 mM. A sulfide RT of 15.4 days was observed for the pulse of 1080 ppm. Nitrate and nitrite breakthrough was similar to the pulse of 800 ppm.

For BV3, continuous injection of 2 mM nitrate was combined with pulsed injection of cocodiamine (Figure 4.3, C). Injection of 12.5 ppm gave only a transient suppression of effluent sulfide production to 1 mM. A dosage of 25 ppm suppressed sulfide production more obviously and gave an RT of 3.3 days and a peak of nitrite at 0.21 mM. Nitrate was completely reduced. The addition of 50 ppm cocodiamine inhibited sulfide production longer for 11.9 days. Nitrate and nitrite breakthrough was the same as at 25 ppm. Cocodiamine pulsed at 100 ppm and 150 ppm displayed similar results. The RT was 9.7 and 10.8 days, respectively. Peaks of nitrate and nitrite were observed with one occurring during treatment and another following the stop of cocodiamine treatment, demonstrating nitrate reduction was partially inhibited.

A summary of biocide concentrations and corresponding sulfide recovery times for all bioreactors is displayed in Table 4.2. In Figure 4.4 B, sulfide recovery times are plotted against the corresponding biocide concentrations.

4.4 Discussion and conclusions

As a strategy to control sulfide production, biocides (Glut, BAC and cocodiamine) were pulsed for 5 days into BV1, BV2 and BV3 that were continuously injected with 2 mM sulfate without nitrate (Section 4.3.2) and with continuous 2 mM nitrate (Section 4.3.3). On a weight concentration basis, 5-day biocide treatments without nitrate and with 2 mM nitrate to control


[A] BV-1 Glut/ 2 mM nitrate
sulfate
sulfide
nitrate
nitrite
increasing nitrate
2
4
8
13.3
2
400 ppm
600 ppm
1000 ppm
300 ppm
200 ppm
68.8 h
244.5 h
588.7 h
147.2 h
Concentration (mM)
Time (days)
[B] BV-2 BAC/ 2 mM nitrate
increasing nitrate
2
4
8
100 ppm
2
800 ppm
1080 ppm
360 ppm
622.8 h
370.5 h
608.2 h
Concentration (mM)
Time (days)

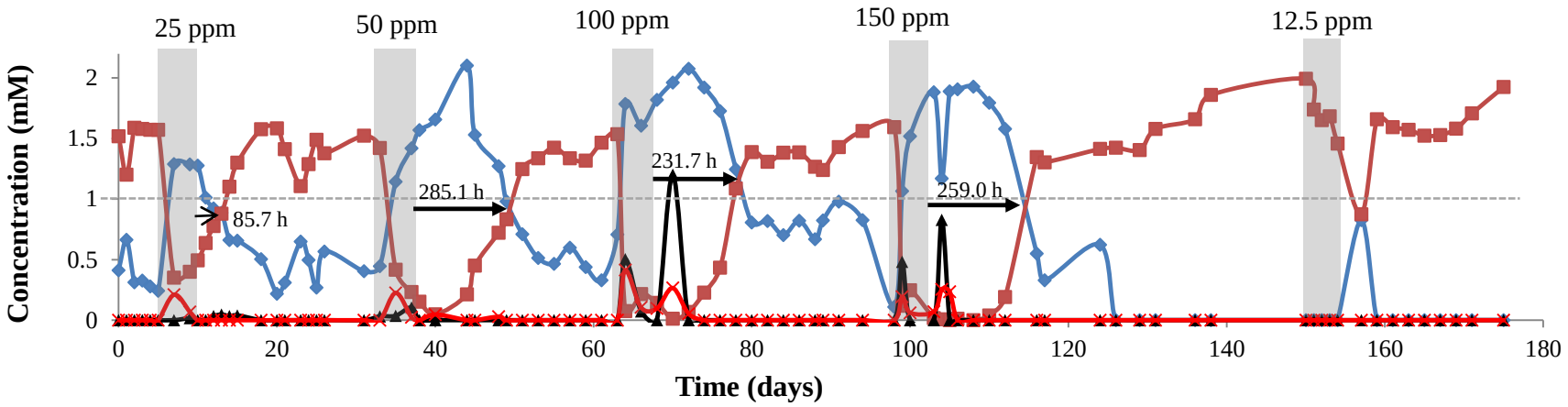


Figure 4.3 The effect of nitrate injection combined with 5-day pulse of biocide on sulfide production. [A] BV1 was pulsed with Glut. An increasing concentration of nitrate (2, 4, 8 or 13.3 mM) was continuously injected and decreased to 2 mM. Glut was then pulsed together with nitrate. [B] BV2 was pulsed with BAC. The effect of nitrate injection (2, 4 or 8 mM) on sulfide production was tested. Continuous injection of 2 mM nitrate was then combined with 100, 360, 800 or1080 ppm of BAC. [C] BV3 was continuously injected with 2 mM nitrate and pulsed with cocodiamine of 12.5, 25, 50, 100 or 150 ppm, respectively. Blue diamonds represent the concentrations of sulfate; Orange squares represent the concentrations of sulfide; **Black triangles** represent the concentrations of nitrate and red crosses represent the concentrations of nitrite.

Table 4.2 Sulfide recovery times (RT) for bioreactors BV1, BV2 and BV3

				No nitrate		Continuous 2 mM nitrate	
BV #	Biocide	†C (mM; ppm)	biocide/500 mL medium	RT (days)	RT (hours)	RT (days)	RT (hours)
BV1	Glut	0.5; 50	45.3 µL	0	0	N/A	N/A
		1; 100	90.5 µL	0	0	N/A	N/A
		2; 200	181.1 µL	0	0	0	0
		3; 300	271.6 µL	N/A	N/A	6.1	147.2
		4; 400	362.0 µL	3.1	73.2	2.9	68.8
		6; 600	543.2 µL	5.4	130.8	10.2	244.5
		10; 1000	905.4 µL	10.2	245.9	24.5	588.7
BV2	BAC	0.1; 36	1.0 mL*	0	0	N/A	N/A
		0.28; 100	2.8 mL*	7.6	183.3	0	0
		0.5; 180	5.0 mL*	8.7	209.8	N/A	N/A
		1; 360	0.18 g	9.3	223	25.3	608.2
		2.24; 800	0.4 g	10.4	249.2	25.9	622.8
		3; 1080	0.54 g	13.1	314.8	15.4	370.5
		4; 1440	0.72 g	19.7	472.1	N/A	N/A
BV3	Cocodiamine	0.025; 12.5	22.5 µL	0	0	0	0
		0.05; 25	45.0 µL	7	168.5	3.6	85.7
		0.1; 50	90.0 µL	12.5	300.9	11.9	285.1
		0.2; 100	180.0 µL	9.5	228.7	9.7	231.7
		0.3; 150	270.0 µL	11	264.8	10.8	259.0

*added from 50 mM BAC stock solution, refer to Section 3.5 for the method to make the stock solution
†C= biocide concentration;
"RT=0" means that no inhibition of sulfide production was observed;
"N/A" means not applicable, because indicated concentration was not tested.

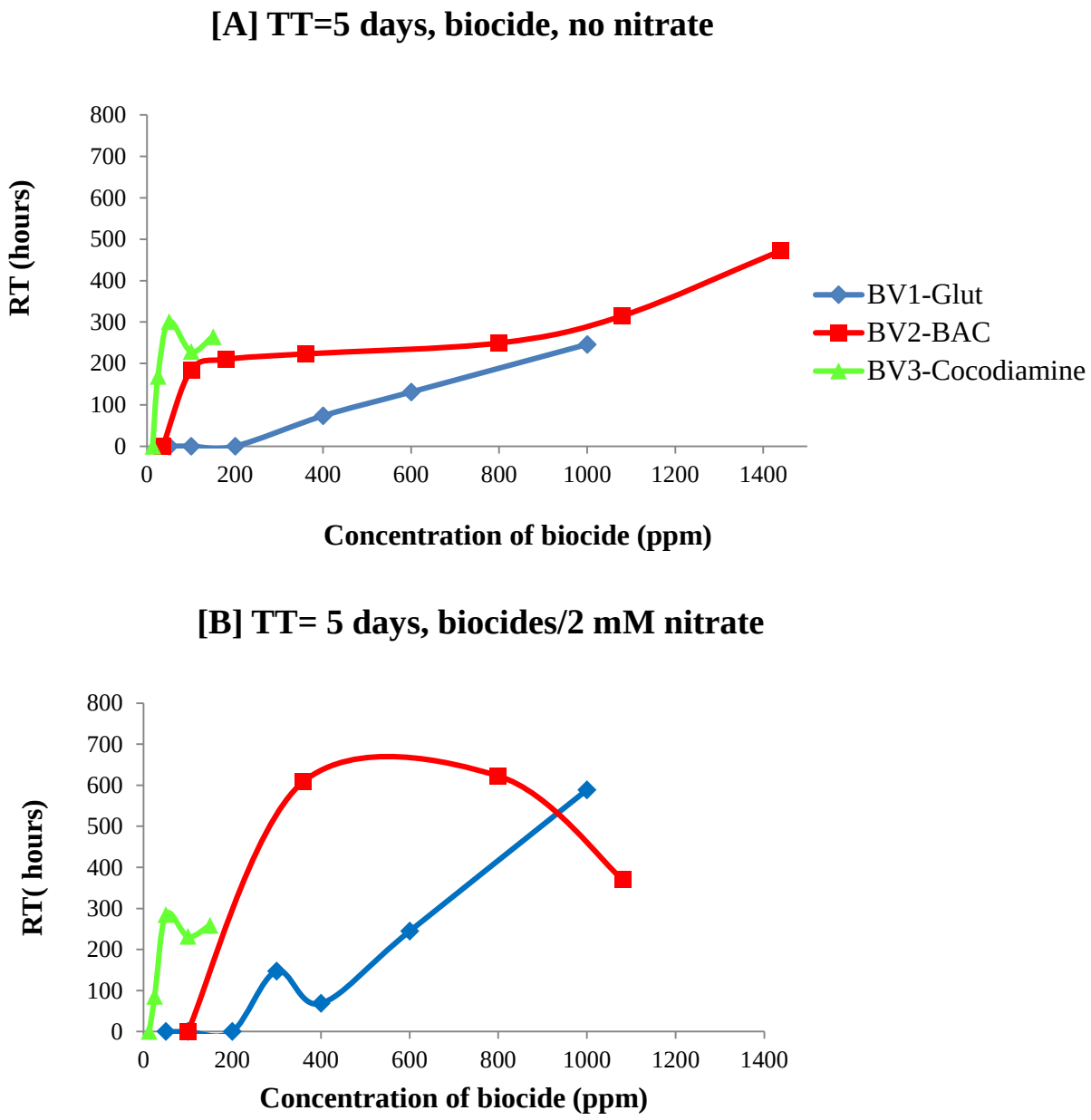


Figure 4.4 Sulfide recovery time (RT) as a function of pulsed biocide concentration. Bioreactors BV1, BV2 and BV3 were [A]continuously injected with 2 mM sulfate without nitrate, or [B] continuously injected with 2 mM sulfate and 2 mM nitrate. Biocides were all pulsed for 5 days.

sulfide production were similarly effective: cocodiamine > BAC> Glut (Figure 4.4).

In the range of 200-1000 ppm, Glut displayed a good linear correlation ($y = 0.3035x - 54.434$; $R^2 = 0.997$) between sulfide recovery time and applied biocide concentration when used without nitrate (Figure 4.4, A). This suggests that increasing the concentration of Glut can proportionally extend sulfide recovery time, even though we still do not know whether this proportional relationship depends on the treatment time or not. A similar trend was also observed for Glut from 400-1000 ppm combined with nitrate (Figure 4.4, B). Surprisingly, a plateau was observed with BAC (100-800 ppm in Figure 4.4, A; 360- 800 ppm in B) and with cocodiamine (50-150 ppm; Figure 4.4) in both with and without nitrate treatment. This implies that increased biocide concentrations did not extend the sulfide recovery times. The mechanism of biocide action may explain these different observations for Glut and for BAC, cocodiamine. Glut is a cross-linking agent that irreversibly reacts with amino and sulfhydryl groups of proteins and nucleic acids, while BAC and cocodiamine are both quaternary cationic surfactants that physically interact with cell membranes and cause rupture of the cells (Greene et al. 2006). The RT plateau for treatment with BAC or cocodiamine indicates that at low concentrations biocide is active, but at high concentrations micelles may be formed so that the biocidal effect does not increase as the concentration of biocide increases.

In Figure 4.5, the two strategies, pulsed biocide without nitrate and pulsed biocide with continuous 2 mM nitrate are compared to investigate if the combination of nitrate and biocide can give more effective souring control. Individual injection of 2 mM nitrate did not give sulfide inhibition (Figure 4.3), which means RT=0. However, when pulsed with biocide, the continuous addition of 2 mM nitrate greatly increased the sulfide recovery time for Glut (Figure 4.5, A) and BAC (Figure 4.5, B). In other words, pulsed Glut and BAC are both synergistic with continuous

2 mM nitrate in controlling sulfide production based on the typical definition of synergy, the combined effect is better than the sum of individual effects of either agent (or $RT_{[biocide+nitrate]} > RT_{[biocide]} + RT_{[nitrate]}$, in this case $RT_{[nitrate]} = 0$, the equation is $RT_{[biocide+nitrate]} > RT_{[biocide]}$; if $RT_{[biocide+nitrate]} < RT_{[biocide]}$, it is antagonistic; if $RT_{[biocide+nitrate]} \approx RT_{[biocide]}$, it is indifferent). The addition of 2 mM nitrate had no effect when combined with pulsed cocodiamine to mitigate souring (Figure 4.5, C), therefore pulsed cocodiamine and continuous nitrate are indifferent. These results indicate that synergy is not caused by whether the biocide is physically- or chemically-reactive. For example, BAC and cocodiamine, which are both physically-reactive biocides, gave different results.

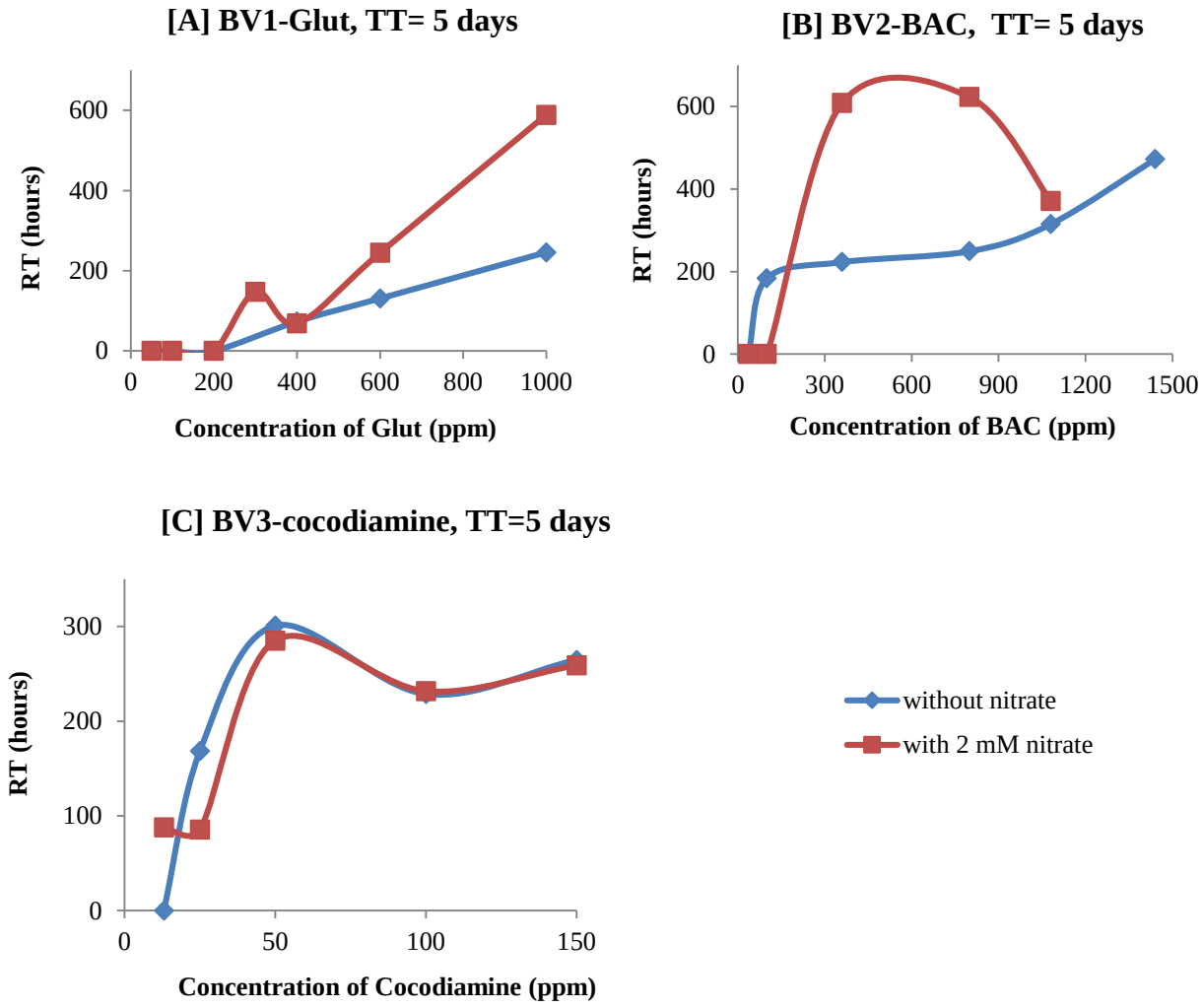


Figure 4.5 Comparison of the efficacy of 5-day pulse of biocide with and without nitrate to control sulfide production

Chapter 5: **Biocides Pulsed for 1 hour to Control Souring**

5.1 Introduction

In Chapter 4, biocides pulsed for a long time (5 days) at low concentrations (L-L) were tested to control sulfide production with and without nitrate. Glut and BAC were found synergistic with continuous injection of 2 mM nitrate in controlling the production of sulfide, while cocodiamine was indifferent to nitrate addition. However, cocodiamine was the most effective biocide to control souring in the L-L strategy. With the aim to figure out if the synergistic relationship between biocide and nitrate relies on biocide treatment time, biocides were tested in this chapter in another strategy: short duration at high concentration (S-H).

Here, bioreactors were pulsed with biocides for only one hour but at high concentration during the experiments in this chapter. One reason to do so was to speed up the experiment by shortening the injection period. Also in actual applications biocides used to mitigate souring are usually pulsed for short duration (e.g. several hours) to decrease costs and avoid the development of antimicrobial resistance. For example, glutaraldehyde treatment at both Veslefrikk and Gullfaks oil fields prior to nitrate injection was 500 ppm of 1 h per week (Bødtker et al. 2008). A glutaraldehyde stability study conducted by McGinley et al. (2011) revealed that high concentration batch dosing was more effective than continuous low concentration doses because lower concentrations of glutaraldehyde were degraded more than higher concentrations. Grobe and Stewart (2000) also observed a similar result in treating *Pseudomonas aeruginosa* biofilms. Batch dosing with higher concentration biocides may thus be more effective in controlling SRB biofilms. However, more studies are needed to confirm this and is addressed here.

5.2 Materials and methods

5.2.1 Medium, SRB enrichment, bioreactor setup, establishment of biofilm and chemical analysis

Bioreactors were set up following the method indicated in Section 3.3. Borosilicate glass syringe barrels (Micro-Mate® interchangeable syringes) without piston, 30 mL, were used as bioreactor columns. The medium, SRB enrichment, and the chemical analyses used were as described in Sections 3.2 and 3.4.

5.2.2 Biocide and nitrate injection

Similar to BV1, BV2 and BV3 (Chapter 4), CSBA medium containing 10 mM sulfate and 8 mM VFA was used initially to establish SRB biofilms and CSBA-S medium containing 2 mM sulfate and 3 mM VFA was used to develop souring. When columns consistently produced 2 mM sulfide, a certain concentration of biocide (Table 5.2) was amended into 50 ml CSBA-S medium and injected into the bioreactors for 1 hour (treatment time TT=1 h). Biocide treatment was stopped one hour later by switching back to CSBA-S medium without biocide. Injection of CSBA-SN medium containing 2 mM sulfate and 2 mM nitrate was used to achieve the establishment of microbial zonation by nitrate addition to control souring. Once 2 mM of sulfide was consistently produced from the effluent, biocide amended into 50 ml CSBA-SN medium (Table 5.3) was injected for 1 hour followed by switch back to CSBA-SN medium without biocide. The next pulse was initiated after sulfide had recovered to 2 mM for at least 4 days. Medium composition is given in Table 3.1.

5.3 Results

5.3.1 Bioreactor parameters

Five bioreactors BV4, BV5, BV6, BV7 and BV8 were set up and run in parallel. Bioreactor parameters are listed in Table 5.1.

5.3.2 Effect of 1-h pulses of biocide on souring control in the absence of nitrate

Bioreactors BV4, BV5, BV6, BV7 and BV8 were pulsed with biocide for 1 hour. BV4 was pulsed with Glut, BV5 with BAC. BV6 with a combination of Glut and BAC, BV7 with THPS, and BV8 with cocodiamine. Table 3.5 lists the biocide information. The effects of biocide treatment on effluent sulfide concentration in the absence of nitrate are seen in Figure 5.1.

BV4 continuously injected with 2 mM sulfate was pulsed with Glut for 1 hour (Figure 5.1, A). Glut started inhibiting sulfide production since the concentration of 500 ppm, gave a very short sulfide RT, 15.0 hours. The pulses of 1000 ppm, 2000 ppm, 4000 ppm and 5000 ppm were all able to lower sulfide concentration to zero and gave a sulfide RT of 84.7 h, 135.4 h, 214.6 h and 234.9 h, respectively. However, after the pulse of 5000 ppm, sulfide could not recover to the initial 2 mM like other pulses, hovering at around 1.5 mM as long as half a month.

BV5 continuously injected with 2 mM sulfate was pulsed with BAC for 1 hour (Figure 5.1, B). One hour pulse of BAC could not inhibit sulfide production until the concentration was raised to 2500 ppm (RT= 159.5 h), which slowly reduced sulfide to 0.84 mM in 5 days. The first complete inhibition of sulfate reduction was observed 98 hour after cessation of the pulse of 3000 ppm BAC. A relatively long sulfide recovery time was recorded at 250.4 h. Increasing concentration of BAC to 3500 ppm expended the sulfide RT to 381.2 h. Complete sulfide inhibition was maintained for 9 days.

BV6 continuously injected with 2 mM sulfate was pulsed with Glut blended with BAC (Glut/BAC) for 1 hour (Figure 5.1, C). The addition of 588 ppm Glut/BAC had no inhibitory effect. The addition of 1177 ppm and 2353 ppm of Glut/BAC reduced sulfate partially to 0.58 mM and 0.31 mM sulfide, respectively. Sulfide was then recovered with the RT of 45.6 h and 92.6 h. Both pulses of 3531 ppm and 4118 ppm Glut/BAC completely inhibited sulfate reduction and kept sulfide level at zero for a couple of days, especially the pulse of 4118 ppm kept it for nearly half a month. The sulfide RT for these was 269.7 h and 379.6 h, respectively.

BV7 continuously injected with 2 mM sulfate was pulsed with THPS for 1 hour (Figure 5.1, D). THPS did not inhibit sulfide production greatly until the concentration was increased to 1500 ppm that decreased sulfide immediately to 0.43 mM. Sulfide recovery time was 31.6 h. As the concentration increased to 2500 ppm, RT was extended to 128.2 h, but sulfide was still not completely inhibited until the concentration raised to 3000 ppm. RT was 320.8 h.

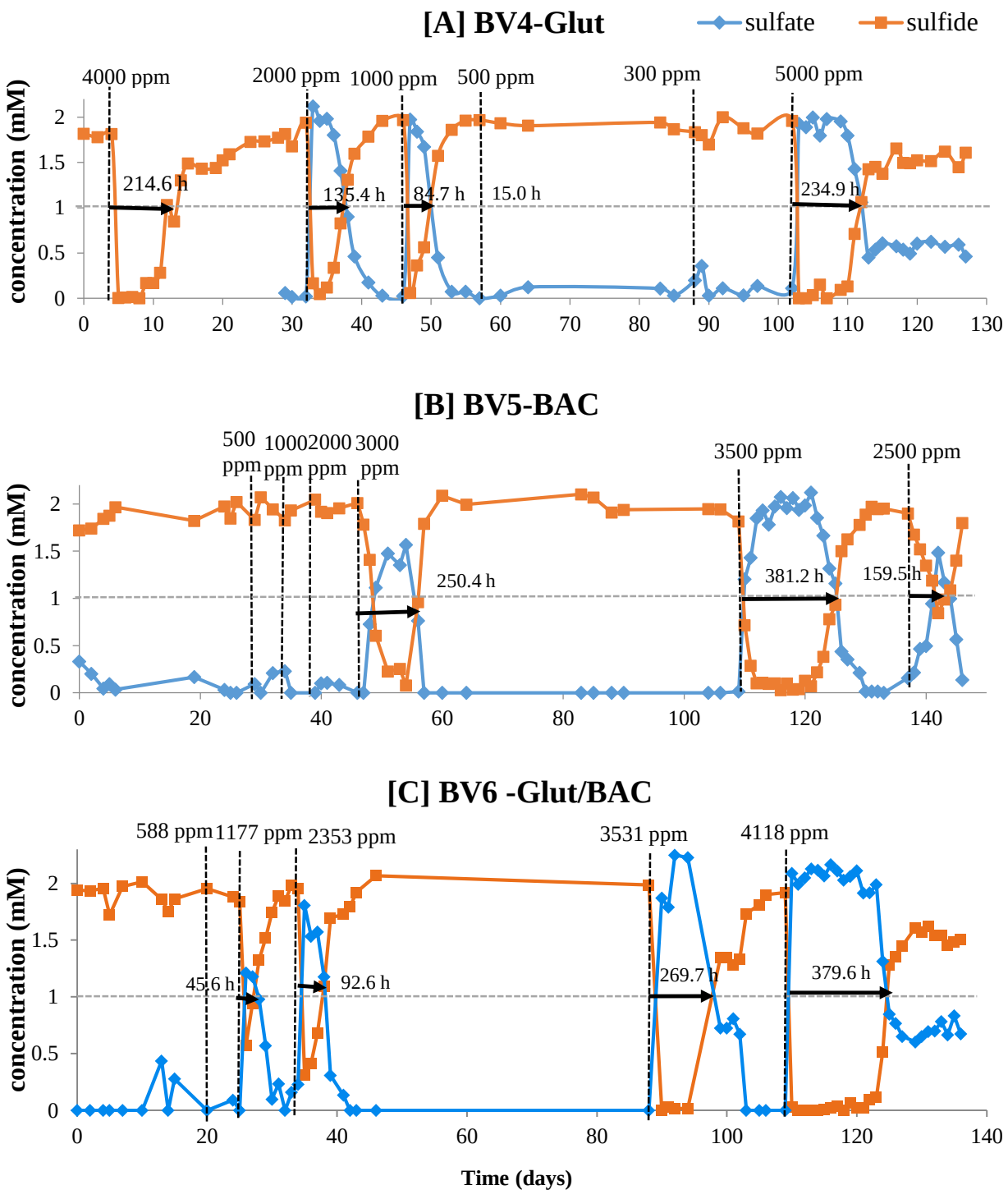
BV8 continuously injected with 2 mM sulfate was pulsed with cocodiamine for 1 hour (Figure 5.1, E). Increasing concentrations of cocodiamine (100, 200, 400, 800 and 1000 ppm) did not inhibit sulfide production until 2000 ppm was pulsed into the column. It seems that cocodiamine was not a fast acting biocide so that the addition of 2000 ppm slowly reduced sulfide below 1 mM in 19 hours, but sulfide recovered quickly to give an RT of 40.4 h. However, it took nearly 200 hours for sulfide to recover from 1.5 mM to 2 mM. Even though the pulse of 3000 ppm gave a longer RT of 58.4 hours, it still could not completely inhibit sulfide production and a longer prolonged tail was observed when sulfide reached 1.5 mM. Injection of 4000 ppm cocodiamine completely inhibited 2 mM sulfide with RT of 109.1 hours. Similar to the previous two pulses, a prolonged tail appeared.

Sulfide recovery times and the corresponding biocide concentrations are summarized in

Table 5.1 Typical parameters of 30 ml syringe bioreactors

Column size L*D (cm)	Pore volume (PV, mL)	Total volume (mL)	Porosity (%)	Flow rate (mL/ h)	Retention time (h)	Velocity (cm/h)
9.8*2	10.9	30.8	35.4	2.7	4.0	2.45

Refer to Table 4.1 for the detailed calculations.



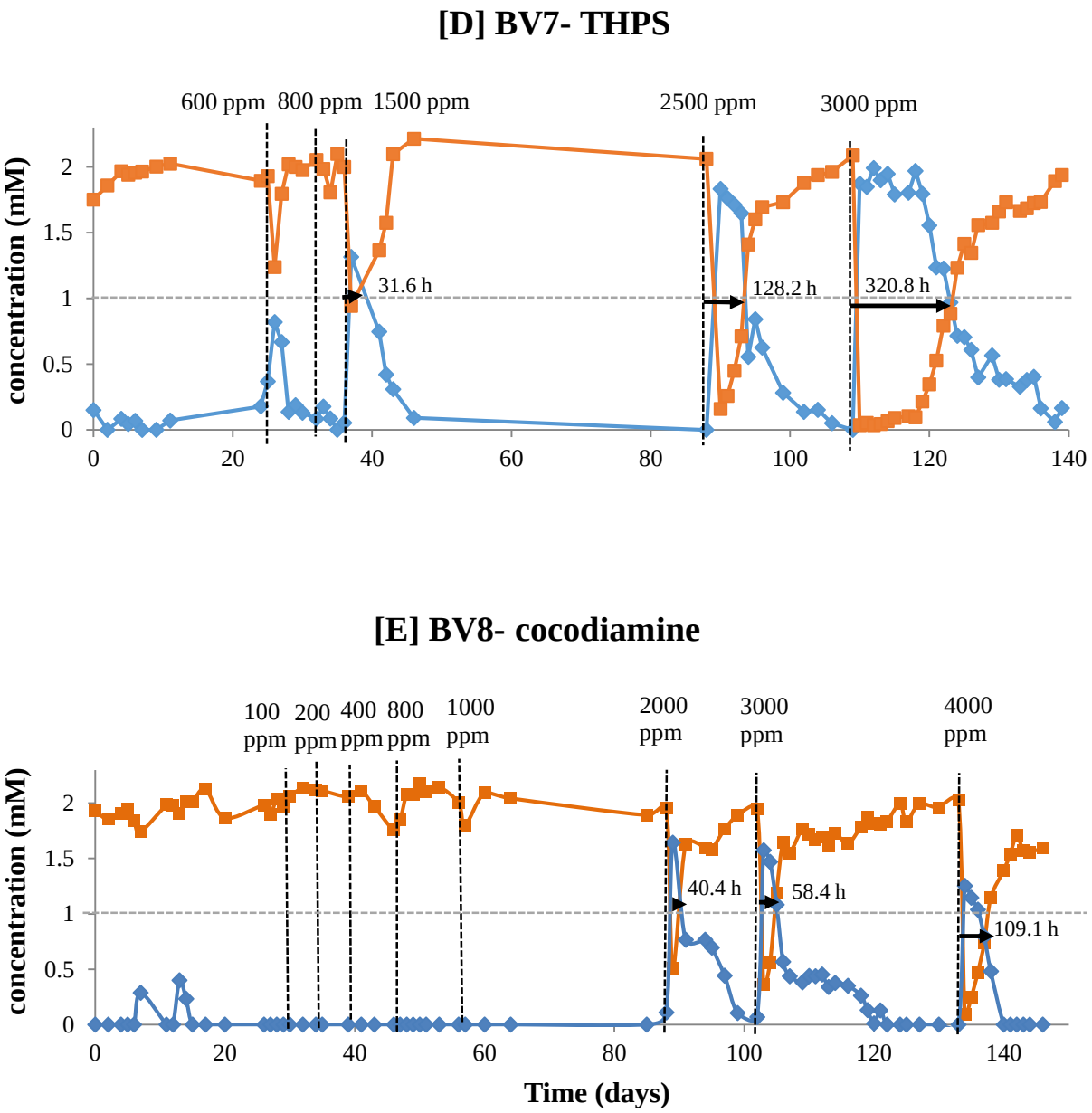


Figure 5.1 The effect of 1-h biocide treatment on sulfide production in the absence of nitrate. Vertical dashed lines indicate the 1-h time periods for pulsing biocides. The biocide concentration is indicated above the lines. Sulfide recovery times (RT) are indicated by black arrows and numerically presented with specific numbers above them. All the RTs are summarized in Table 5.2.

Table 5.2 and graphically presented in Figure 5.3 A by plotting sulfide recovery times against the biocide concentrations.

5.3.3 Effect of 1-h pulses of biocide and continuous nitrate on souring control

CSBA-SN medium containing 2 mM sulfate and 2 mM nitrate was continuously injected into bioreactors BV4, BV5, BV6, BV7 and BV8 after all biocides had been tested in the absence of nitrate (Section 5.3.2). Glut, BAC, Glut/BAC, THPS and cocodiamine were pulsed for 1 hour to inhibit sulfide production. The results for each bioreactor are shown in Figure 5.2. Since 1-hour pulse was very short, samples were taken more frequently than 5-day pulse in order to capture the transient formation of nitrite. Beside the overall result showed on a "days" scale, the detailed result for each pulse based on an "hours" scale is also provided in Appendix.

BV4 was pulsed with Glut (Figure 5.2, A and Figure B1). Adding 500 ppm had no notable inhibitory effect on sulfide production (RT=0). A short sulfide RT of 21.7 hours representing partial inhibition of sulfate reduction resulted from the addition of 1000 ppm Glut. Nitrate was partially reduced without production of nitrite (Appendix B, Figure B1, B). A dose of 2000 ppm extended the RT to 93.4 h. Sulfate reduction was still partially inhibited with breakthroughs of 1.02 mM nitrate and 0.21 mM nitrite measured. A similar result was observed with a pulse of 3000 ppm (RT= 109.6 h, nitrate 0.91 mM and nitrite 0.25 mM). A further increased concentration of 4000 ppm completely inhibited sulfide production with a transient sulfate breakthrough. A transient accumulation of 0.88 mM nitrate and 0.69 mM nitrite was also detected in the effluent (Appendix B, Figure B1, E). However, the RT decreased to 86.9 h.

BV5 was pulsed with BAC (Figure 5.2, B and Figure C1). BAC began partially inhibiting sulfide production at a concentration of 2500 ppm and sulfide recovered at 97.8 hour after stopping BAC treatment. A nitrite peak (0.43 mM) following a nitrate peak (0.82 mM) was

seen during the sulfide suppression period. A pulse of 3000 ppm extended RT to 150.9 h with 1.17 mM nitrate but not much nitrite (0.1 mM) breakthrough. A similar result was found with 3500 ppm injection: RT was 117.7 hours, nitrate breakthrough was at 1.12 mM, and no nitrite was observed.

BV6 was pulsed with Glut/BAC (Figure 5.2, C and Figure D1). Adding 1177 ppm Glut/BAC inhibited 92% of sulfate reduction and 22% of nitrate reduction resulting in an RT of 52.7 h and 0.15 mM nitrite produced (Appendix D, Figure D1, A). A transient production of 1.08 mM nitrate and 1.61 mM sulfate resulted from the pulse of 2353 ppm Glut/BAC, which led to sulfide recovery at 57.3 h and a long period (over 40 h) of nitrite accumulation (peak concentration was 0.87 mM) (Appendix D, Figure D1, B). Increasing the concentration to 3531 ppm suppressed over 77% nitrate reduction for about 40 hours, but only 0.65 mM nitrite was transiently detected. Sulfide was completely inhibited and RT was 331.9 h. The last pulse was 4118 ppm, which surprisingly inhibited only 16% of nitrate reduction (no nitrite) but nearly all sulfate reduction. RT was 114.9 hours.

BV7 was pulsed with THPS (Figure 5.2, D and Figure E2). The addition of 1000 ppm resulted in a breakthrough of 0.23 mM nitrate, which lowered sulfide to 1.62 mM (RT=0). The pulse of 1500 ppm THPS inhibited 1.34 mM sulfate reduction (RT= 61.1 hours) and 0.65 mM nitrate reduction (nitrite = 0.24 mM). Increasing the THPS concentration to 2500 ppm gave a slightly decreased RT of 39.5 h while 0.71 mM nitrate broke through, no nitrite was observed. With the 3000 ppm treatment, sulfide production was only partially inhibited resulting in a very short RT (24.0 hours), nitrate breakthrough was at 0.43 mM (Figure E2, D). No nitrite was observed.

BV8 was pulsed with cocodiamine (Figure 5.2, E and Figure F1). The injection of 400 or

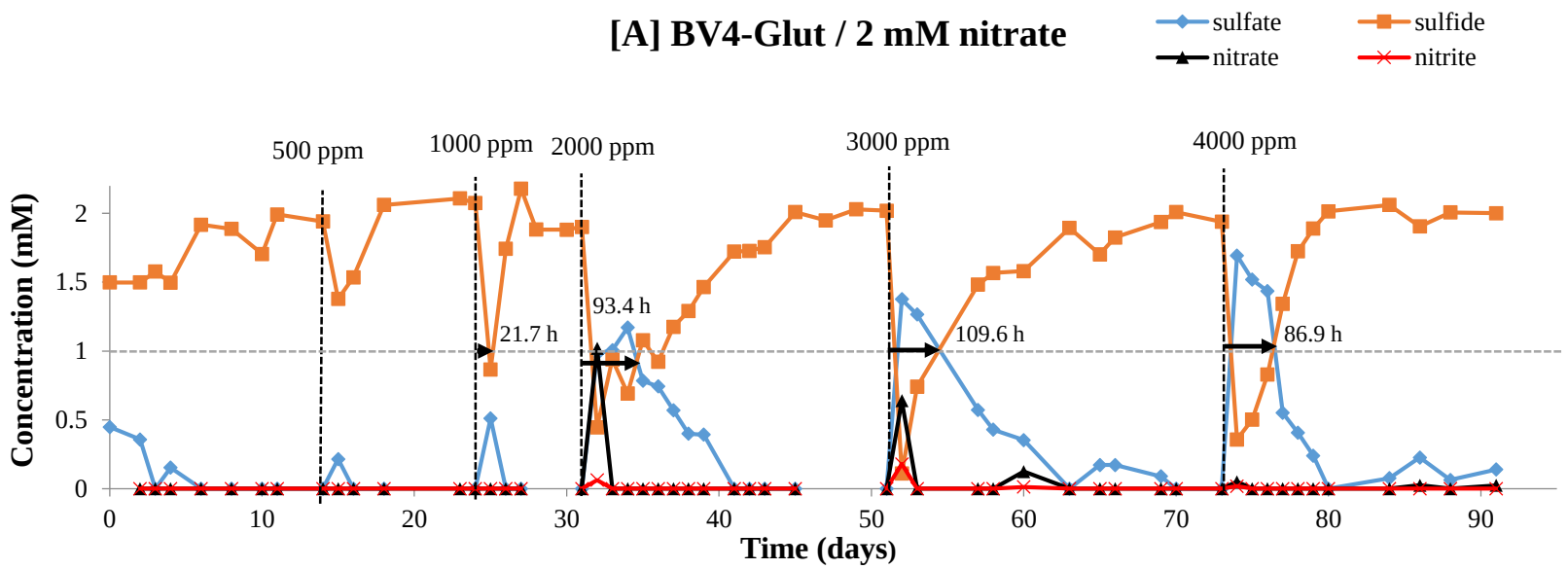
500 ppm cocodiamine was not enough to inhibit sulfide production. The addition of 1000 ppm suppressed sulfide for 68.5 hours with 1.13 mM nitrate and 0.21 mM nitrite breakthrough. As the concentration of cocodiamine increased to 2000 ppm, RT did not change much (57.5 hours). Nitrite breakthrough was at 0.64 mM, while nitrate was at 0.47 mM. An injected concentration as high as 3000 ppm still did not completely inhibit sulfide production but gave a longer RT of 127.3 hours. Nitrate reduction was inhibited by 61% and 0.44 mM nitrite was produced. The inhibitory effect for the pulse of 4000 ppm was similar to 2000 ppm: RT was 79.7 hours, nitrite breakthrough was at 0.59 mM, while nitrate was at 0.23 mM.

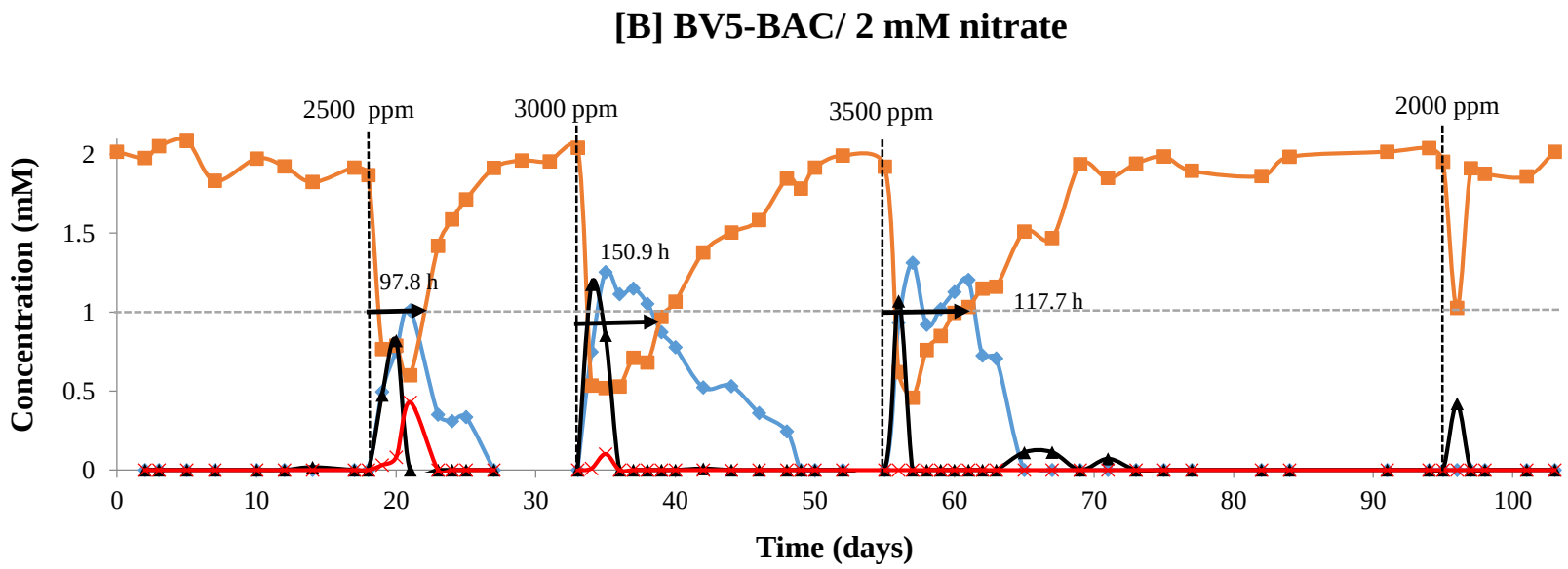
Sulfide recovery times and the corresponding biocide concentrations are summarized in Table 5.2 and graphically presented in Figure 5.3 B by plotting sulfide recovery times against the biocide concentrations.

5.4 Discussion and conclusions

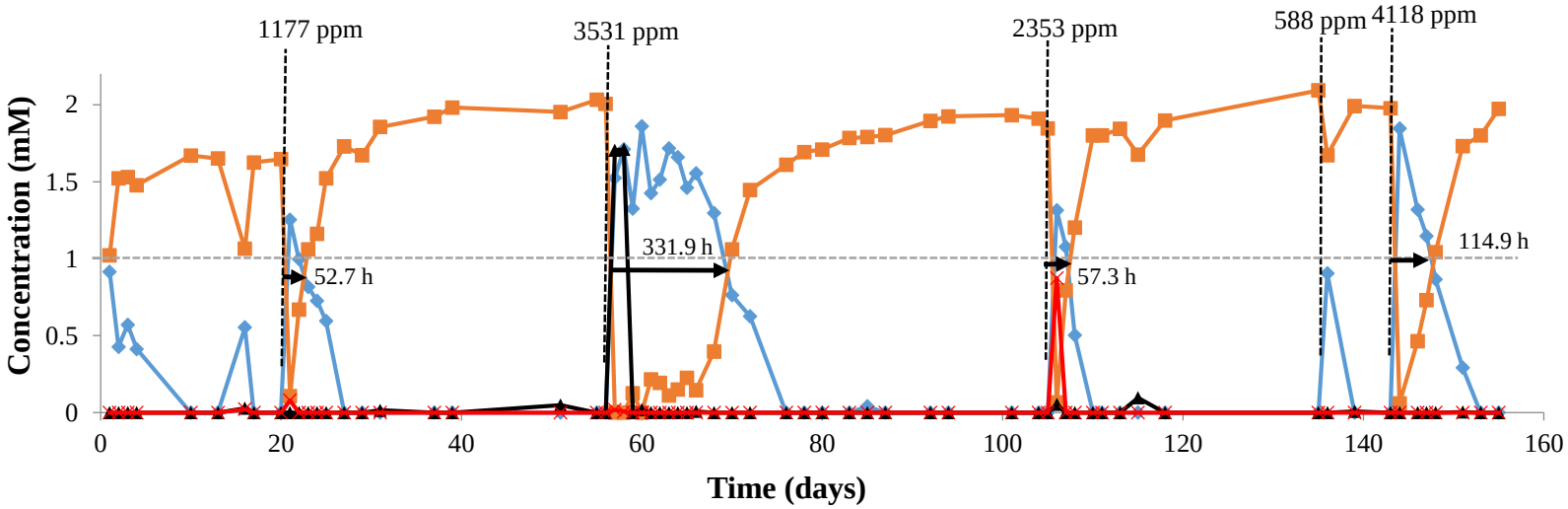
5.4.1 1-h pulse of biocide treatment to control souring with and without nitrate

As a strategy to control sulfide production, relatively large concentrations of biocides (Glut, BAC, Glut/BAC, THPS and cocodiamine) were pulsed for 1 hour into bioreactors BV4, BV5, BV6, BV7 and BV8 that were continuously injected with 2 mM sulfate without nitrate (Section 5.3.2) or with 2mM sulfate and 2 mM nitrate (Section 5.3.3). The effectiveness of 1-hour biocide treatment alone to control souring for biocide concentration below 2000 ppm was in the order: Glut > Glut/BAC > THPS> cocodiamine > BAC. For concentrations over 2500 ppm, the order was: THPS> BAC> Glut /BAC > Glut> cocodiamine (Figure 5.3, A). The continuous injection of 2 mM nitrate decreased the differences in sulfide recovery times following different pulsed biocide treatments (Figure 5.3, B). In the presence of nitrate, all RTs, except one, were between 0 and 150 h, whereas in the absence of nitrate six RTs were above 150 h (Figure 5.3).

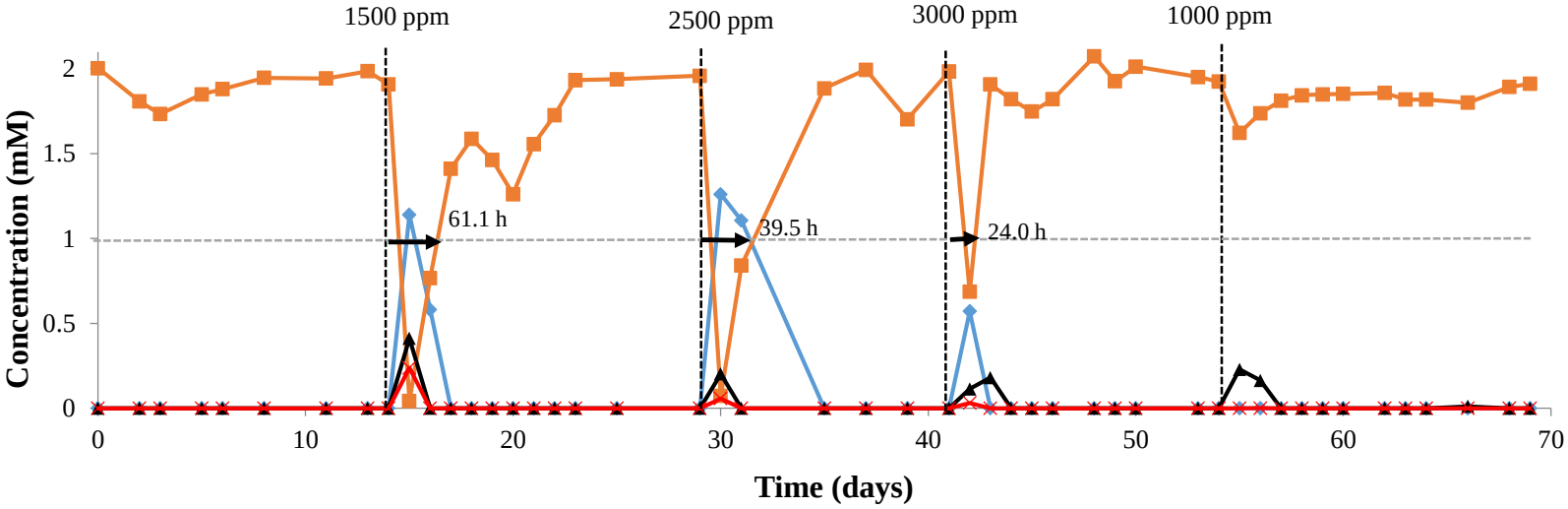

[A] BV4-Glut / 2 mM nitrate
sulfate
sulfide
nitrate
nitrite
500 ppm
1000 ppm
2000 ppm
3000 ppm
4000 ppm
21.7 h
93.4 h
109.6 h
86.9 h
Concentration (mM)
Time (days)


[B] BV5-BAC/ 2 mM nitrate
2500 ppm
3000 ppm
3500 ppm
2000 ppm
97.8 h
150.9 h
117.7 h
Concentration (mM)
Time (days)

[C] BV6-Glut/BAC/ 2 mM nitrate



[D] BV7-THPS/ 2mM nitrate



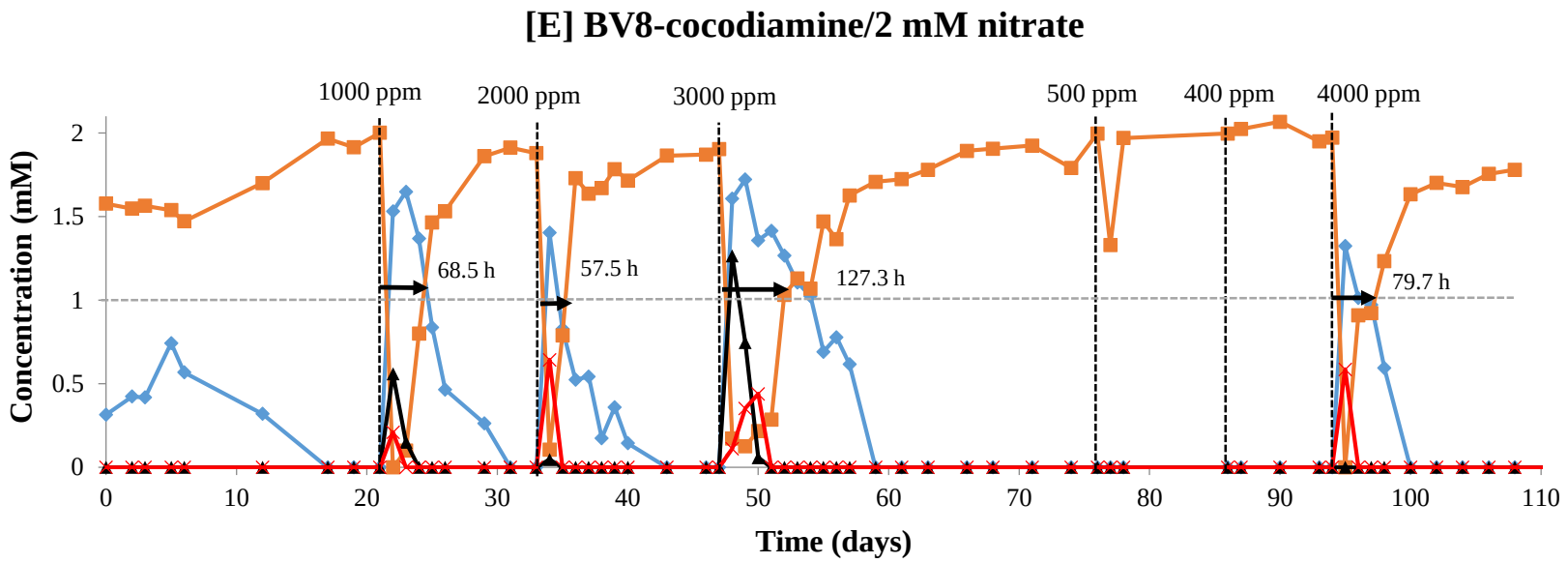


Figure 5.2 The effect of nitrate injection combined with 1-h pulse of biocide on sulfide production. Orange squares are sulfide, blue diamonds are sulfate, **black triangles** are nitrate and red crosses are nitrite. Black arrows and the numbers above indicated sulfide recovery times. The dashed lines represent the time points when biocides were pulsed for one hour, above which the pulsed concentrations are listed.

Table 5.2 Sulfide recovery times (RT) for bioreactors BV4, BV5, BV6, BV7 and BV8

				No nitrate (CSBA-S)		Continuous 2 mM nitrate (CSBA-SN)	
BV #	**Biocide**	***C (mM; ppm)**	**µl (g)/ 50 mL medium**	**RT (days)**	**RT (hours)**	**RT (days)**	**RT (hours)**
BV4	**Glut**	3; 300	27.2 µl	0	0	N/A	N/A
		5; 500	45.3 µl	0.6	15	0	0
		10; 1000	90.5 µl	3.5	84.7	0.9	21.7
		20; 2000	181.0 µl	5.6	135.4	3.9	93.4
		30; 3000	271.5 µl	N/A	N/A	4.6	109.6
		40; 4000	362.0 µl	8.9	214.6	3.6	86.9
		50; 5000	452.5 µl	9.8	234.9	N/A	N/A
BV5	**BAC**	1.39; 500	0.025 g	0	0	N/A	N/A
		2.78; 1000	0.05 g	0	0	N/A	N/A
		5.56; 2000	0.1 g	0	0	0	0
		6.95; 2500	0.125 g	6.6	159.5	4.1	97.8
		8.34; 3000	0.15 g	10.4	250.4	6.3	150.9
		9.73; 3500	0.175 g	15.9	381.2	4.9	117.7
BV6	**Glut/BAC**	5 + 0.25 ; 588	56.8 µl	0	0	0	0
		10 + 0.49; 1177	113.6 µl	1.9	45.6	2.2	527
		20 + 0.98; 2353	227.2 µl	3.9	92.6	2.4	57.3
		30 + 1.47 ; 3531	340.8 µl	11.2	269.7	13.8	331.9
		35+ 1.72; 4118	397.6 µl	15.8	379.6	4.8	114.9
BV7	**THPS**	1.5; 600	28.6 µl	0	0	N/A	N/A
		2; 800	38.1 µl	0	0	N/A	N/A
		2.5; 1000	47.6 µl	N/A	N/A	0	0
		3.75; 1500	71.4 µl	1.3	31.6	2.5	61.1
		6.25; 2500	119.0 µl	5.3	128.2	1.6	39.5
		7.4; 3000	142.8 µl	13.4	320.8	1	24
BV8	**Cocodiam-ine**	0.2; 100	18.4 µl	0	0	N/A	N/A
		0.4; 200	36.7 µl	0	0	N/A	N/A
		0.8; 400	73.4 µl	0	0	0	0
		1.0; 500	91.8 µl	N/A	N/A	0.5	12.3
		1.6; 800	146.9 µl	0	0	N/A	N/A
		2.0; 1000	183.6 µl	0	0	2.9	68.5
		4.0; 2000	367.2 µl	1.7	40.4	2.4	57.5
		6.0; 3000	550.8 µl	2.4	58.4	5.3	127.3
		8.0; 4000	734.4 µl	4.5	109.1	3.3	79.7

*C= biocide concentration;
"RT=0" means that no inhibition of sulfide production was observed;
"N/A" means not applicable, because indicated concentration was not tested.

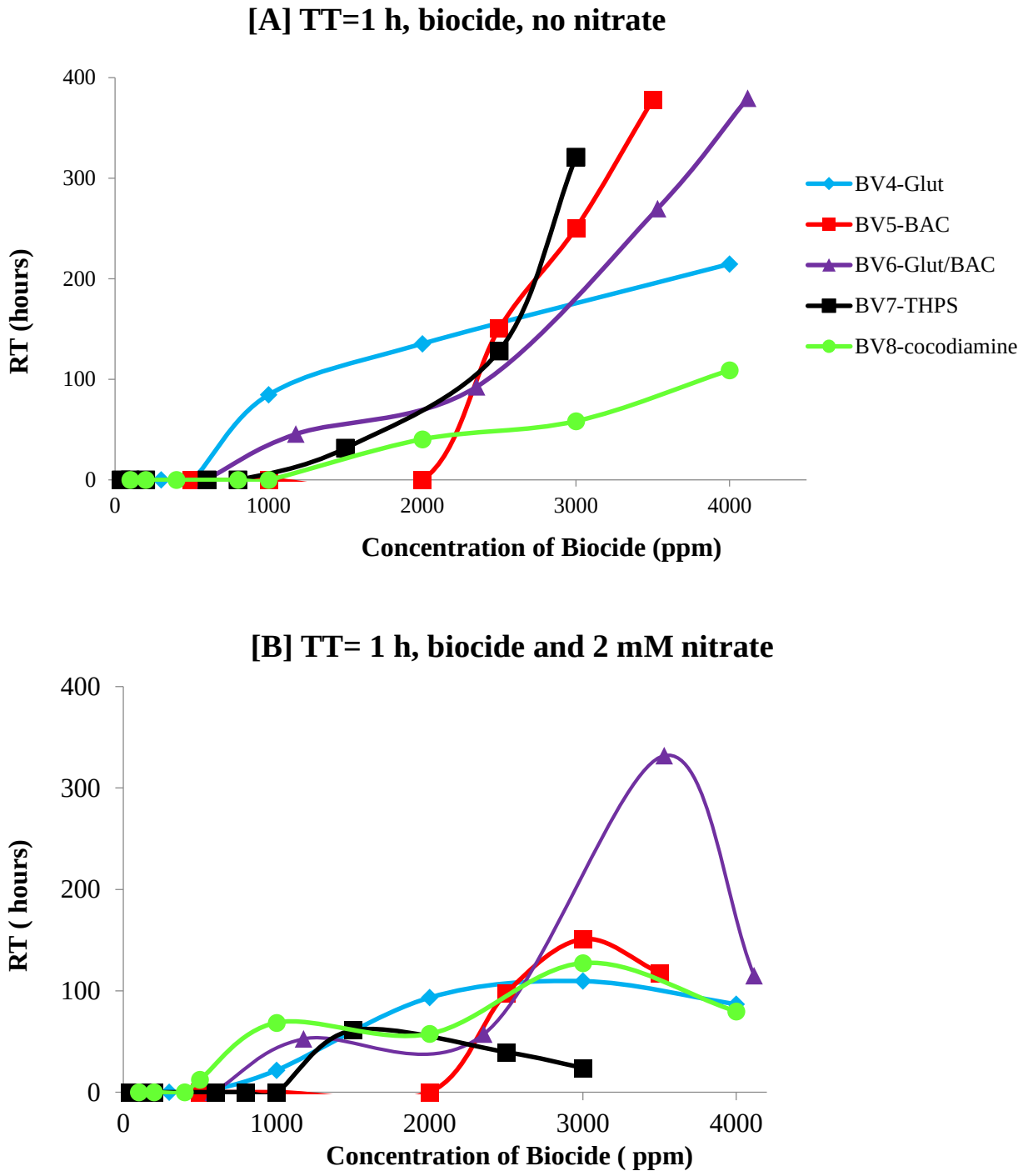


Figure 5.3 Sulfide recovery time (RT) as a function of 1-h pulsed biocide concentration. Bioreactors BV4, BV5, BV6, BV7 and BV8 were [A] continuously injected with 2 mM sulfate without nitrate, or [B] continuously injected with 2 mM sulfate and 2 mM nitrate. Biocides were all pulsed for 1 hour.

When biocides were pulsed alone without nitrate, the sulfide recovery times showed a good positive correlation with biocide concentrations once the concentration exceeded a threshold value (Figure 5.3, A). For example, a linear relationship between sulfide recovery times and Glut concentrations was exhibited when the Glut concentration was above 1000 ppm ($y= 0.0428x+45.1$, $R^2= 0.9959$). This was also true for BAC, Glut/BAC and cocodiamine, when the concentration of BAC was over 2000 ppm ($y=0.247x-484.42$, $R^2=0.9944$), Glut/BAC was over 2353 ppm ($y=0.1608x-288.96$, $R^2= 0.9968$), and cocodiamine was over 1000 ppm ($y= 0.0345x-34.35$, $R^2=0.971$), respectively (Table 5.3). However, THPS did not show a linear relationship between RT and THPS concentration. Nevertheless, most positive correlations indicated that more efficient souring control was obtained by increasing the biocide concentration when biocide was pulsed for 1 hour.

When biocide was pulsed with continuous nitrate injection to control souring, the addition of 2 mM nitrate changed the positive linear trend that was observed with biocides alone into a plateau at RT=100 hours except Glut/BAC pulsed at 3531 ppm (Figure 5.3, B). In this case, increasing the biocide concentrations did not always lead to a corresponding increase of sulfide recovery times.

A sudden rise of total sulfur species (sulfate and sulfide) around the fourth hour following the injection of THPS was found in the pulse of 1000, 2500 and 3000 ppm in the absence of nitrate (Figure E1, B and C) and the pulse of 2500 and 3000 ppm in the presence of nitrate (Figure E2, C and D). The seeming loss of sulfur balance (S-species > 2 mM) was caused by the addition of THPS that contains sulfate anions. Based on the structure of THPS shown in Figure 1.7, 1 mole THPS can provide 1 mole sulfate. The concentration of injected THPS expressed as mM was also listed in Table 5.2, which equals the injected concentration of sulfate.

Table 5.3 The linear relationship between sulfide recovery times and 1-h pulsed biocide concentrations

Biocide	Linear equation	R^2	Concentration range
Glut	y = 0.0428x + 45.1 R	0.9959	1000- 4000 ppm
BAC	y = 0.247x - 484.42	0.9944	2000-3500 ppm
Glut/BAC	y = 0.1608x - 288.96	0.9968	2353-4118 ppm
THPS	N/A*	N/A*	800-3000 ppm
Cocodiamine	y = 0.0345x - 34.35	0.971	1000- 4000 ppm

*N/A means THPS treatment did not show a good linear relationship based on the current data.

The injection flow rate for BV7 was 2.7 mL/h which equals 6 PV/day. It means 4 hours was needed to elute one PV. THPS was pulsed for only 1 hour. The retention time for THPS in the bioreactor column was, therefore, 4 hours (elution time), which is exactly the time that the S-peaks appeared. This can also explain that the peak was not found in the day-scale figure (Figure 5.1, D and Figure 5.2, D), but in the hour-scale figures (Appendix E). If THPS was also tested in the pulse of 5 days, the sulfate peak will be shown in the day-scale graphs, as had been previously observed in Callbeck's work (2012).

5.4.2 Synergy between biocide and nitrate

In Figure 5.4, the two ways to control souring, pulsed biocide without nitrate and pulsed biocide with continuous injection of 2 mM nitrate, are compared to investigate if the combination of nitrate and biocide can give more effective souring control. Injection of 2 mM nitrate alone did not control souring (Figure 5.2), which means $RT_{[nitrate]}=0$. The synergy between pulsed biocide and continuous nitrate can be then obtained if $RT_{[biocide+nitrate]} > RT_{[biocide]}$. Only cocodiamine showed synergy (Figure 5.4, E) because $RT_{[biocide+nitrate]} > RT_{[biocide]}$. Glut, BAC and THPS were all antagonistic with nitrate (Figure 5.4, A, B and D) where $RT_{[biocide+nitrate]} < RT_{[biocide]}$. Pulsed Glut/BAC and continuous nitrate were indifferent (Figure 5.4, C) where $RT_{[biocide+nitrate]} \approx RT_{[biocide]}$.

The results confirmed what was found in 5-day treatment, which is that synergy is not caused by whether the biocide is physically- or chemically-reactive. BAC and cocodiamine, which are both physically-reacting biocides, gave different results; whereas Glut and BAC, which are different types of biocides gave the same result. Also, the mixture of Glut and BAC did not show the same antagonism that was shown by individual Glut or BAC treatments.

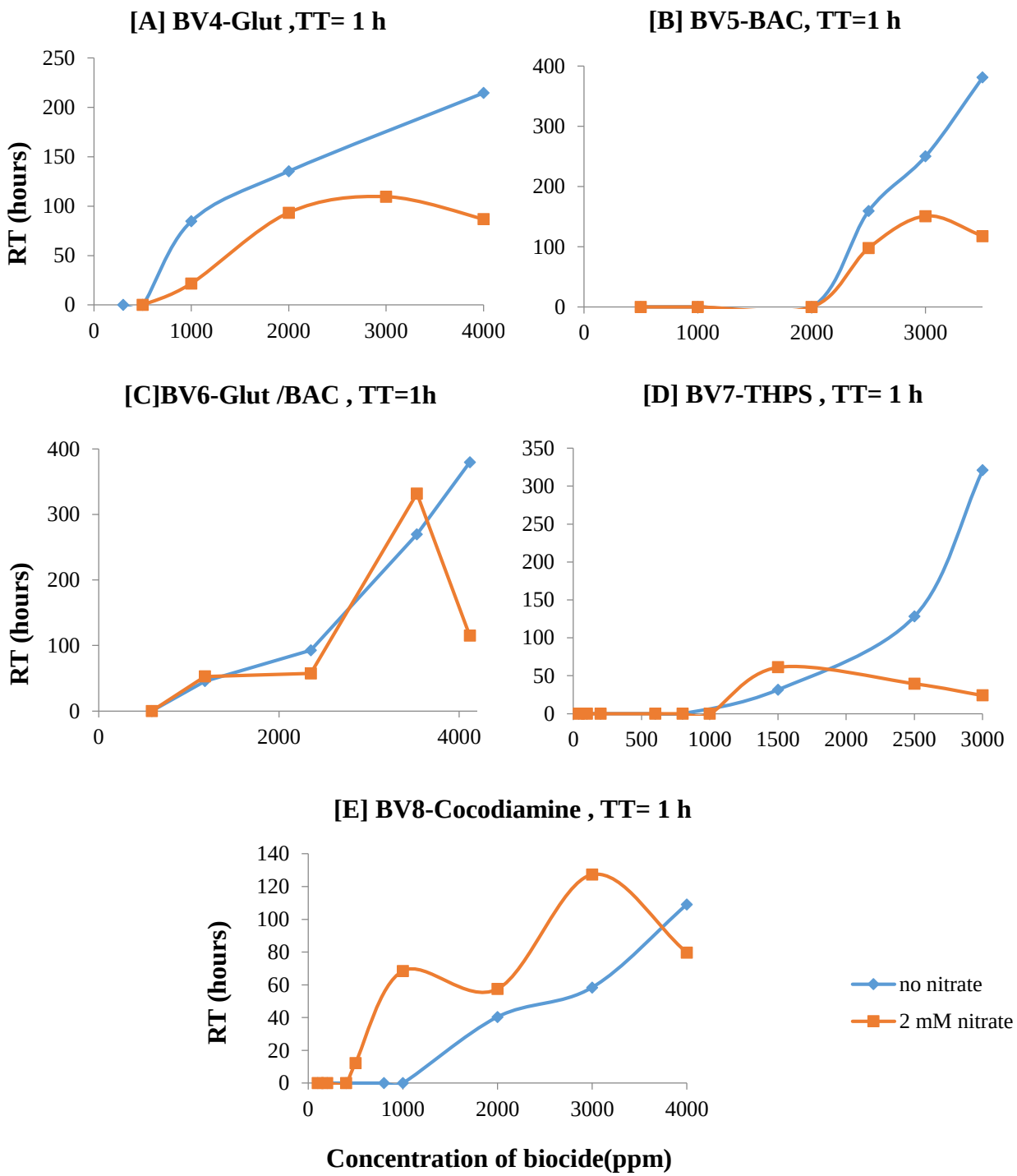


Figure 5.4 Comparison of the efficacy of 1-h pulse of biocide on souring control. The orange squares represent the treatment with continuous injection of nitrate. The blue diamonds represent the treatment in the absence of nitrate.

Chapter 6: **Discussion and Conclusions**

6.1 Discussion

The production of sulfide by sulfate-reducing bacteria (SRB) can be remediated by nitrate injection, a relatively new and "green" method to control souring. Success has been achieved in high-temperature oil reservoirs with nitrate. However, its application in the MHGC (Section 1.4.2.3), a low temperature oil field, poses a challenge because sulfide production resumed to pre- nitrate treatment levels after a transient inhibition. Voordouw et al., (2009) credited this observation to microbial zonation in which SRB move away from NRB zone into deeper reservoir where sulfate is still available and NRB inhibition and temperature limits do not exist. Callbeck et al. (2011) also demonstrated a microbial succession along bioreactor path by integrating the result of chemical assay and community analysis. Disrupting the zone or succession is thus crucial to improve low-temperature souring control. Although pulsed injection of high concentration of nitrate has shown promising results in the field to lower sulfide, it was only observed for a single well, not in other neighbouring wells (Voordouw et al. 2009).

Biocides are routinely used above ground to control biofouling and biocorrosion, but rarely injected into actual reservoir to control souring. The combination of two biocides or a biocide and a metabolic inhibitor has been studied to overcome bacterial resistance and enhance the efficiency of souring control (Gieg et al. 2011). However, a combination of biocide and nitrate has never been investigated before. This work was initiated based on the hypothesis that the synergy between biocide and nitrate can disrupt microbial zonation and lead to more effective souring control. Based on the work of Callbeck (2012), the synergistic biocides were expected to be physically-reactive biocides, not chemically-reactive biocides, because the surfactant trait of physically-reactive biocides was thought to be able to protect the biocide from

being inactivated by chemical reactions with biomass.

In the current work, up-flow sand-packed bioreactors injected with excess VFA and limited concentration of sulfate and/or nitrate were employed to test the hypothesis. Biocides were pulsed into bioreactors by using two strategies: long duration (5 days) low concentration (L-L) and short duration (1 hour) high concentration (S-H). Sulfide recovery time was used to quantify the rate of re-establishment of SRB activity. The synergy between pulsed biocide and continuous nitrate was obtained by comparing the sulfide recovery times acquired from the bioreactors continuously injected with 2 mM sulfate and the sulfide recovery times acquired from the bioreactors continuously injected with 2 mM sulfate and 2 mM nitrate.

Continuous injection of 2 mM nitrate did not inhibit sulfide production, which is reasonable because the excess electron donors exclude the competitive mechanism between NRB and SRB. The complete reduction of nitrate to N_2 suggests that the only effect of injecting 2 mM nitrate was to stimulate the growth of NRB in the NIWR, establishing microbial zonation. Because no separated NRB enrichment was inoculated into the column, NRB that were originally from produced water were believed to ferment organic acids when nitrate was absent (Callbeck et al. 2013). Nitrate did not inhibit sulfide production until the concentration was increased to 13.3 mM (Figure 4.4, A), in which the inhibition mechanism is competitive exclusion according to the stoichiometry in Table 3.4. Reducing 2 mM sulfate needs 1.6 mM VFA, the remaining 1.4 mM VFA (3 mM in total) is enough to reduce 11.76 mM nitrate. Therefore, any concentration of nitrate above 11.76 mM will cause competition for carbon source with SRB. This explains why 13.3 mM nitrate could inhibit sulfate reduction. No nitrate or nitrite was observed in the effluent of a column injected with 13.3 mM nitrate suggesting that nitrate was completely reduced. However, the effluent did contain 0.17 mM sulfate. It indicates

that nitrate reduction was preferable compared to sulfate reduction.

6.1.1 Comparison of 5-day and 1-hour biocide treatments

The efficacy of using biocide to control souring by using these two strategies, biocide pulsed for 5 days (L-L) and biocide pulsed for 1 hour (S-H), is compared in Figure 6.1. With 5 days of biocide treatment, the injection flow rate was 3 PV per day and the volume of biocide used was actually 15 PV; whereas in the strategy of 1-hour biocide treatment, the injection flow rate was 6 PV/day and the volume of biocide used was 1/4 PV. Larger columns (PV=25.7 mL) were also used for the 5-day treatments than for the 1-h treatments (PV= 10.9 mL), Therefore, we corrected this to compare the effectiveness of the two strategies by plotting RT against the dose of biocide (mg) divided by the pore volume (mg/mL) (Figure 6.1).

In the absence of nitrate, the results indicate that when using the same amount of biocide per mg of PV, the 5-day treatment (L-L) strategy gave shorter sulfide recovery times than 1-hour treatment (S-H) strategy for both Glut and BAC (Figure 6.1, A and B, blue and green lines). However, the opposite was true for cocodiamine (Figure 6.1, C, blue and green lines). This suggests that short duration high concentration (S-H) is more effective than long duration low concentration (L-L) when Glut or BAC is used to control souring, while L-L is preferred over S-H when cocodiamine is chosen to control souring. When biocide was used together with continuous nitrate injection (2 mM) to control souring, a similar result was found (Figure 6.1, red and purple lines).

6.1.2 Synergy between nitrate and biocide

Continuous injection of nitrate combined with pulsed biocide for 5 days or for 1 hour duration were studied in this work to control souring. The results of synergy tests for both 1-h

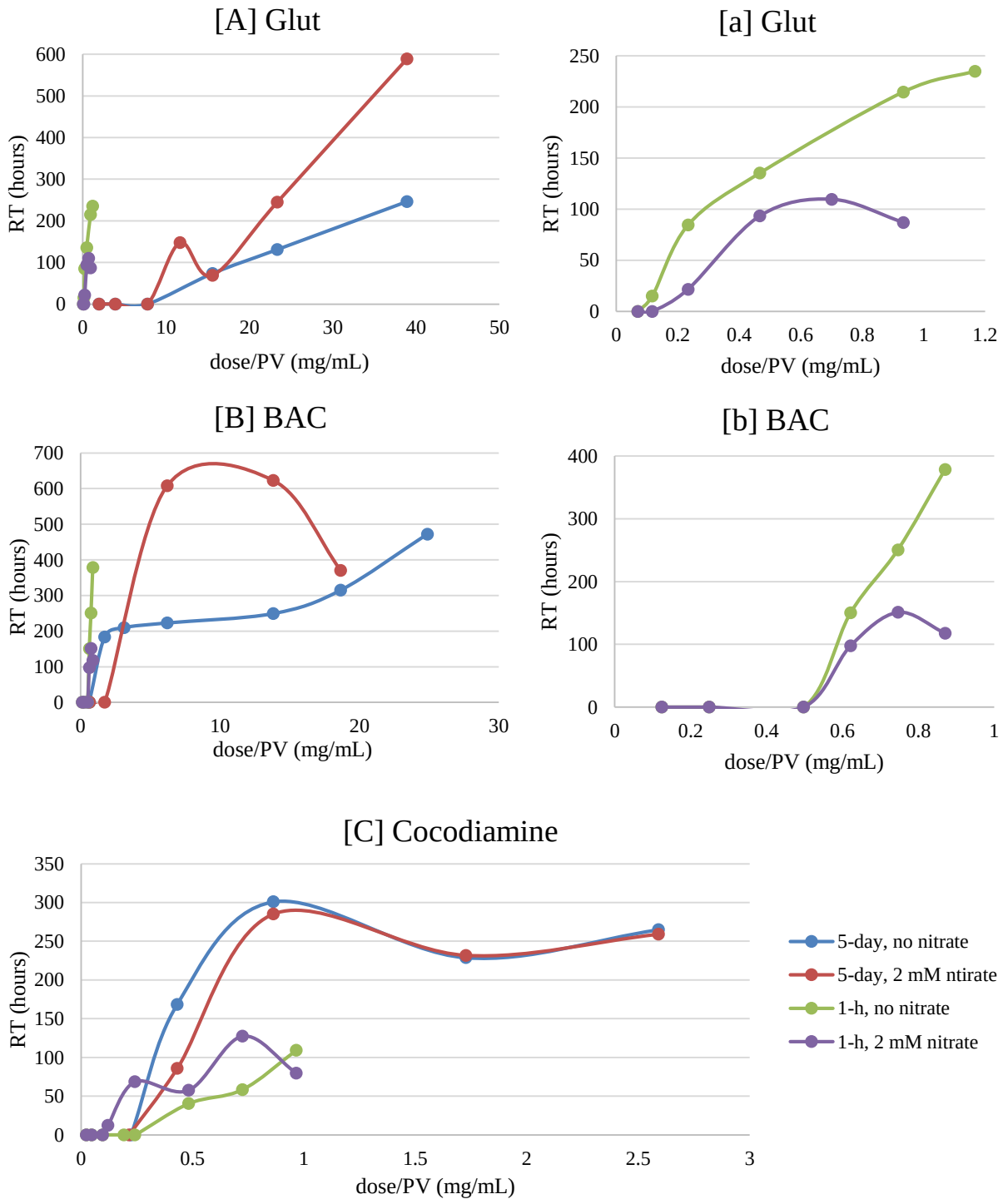


Figure 6.1 Comparison of 5-day and 1-h pulsed strategies to control souring. [A] is with Glut, [B] is with BAC and [C] is with cocodiamine. Blue lines are 5-day treatments without nitrate, red lines are 5-day treatments combined with 2 mM nitrate, green lines are 1-h treatments without nitrate and purple lines are 1-h treatments combined with 2 mM nitrate. Since 1-h treatments with and without nitrate for Glut and BAC are clustered together on the current scale, they were zoomed in to give a clearer view on a smaller scale in [a] and [b], respectively.

and 5-day treatments are compared in Table 6.1. Both Glut and BAC were found to be synergistic with nitrate when pulsed for 5 days, but antagonistic when pulsed for 1 hour. The addition of nitrate had no effect on 5-day cocodiamine treatment, yet enhanced the inhibitory effect of 1-h pulses. Consequently, beside the kind of biocides used, the synergy between pulsed biocide and continuous nitrate to control souring also depends on the biocide treatment strategy (L-L or S-H).

The most important discovery of this research is that pulsed biocide can be synergistic with continuous nitrate. The synergy between two biocides or biocide and metabolic inhibitor (nitrite and molybdate) has been studied before (Greene et al. 2006; Nemati et al. 2001; Mustafa et al. 1996). However, no previous studies are seen on the combination of nitrate and biocides to control souring. Theoretically, the synergy between nitrate and biocide is believed impossible. Firstly, nitrate, unlike nitrite, has no direct impact on SRB. Secondly, using biocide to control SRB that are living in the zone of an oil reservoir following the NRB zone during nitrate injection may not be expected, because NRB can protect SRB by inactivating the incoming biocides. However, the synergy between nitrate and biocide was indeed observed in this research.

The synergy between nitrate and biocide was possibly caused by the formation of nitrite that may either be synergistic with biocide or directly inhibit SRB activity making SRB more vulnerable during biocide treatment. The mechanism of nitrite controlling sulfide production is well-known. Nitrite outcompetes sulfite in binding to Dsr, inhibiting sulfite reduction. Nitrite can also act as sulfide scavenger to remove sulfide abiotically via the following reaction (Sturman et al. 1999; Reinsel et al. 1996) :

$$3\ H_2S + 3\ HS^- + 4\ NO_2^- + 7\ H^+ \rightarrow 0.75\ S_8^0 + 2\ N_2 + 8\ H_2O$$

Greene et al. (2006) have proved that nitrite was synergistic with all tested biocides including Glut, BAC and cocodiamine except THPS (indifferent) to control microbial sulfide production. Since 2 mM nitrate was completely reduced to N_2 without accumulation of nitrite, the produced nitrite during or after biocide treatment is attributed to the impact of biocide on NRB. Information on nitrite production during or after biocide treatment with BAC and cocodiamine is listed in Table 6.2. The area of the nitrite peak was calculated as an indicator of the amount of produced nitrite. The nitrite production resulting from biocide treatment can be thus expressed graphically by plotting nitrite peak area (or nitrite amount) against biocide dose to bioreactor pore volume (mg/mL) (Figure 6.2, A and C). Also, they are compared with the sulfide inhibition resulting from BAC and cocodiamine treatment in the presence of nitrate that are originally from Figure 6.1 (Figure 6.2, B and D). The similar trend in nitrite production and sulfide inhibition suggests that the produced nitrite may cause sulfide inhibition.

Although the concentrations of nitrite produced from nitrate reduction in BAC and cocodiamine columns are very low (0.01-0.64 mM), the potential to impact SRB or synergistically inhibit SRB with biocides is not negligible. Reinsel et al. (1996) used sandstone columns inoculated with oilfield produced water to study the injection of nitrate, nitrite and glutaraldehyde on souring control at 60^{o}C. The study showed that 0.57 mM nitrite resulting from 0.71 mM nitrate addition could completely suppress sulfate reduction, while a direct injection of 0.71 mM nitrite could only suppress 90% sulfide, and a concentration of 0.86 mM nitrite was needed to give a complete souring control. In this case, a low concentration of nitrite was proved to be effective to control souring and the biologically produced nitrite also seemed to be more effective than direct nitrite injection.

Interestingly, a synergy between nitrate and biocide (5 days) was also found in the Glut-

Table 6.1 Comparison of the synergy results of 5-day and 1-h pulsed biocide treatments to control souring

Biocides	5-day	1-h
Glut	+	-
BAC	+	-
Cocodiamine	≈	+
Glut/BAC	N/A	≈
THPS	N/A	-

"+" means synergistic; "-" means antagonistic;
"≈"means indifferent; "N/A" means no test was conducted.

Table 6.2 Information of nitrite produced during or after biocide treatment

BV-biocide	Synergy result with nitrate	Biocide pulse (ppm)	Nitrite lasting period (days)	Nitrite concentration range (mM)	Area of nitrite peak (h*mM)
BV2-BAC (5-day)	synergy	100	0	0	0
		360	8	0.01-0.21	25.85
		800	13	0.04-0.53	64.81
		1080	12	0.05-0.64	46.28
BV5-BAC (1-h)	antagonistic	2000	< 1	0.17	0
		2500	1	0.08-0.43	19.25
		3000	1	0.01-0.10	1.63
		3500	<1	0.06	0
BV3-Cocodiamine (5-day)	indifferent	12.5	0	0	0
		25	3	0.1-0.2	12.62
		50	6	0.02-0.23	15.14
		100	9	0.1-0.3	40.04
		150	7	0.1-0.25	22.8
BV8-Cocodiamine (1-h)	synergy	400	0	0	0
		500	0	0	0
		1000	1.7	0.1-0.21	4.72
		2000	2	0.02-0.64	8.43
		3000	2.7	0.03-0.44	19.9
		4000	<1	0.1-0.60	14.3

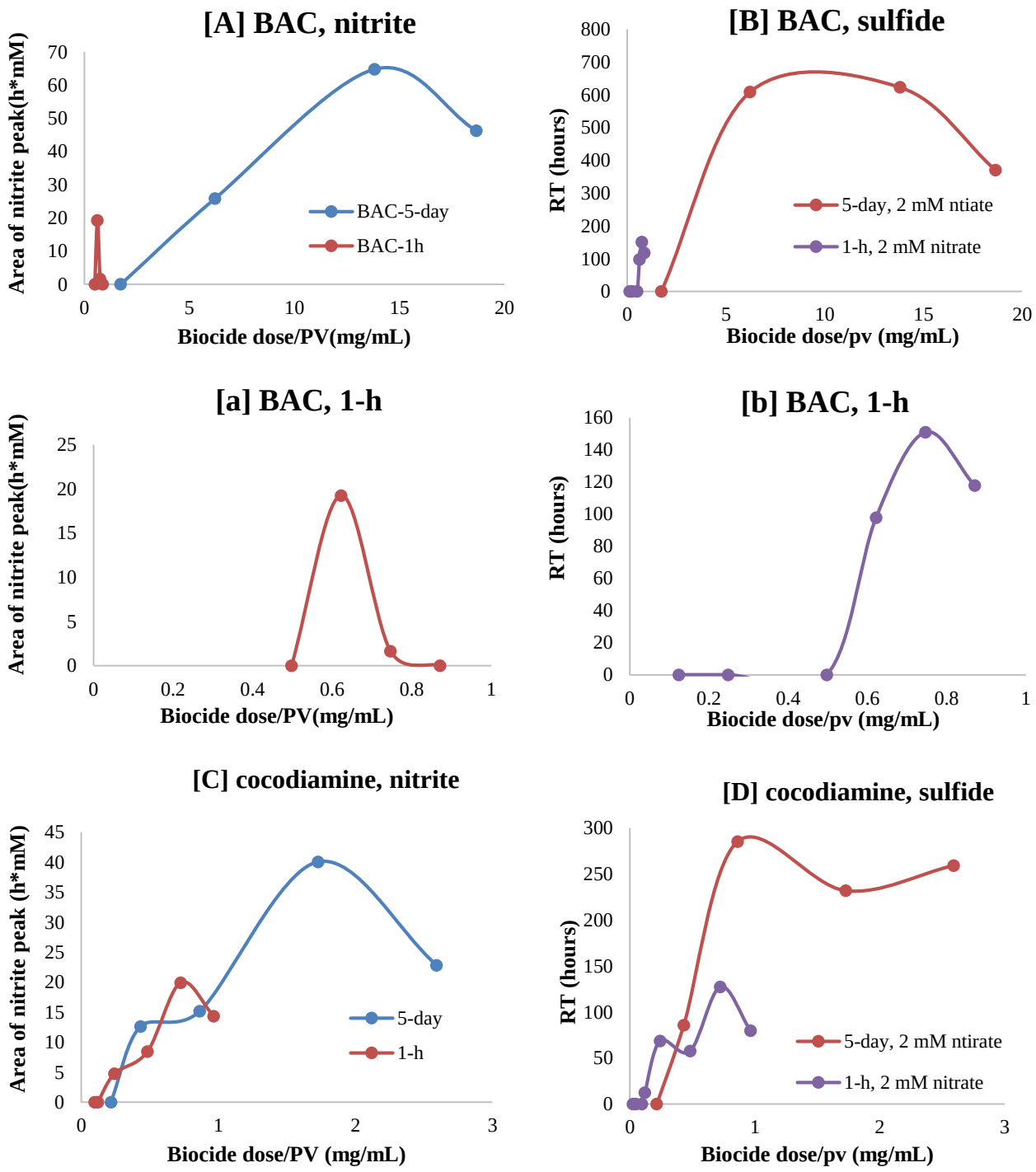


Figure 6.2 Comparison of nitrite production and sulfide inhibition for BAC and cocodiamine treatments. Nitrite production (quantified by peak area) from [A] BAC and [C] cocodiamine treatment is plotted against biocide dose to PV. Sulfide inhibition (quantified by RT) due to biocide treatments of [B] BAC and [D] cocodiamine is from Figure 6.1, B and C. Since 1-h pulses of BAC in [A] and [B] are too small on the current scale, they are zoomed in to give a clear view on a smaller scale in [a] and [b], respectively.

treated column that did show no or little nitrite breakthrough in the effluent. The synergy does not seem to be caused by nitrite. A possible explanation is that long-term injection of nitrate to control souring may lead to a rise of redox potential in the column, which is detrimental for sulfate reduction. Reinsel et al. (1996) showed that after nitrate addition the redox potential increased to above zero with the lowest potential occurring in the first 5 cm of the column. However, the decrease was concomitant with the inhibition of sulfide, which was not the case in our 2 mM nitrate injection. Nevertheless, the mechanism for synergy between Glut and nitrate is still unclear.

Surprisingly, no synergy was observed for nitrate and the blend of Glut and BAC that is usually used as a mixture due to their enhanced biocidal effect. Additionally, the reason why biocide pulse strategy (L-L or S-H) can alter the synergy condition between nitrate and biocide is also mysterious. It was originally thought that it was because long time biocide treatment was able to give a corresponding long lasting presence of nitrite. However, a longer lasting presence of nitrite was observed in 5-day pulse of cocodiamine that was indifferent to nitrate addition compared to the 1-h pulse of cocodiamine that was synergistic to nitrate (Table 6.2).

If the L-L and S-H comparison results (Section 6.1.1) and the synergy results (Table 6.1) are combined, it is found that the synergy for Glut, BAC and cocodiamine were all associated with the less effective treatment strategy. For example, both Glut and BAC were demonstrated to be synergistic with nitrate with the 5-day treatment (or L-L strategy) that was found to be less effective compared to S-H. As for cocodiamine, the synergy with nitrate was observed with 1-hour pulse (or S-H) that was less effective than L-L treatment strategy. Even though we are not sure if this correlation is significant, this discovery may suggest that a higher biocide efficiency and synergy with nitrate may not be achieved simultaneously, which is not a good sign for

industrial operations. THPS and a Glut/BAC blend were not tested with a 5-day pulse, so that this correlation cannot be verified to further test this hypothesis.

6.2 Conclusions and future work

Overall, the conclusion that can be drawn from this work is that pulsed biocide and continuous nitrate can achieve synergy in controlling souring. However, it depends on the biocide pulse strategy: long time low concentration or short time high concentration. The pulse strategy also affects the efficacy of a biocide. The difference between physically-reactive biocides and chemically-reactive biocides is not the cause of synergy between nitrate and biocide in a zoned sour reservoir.

Microbial community analysis will be done next by dismantling the column and collecting samples from the bottom part (1/3) of the entire sand core, which is believed to contain most of biomass (Callbeck et al. 2011). Microbial community structure change under biocide stress will be examined by comparing against the samples from the control column that was injected with 2 mM sulfate and then switched to 2 mM sulfate and 2 mM nitrate. The community study can help us gain an insight into the molecular aspect of biocide treatment effects on microbial consortia.

This work has examined the effect of combined nitrate and biocide on souring mitigation in an attempt to break microbial zonation and enhance souring control. Although synergy was found between nitrate and biocide, the mechanism behind that still needs to be further investigated. Since nitrite was suspected to be the major reason causing synergy between nitrate and biocide, its production under biocide stress and synergistic effect with biocide on SRB biofilm in a sand-packed column must be studied to gain a better understanding of the reactions in this complex system.

To sum up, the major finding of this work is that nitrate and biocide can be synergistic to control microbial sulfide production. However, the synergy depends on biocide pulse strategy. Nitrite formation from nitrate reduction by NRB can be a possible mechanism for the synergy. The synergy between nitrate and biocide tends to be caused by a complex interaction amongst biocide, NRB, SRB and their metabolites and the finding will change the common view on simultaneous injection of biocide and nitrate, which is the existence of a conflict between the encouragement of NRB growth and the inhibition by biocide.

References

Augustinovic Z, Birketveit Ø, Clements K, Freeman M, Gopi S, Ishoey T, Jackson G, Kubala G, Larsen J, Marcotte BWG, Scheie J, Skovhus TL, Sunde E (2012) Microbes-oilfield enemies or allies? Oilfield Review 24:4–17.

Bachmann RT, Johnson AC, Edyvean RGJ (2014) Biotechnology in the petroleum industry: An overview. Int Biodeterioration & Biodegradation 86:225–237.

Barton LL, Fauque GD (2009) Biochemistry, physiology and biotechnology of sulfate-reducing bacteria. Adv Appl Microbiol 68:41–98.

Beauchamp RO, Bus JS, Popp JA, Boreiko CJ, Andjelkovich DA (1984) A critical review of the literature on hydrogen sulfide toxicity. CRC Crit Rev Toxicol 13:25–97.

Bødtker G, Thorstenson T, Lillebø B-LP, Thorbjørnsen BE, Ulvøen RH, Sunde E, Torsvik T (2008) The effect of long-term nitrate treatment on SRB activity, corrosion rate and bacterial community composition in offshore water injection systems. J Ind Microbiol Biotechnol 35:1625–1636.

Bonapace CR, White RL, Friedrich L V, Bosso JA (2000) Evaluation of antibiotic synergy against *Acinetobacter baumannii*: a comparison with Etest, time-kill, and checkerboard methods. Diagn Microbiol Infect Dis 38:43–50.

Borzenkov I, Belyave S, Miller Y, Davydova I, Ivanov M (1997) Methanogenesis in the highly mineralized stratal waters of the Bondyuzhskoe oil field. Microbiology (Mikrobiologiya) 66:104–110.

Brock TD, Madigan MT, Martinko JM, Parker J (1994) Chapter 16 Metabolic diversity among the microorganisms. In: Brake DK (ed) Biology of Microorganisms, 7th ed. Prentice-Hall, Inc, Englewood Cliffs, NJ, pp 575–622.

Callbeck CM (2012) Souring control by nitrate and biocides in up-flow bioreactors simulating oil reservoirs. Master thesis, University of Calgary.

Callbeck CM, Agrawal A, Voordouw G (2013) Acetate production from oil under sulfate-reducing conditions in bioreactors injected with sulfate and nitrate. Appl Environ Microbiol 79:5059–5068.

Callbeck CM, Dong X, Chatterjee I, Agrawal A, Caffrey SM, Sensen CW, Voordouw G (2011) Microbial community succession in a bioreactor modeling a souring low-temperature oil reservoir subjected to nitrate injection. Appl Microbiol Biotechnol 91:799–810.

Chang A-F, Pashikanti K, Liu YA (2012) Characterization, physical and thermodynamic properties of oil fractions. Refinery Engineering: Integrated Process Modeling and

Optimization, 1st ed. Wiley-VCH Verlag Gmbh & Co.KGaA, Weinheim Germany, pp 1–56.

Chen C-I, Reinsel MA, Mueller RF (1994) Kinetic investigation of microbial souring in porous media using microbial consortia from oil reservoirs. Biotechnol Bioeng 44:263–269.

Coombe D, Hubert C, Voordou G (2004) Mechanistic modelling of H_2S souring treatments by application of nitrate or nitrite. Pap. 2004-292, Can. Int. Pet. Conf. Petroleum Society of Canada, Calgary, AB, pp 1–16.

Davidova I, Hicks MS, Fedorak PM, Suflita JM (2001) The influence of nitrate on microbial processes in oil industry production waters. J Ind Microbiol Biotechnol 27:80–86.

Davis RA, McElhiney JE (2002) The advancement of sulfate removal from seawater in offshore waterflood operation. Pap. 02314, Corros. 2002. NACE International, Denver, Colorado, pp 1–13.

Davydova-Charakhchyan IA, Mileeva AN, Mityushina LL, Belyaev SS (1992) Acetogenic bacteria from oil-fields of Tataria and western Siberia. Microbiology 61:208–216.

Downward BL, Talbot RE, Haack TK, Allen G (1997) Tetrakishydroxymethylphosphonium sulfate (THPS) a new industrial biocide with low environmental toxicity. Pap. 401, Corros. 97, NACE Conf. Pap. NACE International, New Orleans, Louisiana, pp 1–11.

Enning D, Garrelfs J (2014) Corrosion of iron by sulfate-reducing bacteria: new views of an old problem. Appl Environ Microbiol 80:1226–1236.

Enning D, Venzlaff H, Garrelfs J, Dinh HT, Meyer V, Mayrhofer K, Hassel AW, Stratmann M, Widdel F (2012) Marine sulfate-reducing bacteria cause serious corrosion of iron under electroconductive biogenic mineral crust. Environ Microbiol 14:1772–1787.

Fisher JB (1987) Distribution and occurrence of aliphatic acid anions in deep subsurface waters. Geochimica et Cosmochimica Acta 51:2459–2468.

Ganzer GA, McIlwaine DB, Diemer JA, Freid M, Russo M (2001) Applications of glutaraldehyde in the control of MIC. Pap. 01281, Corros. 2001. NACE International, Houston, TX, pp 1–8.

Gevertz D, Telang AJ, Voordouw G, Jenneman GE (2000) Isolation and characterization of strains CVO and FWKO B , two novel nitrate-reducing , sulfide-oxidizing bacteria isolated from oil field brine. Appl Environ Microbiol 66:2491–2501.

Giangiacomo LA, Dennis DM (1997) Field testing of the biocompetitive exclusion process for control of iron and hydrogen sulfides. SPE 38351, SPE Rocky Mt. Reg. Meet. Society of Petroleum Engineers, Casper, Wyoming, pp 125–135.

Gieg LM, Fowler SJ, Berdugo-Clavijo C (2014) Syntrophic biodegradation of hydrocarbon contaminants. Curr Opin Biotechnol 27:21–29.

Gieg LM, Jack TR, Foght JM (2011) Biological souring and mitigation in oil reservoirs. Appl Microbiol Biotechnol 92:263–282.

Gorman SP, Scott EM, Russell AD (1980) A review antimicrobial activity, uses and mechanism of action of glutaraldehyde. J Appl Bacteriol 48:161–190.

Greene EA, Brunelle V, Jenneman GE, Voordouw G (2006) Synergistic inhibition of microbial sulfide production by combinations of the metabolic inhibitor nitrite and biocides. Appl Environ Microbiol 72:7897–7901.

Greene EA, Hubert C, Nemati M, Jenneman GE, Voordouw G (2003) Nitrite reductase activity of sulphate-reducing bacteria prevents their inhibition by nitrate-reducing, sulphide-oxidizing bacteria. Environ Microbiol 5:607–617.

Grigoryan A, Voordouw G (2008) Microbiology to help solve our energy needs: methanogenesis from oil and the impact of nitrate on the oil-field sulfur cycle. Ann N Y Acad Sci 1125:345–352.

Grigoryan AA, Cornish SL, Buziak B, Lin S, Cavallaro A, Arensdorf JJ, Voordouw G (2008) Competitive oxidation of volatile fatty acids by sulfate- and nitrate-reducing bacteria from an oil field in Argentina. Appl Environ Microbiol 74:4324–4335.

Grobe KJ, Stewart PS (2000) Characterization of glutaraldehyde efficacy against bacterial biofilm. Pap. 00124, Corros. 2000,. NACE International, Orlando, FL, pp 1–11.

Guidotti TL (1994) Occupational exposure to hydrogen sulfide in the sour gas industry: some unresolved issues. Int Arch Occup Environ Health 66:153–160.

Hamilton WA (1983) Sulphate-reducing bacteria and the offshore oil industry. Trends Biotechnol 1:36–40.

Hao OJ, Chen JM, Huang L, Buglass RL (1996) Sulfate-reducing bacteria. Crit Rev Environ Sci Technol 26:155–187.

Haveman SA, Greene EA, Stilwell CP, Voordouw JK, Voordouw G (2004) Physiological and gene expression analysis of inhibition of *Desulfovibrio vulgaris* Hildenborough by nitrite. J Bacteriol 186:7944–7950.

Head IM, Jones DM, Larter SR (2003) Biological activity in the deep subsurface and the origin of heavy oil. Nature 426:344–52.

Hubert C (2010) Microbial ecology of oil reservoir souring and its control by nitrate injection. In: Timmis KN (ed) Handbook of Hydrocarbon and Lipid Microbiology. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 2754–2766.

Hubert C, Nemati M, Jenneman G, Voordouw G (2003) Containment of biogenic sulfide production in continuous up flow packed be bioreactors. Biotechnol Prog 19:338–345.

Javaherdashti R (2011) Impact of sulphate-reducing bacteria on the performance of engineering materials. Appl Microbiol Biotechnol 91:1507–1517.

Jeanthon C, Nercessian O, Corre E, Grabowski-Lux A (2005) Hyperthermophilic and methanogenic archaea in oil fields. In: Ollivier B, Magot M (eds) Petroleum Microbiology. ASM press, Washington, DC, pp 55– 69.

Jenneman GE, Moffitt PD, Bala GA, Webb RH (1999) Sulfide removal in reservoir brine by indigenous bacteria. SPE Prod Facil 14:219–225.

Jones C, Downward B, Edmunds S, Curtis T, Smith F (2012) THPS:a review of the first 25 years, lessons learned, value created and visions for the future. Pap. 1505, Corros. 2012, NACE Conf. Expo. NACE International, Salt Lake City, Utah, pp 1–14.

Jones C, Downward B, Edmunds S, Hernandez K, Curtis T, Smith F (2011) A novel approach to using THPS for controlling reservoir souring. Pap. 11219, Corros. 2011, NACE Conf. Expo. NACE International, Houston, TX, pp 1–11.

Jones CR, Downward BL, Hernandez K, Curtis T, Smith F (2010) Extending performance boundaries with third generation THPS formulations. Pap. 10257, Corros. 2010, NACE Conf. Expo. NACE International, San Antonio, Texas, pp 1–14.

Khatib ZI, Salanitro JP (1997) Reservoir souring:analysis of surveys and experience in sour waterfloods. SPE 38795. Proc. SPE Annu. Tech. Conf. Society of Petroleum Engineers, Rchardson,Tex, pp 449–459.

Kraft B, Strous M, Tegetmeyer HE (2011) Microbial nitrate respiration-genes, enzymes and environmental distribution. J Biotechnol 155:104–117.

Kramer JF (2006) A new high performance quaternary phosphonim biocide for biofouling control in industrial water systems. Pap. 06093, Corros. 2006, 61st Annu. Conf. Expo. NACE International, San Diego, CA, pp 1–14.

Kramer JF, Brien FO, Strba SF (2008) A new high performance quaternary phosphonium biocide for microbiological control in oilfield water systems. Pap. 08660, Corros. 2008, NACE Conf. Expo. NACE International, New Orleans, Louisiana, pp 1–14.

Kramer JF, Srivastava A, Strba SF (2010) Comparative performance of biocides versus corrosion causing biofilms. Pap. 10258, Corros. 2010,NACE Conf. Expo. NACE International, San Antonio, TX, pp 1–13.

Kriel BG, Crews AB, Burger ED, Vanderwende E, Hitzman DO (1993) The efficacy of formaldehyde for the control of biogenic sulfide production in porous media. SPE 25196, SPE Int. Symp. Oilf. Chem. Society of Petroleum Engineers, New Orleans, LA, pp 1–8.

Kuijvenhoven C, Bostock A, Chappell D, Noirot J, Khan A (2006) Use of nitrate to mitigate reservoir souring in Bonga deepwater development, offshore Nigeria. SPE 92795, Prod. Oper. Society of Petroleum Engineers, Houston, Texas, pp 467–474.

Larsen J (2002) Downhole nitrate applications to control sulfate reducing bacteria activity and reservoir souring. Corros. 2002. Pap. 02025. NACE International, Houston, Tex, pp 1–10.

Larsen J, Rod MH, Zwolle S (2004) Prevention of reservoir souring in the Halfdan field by nitrate injection. Pap. 04761, Corros. 2004. NACE International, New Orleans, Louisiana, pp 1–18.

Larsen J, Sanders PF, Talbot RE (2000) Experience with the use of tetrakishydroxymethylphosphonium sulfate (THPS) for the control of downhole hydrogen sulfide. Pap. 00123, Corros. 2000, Maersk Oil Gas, Oil Plus, Albright Wilson. NACE International, Orlando, FL, pp 1–18.

Lee D, Lowe D, Grant P (1996) Microbiology In the Oil Patch: A Review. Pap. 96-109, 47th Annu. Tech. Meet. Pet. Soc. Petroleum Society of Canada, Calgary, AB, pp 1–7.

Liamleam W, Annachhatre AP (2007) Electron donors for biological sulfate reduction. Biotechnol Adv 25:452–463.

Machel HG (2001) Bacterial and thermochemical sulfate reduction in diagenetic settings- old and new insights. Sediment Geol 140:143–175.

Macleod N, Bryan J, Buckley AJ, Talbot RE, Veale MA (1994) A novel biocide for oilfield applications. SPE Conf. Society of Petroleum Engineers, Aberdeen,UK, pp 1–18.

Magot M (2005) Indigenous microbial communities in oil fields. In: Ollivier B, Magot M (eds) Petroleum Microbiology. ASM press, Washington, pp 21–33.

Magot M, Ollivier B, Patel BK. (2000) Microbiology of petroleum reservoirs. Antonie Van Leeuwenhoek 77:103–16.

Maxwell S (2005) Controlling corrosive biofilms by the application of biocides. SPE93172, SPE Int. Symp. Oilf. Corros. Society of Petroleum Engineers, Aberdeen, UK., pp 1–8.

McGinley HR, Enzien M V, Jenneman G, Harris J (2011) Studies on the chemical stability of glutaraldehyde in produced water. SPE 141449, SPE Int. Symp. Oilf. Chem. Society of Petroleum Engineers, Woodlands, TX, pp 1–8.

Mcginley HR, Van Der Kraan GM (2013) Benchmarking of biocidal chemistries for the control of corrosion biofilms. SPE 156036, SPE Int. Conf. Work. Oilf. Corros. Society of Petroleum Engineers, Aberdeen,UK, pp 1–9.

McInerney MJ, Sieber JR, Gunsalus RP (2009) Syntrophy in anaerobic global carbon cycles. Curr Opin Biotechnol 20:623–632.

McInerney MJ, Wofford NQ, Sublette KL (1996) Microbial control of hydrogen sulfide production in a porous medium. Appl Biochem Biotechnol 57/58:933–944.

Moore SL, Cripps CM (2012) Bacterial survival in fractured shale-gas wells of the Horn River basin. J Can Pet Technol July:283–289.

Mustafa CM, Rahman AKMO, Begum DA (1996) Effects of time and temperature on the mild steel corrosion inhibition by molybdate and nitrite. Indian J Chem Technol 3:44–48.

Muyzer G, Stams AJM (2008) The ecology and biotechnology of sulphate-reducing bacteria. Nat Rev Microbiol 6:1–14.

Myhr S, Lillebø BLP, Sunde E, Beeder J, Torsvik T (2002) Inhibition of microbial H_2S production in an oil reservoir model column by nitrate injection. Appl Microbiol Biotechnol 58:400–408.

Nemati M, Mazutinec TJ, Jenneman GE, Voordouw G (2001) Control of biogenic H_2S production with nitrite and molybdate. J Ind Microbiol Biotechnol 26:350–355.

Ollivier B, Cayol J (2005) Fermentative, iron-reducing, and nitrate-reducing microorganisms. In: Ollivier B, Magot M (eds) Petroleum Microbiology. ASM press, Washington, DC, pp 71–88.

Planckaert M (2005) Oil reservoirs and oil production. In: Ollivier B, Magot M (eds) Petroleum Microbiology. ASM press, Washington, DC, pp 3–19.

Rassenfoss S (2011) From bacteria to barrels : microbiology having an impact on oil fields. J Pet Technol 32–39.

Reinsel MA, Sears JT, Stewart PS, Mclnerney MJ (1996) Control of microbial souring by nitrate , nitrite or glutaraldehyde injection in a sandstone column. J Ind Microbiol 17:128–136.

Rempel CL, Evitts RW, Nemati M (2006) Dynamics of corrosion rates associated with nitrite or nitrate mediated control of souring under biological conditions simulating an oil reservoir. J Ind Microbiol Biotechnol 33:878–886.

Riazi MR (2007) Characterization and properties of pure hydrocarbons. Characterization and Properties of Petroleum Fractions, 1st ed. ASTM International, West Conshohocken, PA, pp 30–86.

Roanea JT, Graue A, Lien T (1991) Activity of sulfate-reducing bacteria under simulated reservoir conditions. SPE Prod Eng 217–220.

Robinson; K, Ginty; W, Samuelsen E, Lundgaard T, Skovhus TL (2010) Reservoir souring in a field with sulphate removal: a case study. SPE 132697,Annual Tech. Conf. Exhib. Society of Petroleum Engineers, Florence, Italy, pp 1–15.

Sandrea I, Sandrea R (2007) Global oil reserves – recovery factors leave vast target for EOR technologies. Oil Gas J 105:1–8.

Seto CJ, Beliveau DA (2000) Reservoir souring in the Caroline field. SPE 59778, SPE/CERI Gas Technol. Symp. Society of Petroleum Engineers, Richardson, TX, pp 1–9.

Shartau SLC, Yurkiw M, Lin S, Grigoryan AA, Lambo A, Park H-S, Lomans BP, van der Biezen E, Jetten MSM, Voordouw G (2010) Ammonium concentrations in produced waters from a mesothermic oil field subjected to nitrate injection decrease through formation of denitrifying biomass and anammox activity. Appl Environ Microbiol 76:4977–4987.

Sheng X, Ting Y-P, Pehkonen SO (2007) The influence of sulphate-reducing bacteria biofilm on the corrosion of stainless steel AISI 316. Corros Sci 49:2159–2176.

Sieber JR, McInerney MJ, Gunsalus RP (2012) Genomic insights into syntrophy: the paradigm for anaerobic metabolic cooperation. Annu Rev Microbiol 66:429–452.

Speight JG (2014) Chapter 1 History and terminology. The Chemistry and Technology of Petroleum, 5th ed. CRC press, Boca Raton, FL, pp 3–30.

Speight JG (1999) Chapter 6 Chemical composition. The Chemistry and Techonology of Petroleum, 3rd ed. CRC press, New York, NY, pp 234–259.

Struchtemeyer CG, Davis JP, Elshahed MS (2011) Influence of the drilling mud formulation process on the bacterial communities in thermogenic natural gas wells of the Barnett Shale. Appl Environ Microbiol 77:4744–4753.

Sturman PJ, Goeres DM, Winters MA (1999) Control of hydrogen sulfide in oil and gas wells with nitrite injection. SPE 56772,SPE Annu. Tech. Conf. Exhib. Society of Petroleum Engineers, Houston, Texas, pp 1–7.

Sunde E, Lillebø B-LP, Bødtker G, Torsvik T, Thorstenson T (2004) H_2S inhibition by nitrate injection on the gullfaks field. Pap. 04760, NACE Corros. Annu. Conf. Expo. NACE International, New Orleans, pp 1–14.

Tanji Y, Toyama K, Hasegawa R, Miyanaga K (2014) Biological souring of crude oil under anaerobic conditions. Biochem Eng J 90:114–120.

Teclu D, Tivchev G, Laing M, Wallis M (2008) Bioremoval of arsenic species from contaminated waters by sulphate-reducing bacteria. Water Res 42:4885–4893.

Thorstenson T, Bodtker G, Lillebo BP, Torsvik T, Sunde E, Fores S, Beeder J (2002) Biocide replacement by nitrate in sea water injection systems. Proc. NACE Expo 2002 Annu. Conf. Expo. NACE International, Denver, CO, Paper 02033.

Truper HG, Schlegel HG (1964) Sulphur metabolism in Thiorhodaceae, I. Quantitative measurements on growing cells of *chromatium Okenii*. Antonie Van Leeuwenhoek 30:225–238.

Turkiewicz A (2011) The role of microorganisms in the oil and gas industry. Rocz Ochr Sr 13:227–239.

Vance I, Thrasher DR (2005) Reservoir souring: mechanisms and prevention. In: Ollivier B, Magot M (eds) Pettroleum Microbiology. ASM press, Washington, DC, pp 123–142.

Vik EA, Janbu AO, Garshol F, Henninge LB, Engebretsen S, Kuijvenhoven C, Oliphant D, Hendriks WP, Shell ASN (2007) Nitrate-based souring mitigation of produced water-side effects and challenges from the Draugen produced- water reinjection pilot. SPE 106178, Int. Symp. Oilf. Chem. Society of Petroleum Engineers, Houston, Texas, pp 1–11.

Voordouw G (2003) Oil field biotechnology : should we use nitrate or nitrite to remediate souring. Pap. 2003-144, Pet. Soc. Can. Int. Pet. Conf. Calgary, AB, pp 1–3.

Voordouw G (2011) Production-related petroleum microbiology: progress and prospects. Curr Opin Biotechnol 22:401–405.

Voordouw G, Armstrong SM, Reimer MF, Fouts B, Telang AJ, Shen YIN, Gevertz D (1996) Characterization of 16S rRNA genes from oil field microbial communities indicates the presence of a variety of sulfate-reducing , fermentative , and sulfide-oxidizing bacteria. Appl Environ Microbiol 62:1623–1629.

Voordouw G, Grigoryan AA, Lambo A, Lin S, Park HS, Jack TR, Coombe D, Clay B, Zhang F, Ertmoed R, Miner K, Arensdorf JJ (2009) Sulfide remediation by pulsed injection of nitrate into a low temperature Canadian heavy oil reservoir. Environ Sci Technol 43:9512–9518.

Voordouw G, Nemati M, Jenneman GE (2002) Use of nitrate reducing, sulfide oxidizing bacteria to reduce souring in oil fields: interactions with SRB and effects on corrosion. Pap. 02034, Corros. 2002. NACE International, Denver, Colorado, pp 1–6.

White RL, Burgess DS, Manduru M, Bosso JA, White RL, Burgess DS, Manduru M, Bosso JA (1996) Comparison of three different in vitro methods of detecting synergy : time-kill , checkerboard, and E test. Antimicrob Agents Chemother 40:1914–1918.

Wolicka D, Borkowski A (2012) Microorganisms and crude oil. In: Romero-Zerón L (ed) Introduction to Enhanced Oil Recovery (EOR): Processes and Bioremediation of Oil-contaminated Sites. In Tech, Rijeka, Croatia, pp 113–143.

Yin B, Enzien M, Love D (2012) Biocide formulations with enhanced performance on sessile and planktonic bacteria control. Pap. Corros. 2012, NACE Conf. Expo. NACE International, Salt Lake City, Utah, pp 1–8.

Youssef N, Elshahed MS, Mclnerney MJ (2009) Microbial processes in oil fields: culprits, problems, and opportunities. Adv Appl Microbiol 66:141–251.

Zengler K, Richnow HH, Rosselló-Mora R, Michaelis W, Widdel F (1999) Methane formation from long-chain alkanes by anaerobic microorganisms. Nature 401:266–269.

Zhang S, Zhu G, Liang Y, Dai J, Liang H, Li M (2005) Geochemical characteristics of the Zhaolanzhuang sour gas accumulation and thermochemical sulfate reduction in the Jixian Sag of Bohai Bay Basin. Org Geochem 36:1717–1730.

Zumft WG (1997) Cell biology and molecular basis of denitrification. Microbiol Mol Biol Rev 61:533–616.

Zuo R (2007) Biofilms: strategies for metal corrosion inhibition employing microorganisms. Appl Microbiol Biotechnol 76:1245–1253.

Appendix A: Toxic effects of exposure to H_2S on humans

(adopted from Beauchamp et al. 1984; Guidotti 1994)

ppm	effect
0.02- 0.13 ppmv	detectable by nose
10 ppmv	obvious rotten egg smell; "sore eyes" 8 h occupational exposure limit in Alberta
15 ppmv	15-min occupational exposure limit in Alberta
20 ppmv	ceiling occupational exposure limit and community evacuation level in Alberta, odor very strong
30 ppmv	eyes inflammation
50 ppmv	inflammation and dryness of the respiratory tract
100 ppmv	olfactory fatigue
150 ppmv	olfactory nerve paralysis
200 ppmv	depression of nervous system
250 ppmv	prolonged exposure can be life threatening via pulmonary edema
500 ppmv	serious damage to eyes within 30min; headache, dizziness; lung irritation; unconsciousness and death within 4-8h
>500 ppmv	acute intoxications respiratory failure with consequent asphyxia and cardiac faillure, may cause death
700 ppmv	paralysis of nervous system
> 700 ppmv	shock, convulsions, inability to breathe, coma and rapidly death
1000 ppmv	unconscious at once, followed by death within minutes
5000 ppmv	imminent death

Appendix B: The effect of 1-h pulse of Glut with continuous nitrate injection on sulfide production on an hour-scale

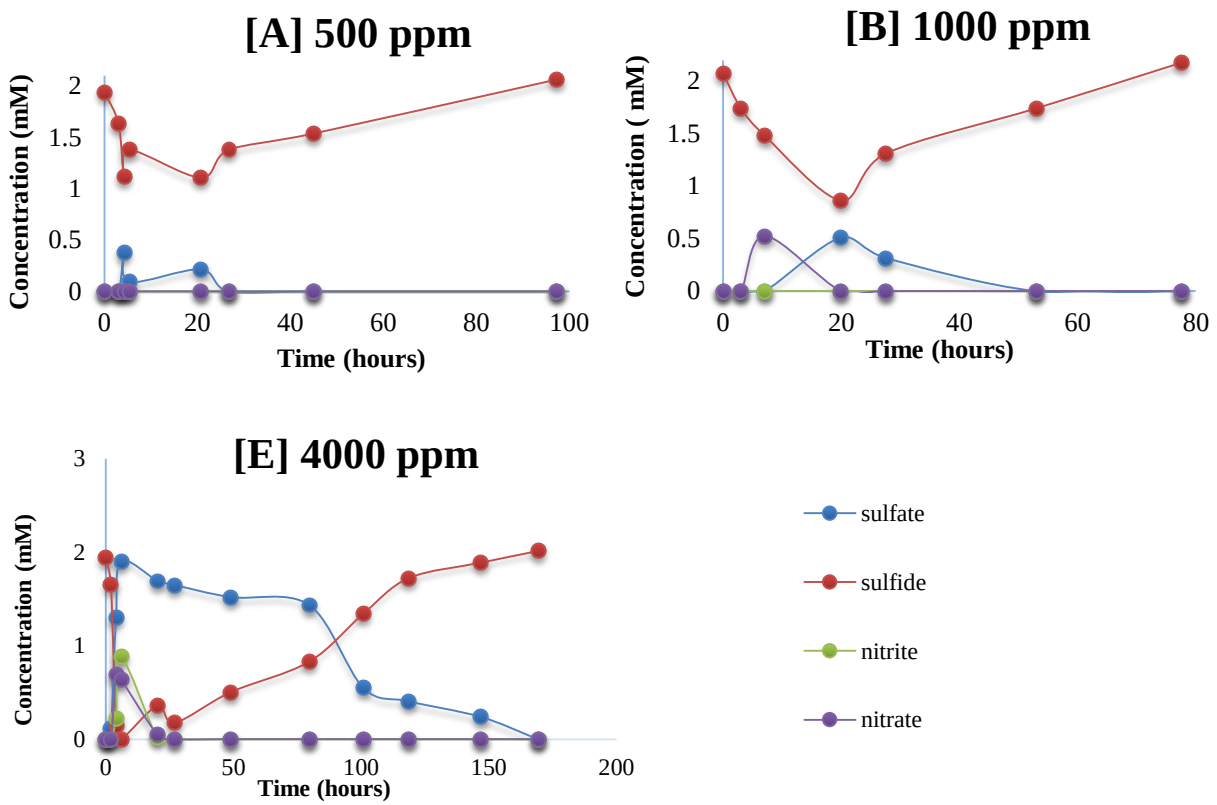


Figure B1 The effect of 1-h pulse of Glut with continuous nitrate injection on sulfide production on an hour-scale. Blue lines are sulfate, red lines are sulfide, green lines are nitrite and purple lines are nitrate. Glut was pulsed in the first hour.

Appendix C: The effect of 1-h pulse of BAC with continuous nitrate injection on sulfide production on an hour-scale

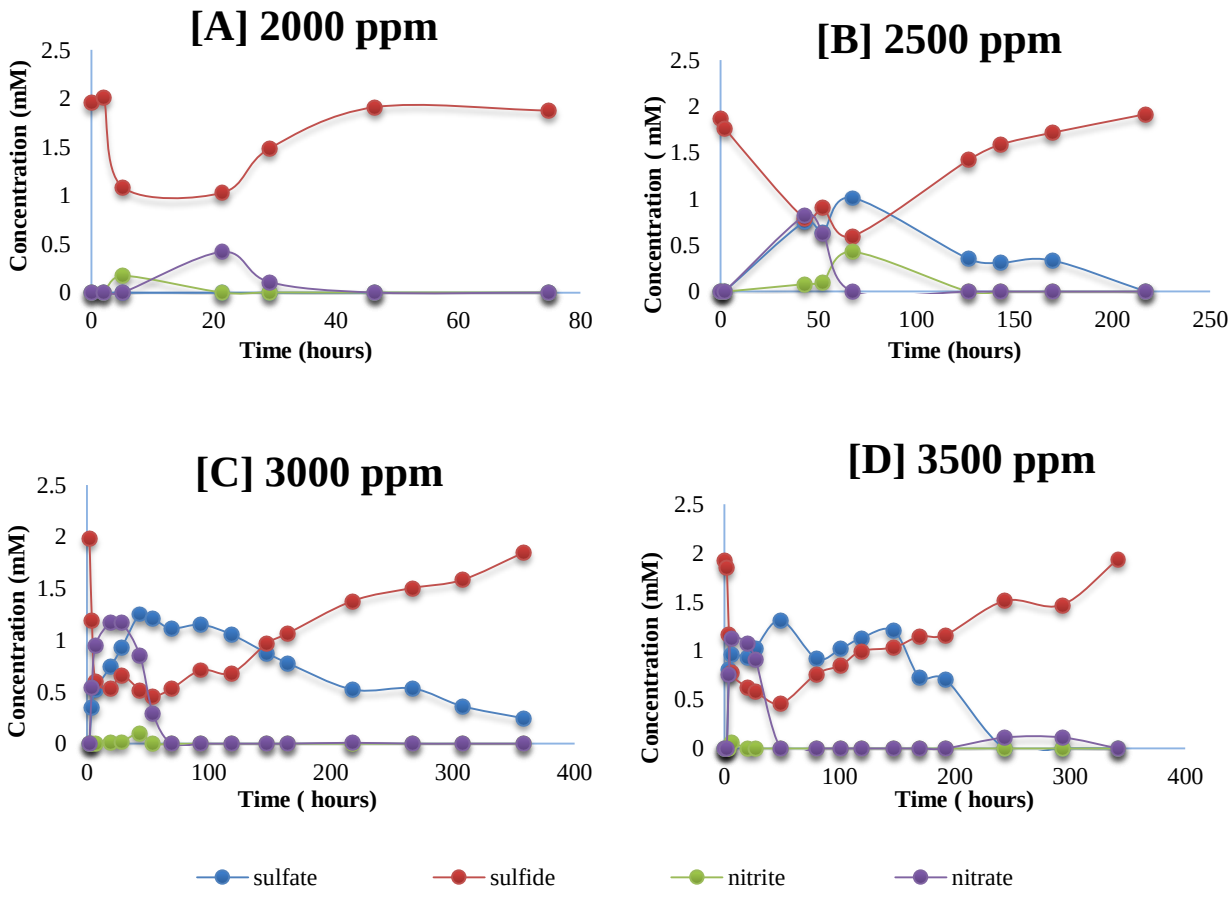


Figure C1 The effect of 1-h pulse of BAC with continuous nitrate injection on sulfide production on an hour-scale. Blue lines are sulfate, red lines are sulfide, green lines are nitrite and purple lines are nitrate. BAC was pulsed in the first hour.

Appendix D: The effect of 1-h pulse of Glut/BAC with continuous nitrate injection on sulfide production on an hour-scale

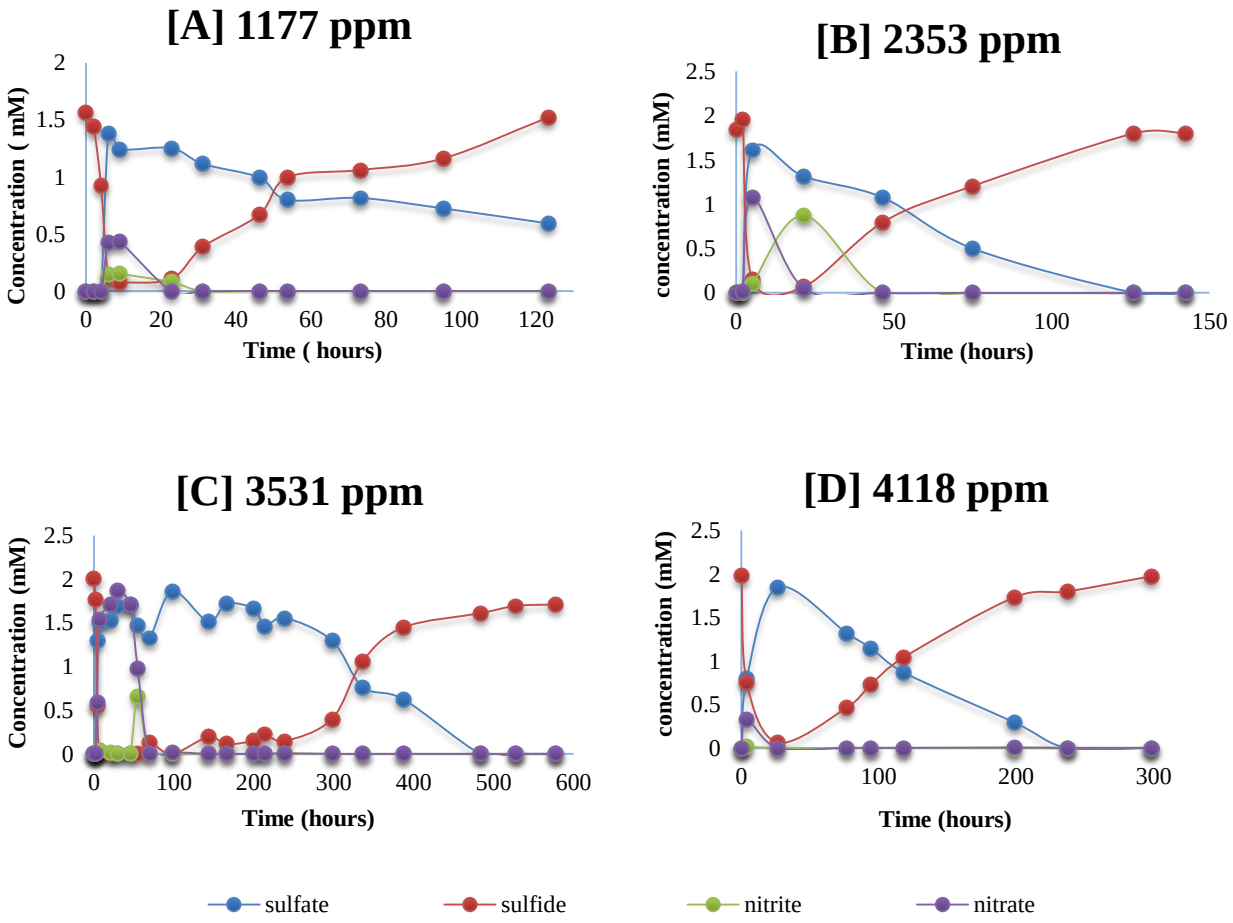


Figure D1 The effect of 1-h pulse of Glut/BAC with continuous nitrate injection on sulfide production on an hour-scale. Blue lines are sulfate, red lines are sulfide, green lines are nitrite and purple lines are nitrate. Glut/BAC was pulsed in the first hour. The pulse of 588 ppm was not shown, because no notable souring control was observed.

Appendix E: The effect of 1-h pulse of THPS on sulfide production on an hour-scale

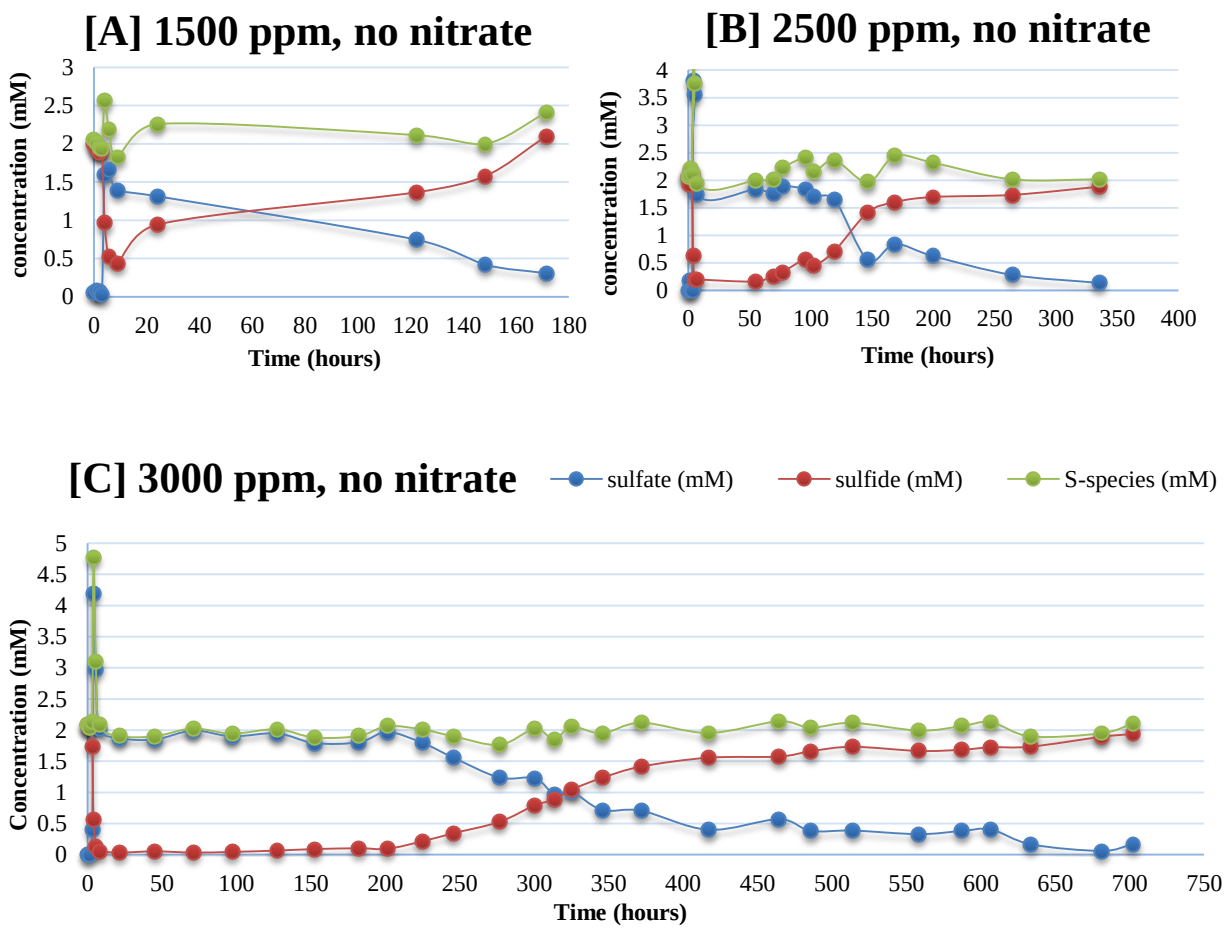


Figure E1 The effect of 1-h pulses of THPS on sulfide production in the absence of nitrate on an "hours" scale. Blue lines are sulfate, red lines are sulfide, and green lines are the total S species. THPS was pulsed in the first hour.

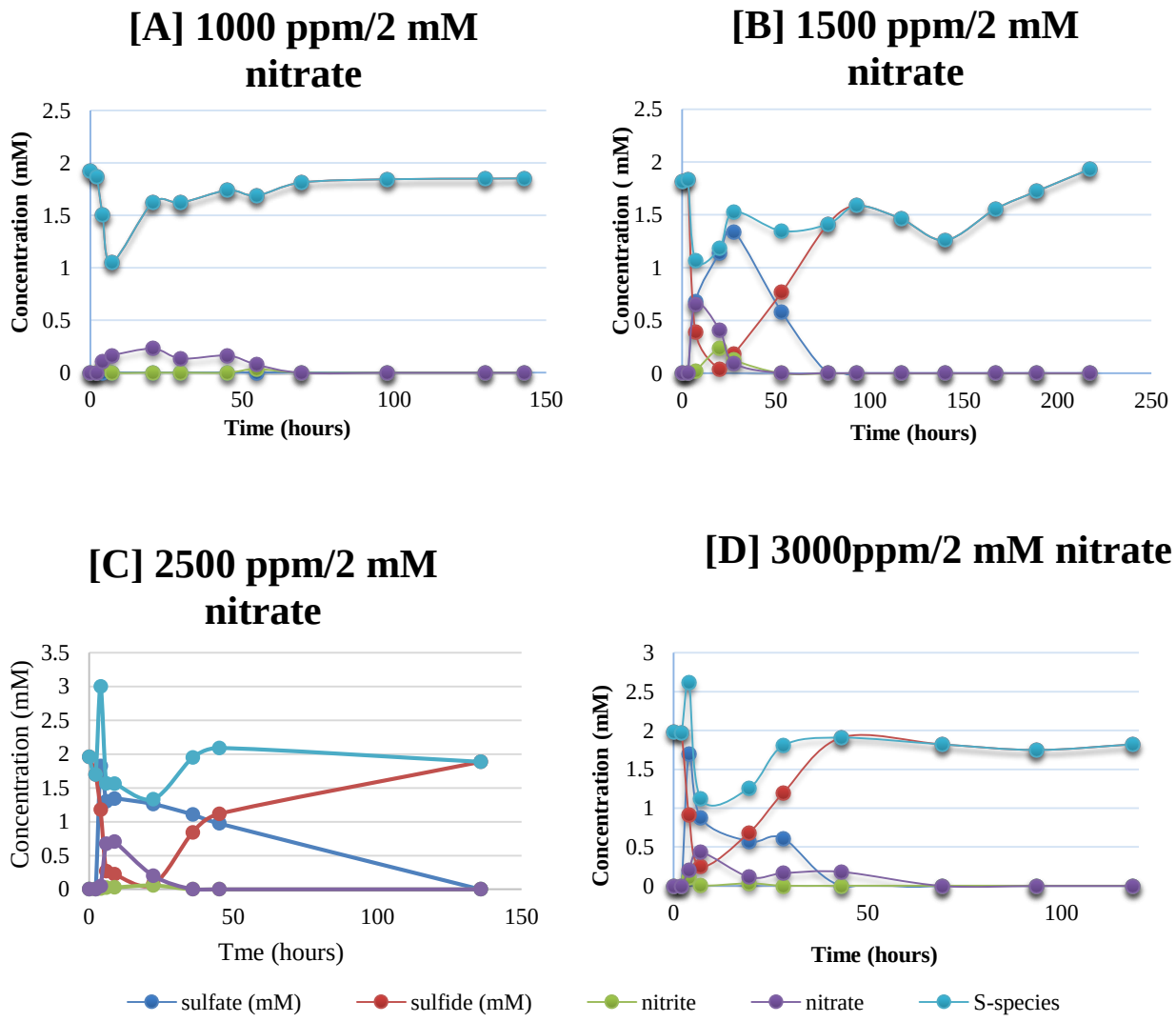


Figure E2 The effect of 1-h pulse of THPS with continuous nitrate injection on sulfide production on an hour-scale. Dark blue lines are sulfate, red lines are sulfide, green lines are nitrite, purple lines are nitrate, and light blue lines are total sulfur species. THPS was pulsed in the first hour.

Appendix F: The effect of 1-h pulse of cocodiamine with continuous nitrate injection on sulfide production on an hour-scale

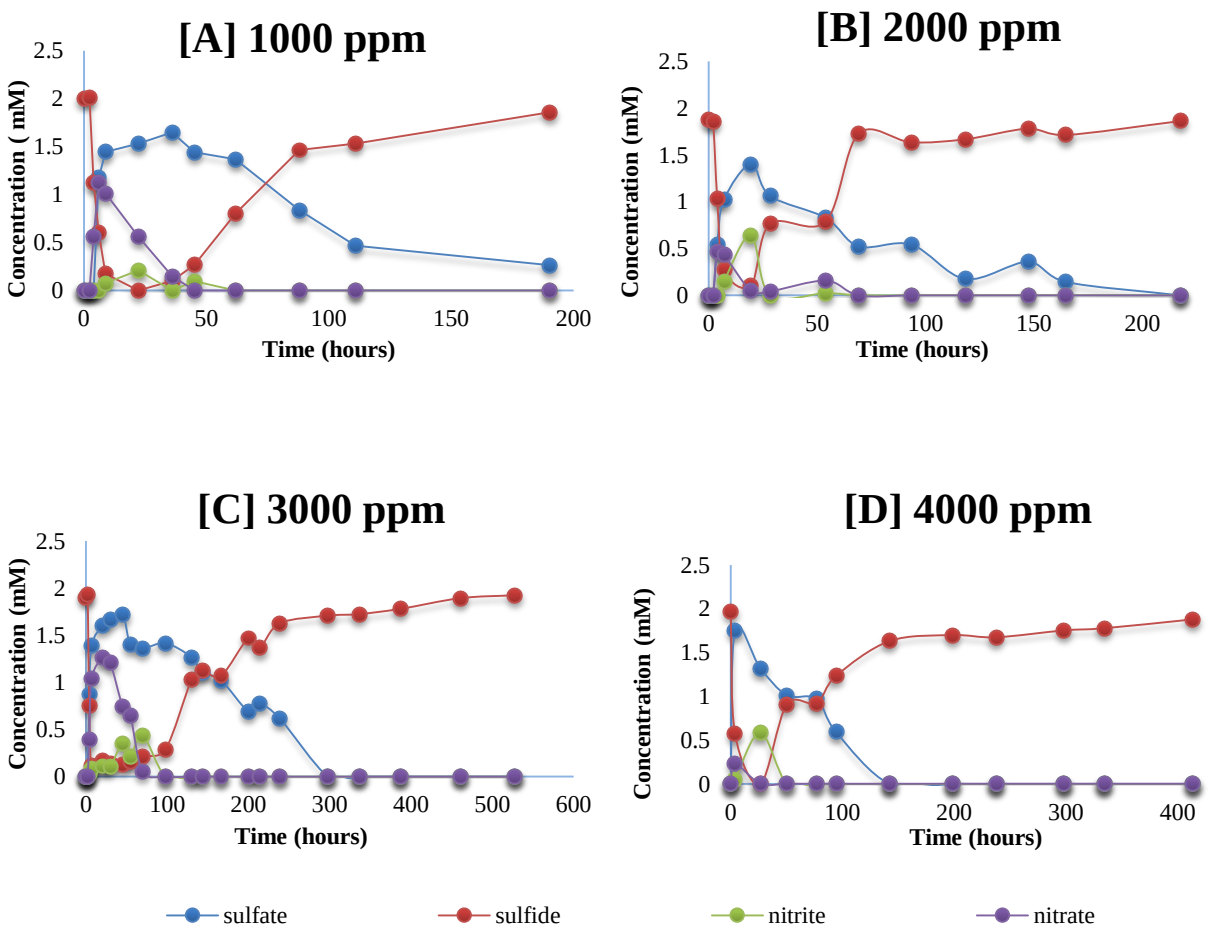


Figure F1 The effect of 1-h pulse of cocodiamine with continuous nitrate injection on sulfide production on an hour-scale. Blue lines are sulfate, **red lines** are sulfide, **green lines** are nitrite and **purple lines** are nitrate. Cocodiamine was pulsed in the first hour. The pulse of 400 and 500 ppm were not shown, because no notable souring control was observed.

Appendix G: Chemical assay profiles for all bioreactors

BV0, the control, no nitrate or biocide

days	sulfate (mM)	sulfide (mM)	sulfate and sulfide (mM)
0	0.300431832	1.56545961	1.865891442
1	0.183220234	1.74187558	1.925095815
2	0.201727329	1.611884865	1.813612194
3	0.244910549	1.624883937	1.869794486
4	0.41764343	1.496750232	1.914393662
5	0.578038248	1.259052925	1.837091173
9	0.726095003	1.143918292	1.870013295
11	0.645897594	1.023212628	1.669110222
13	0.621221468	1.149489322	1.77071079
20	0	1.708449396	1.708449396
22	0.016656385	1.764159703	1.780816088
27	0.028994448	1.639740019	1.668734466
29	0.096853794	1.73630455	1.833158344
31	0.016656385	1.727019499	1.743675884
33	0.016656385	1.676880223	1.693536608
36	0.016656385	1.745589601	1.762245986
42	0	1.675023213	1.675023213
47	0.028994448	1.65088208	1.679876528
49	0.004318322	1.769730734	1.774049056
51	0.022825416	1.711302847	1.734128264
53	0.016656385	1.654357204	1.671013589
55	0.028994448	1.709577222	1.73857167
57	0.096853794	1.621570319	1.718424113
60	0.035163479	1.638826575	1.673990054
64	0.16471314	1.581880932	1.746594072
67	0.035163479	1.694046592	1.729210071
69	0.010487353	1.711302847	1.721790201
74	0	1.740638481	1.740638481
76	0.010487353	1.664710958	1.675198311
95	0.053670574	1.630198447	1.683869021
97	0.090684762	1.709577222	1.800261984
104	0.016656385	1.68714409	1.703800475
106	0.0783467	1.756169111	1.834515811
109	0.022825416	1.718205349	1.741030766
111	0.028994448	1.730284728	1.759279176
117	0.016656385	1.744089733	1.760746117
119	0.010487353	1.666436583	1.676923937
131	0.066008637	1.588783434	1.654792071
136	0.084515731	1.566350302	1.650866033
156	0	1.686363636	1.686363636
158	0	1.702892562	1.702892562
160	0	1.673966942	1.673966942
171	0	1.830991736	1.830991736

173	0	1.744214876	1.744214876
175	0.016656385	1.758677686	1.775334071
178	0.202463818	1.81446281	2.016926628
180	0.022825416	1.72768595	1.750511367
182	0	1.802066116	1.802066116
184	0	1.775206612	1.775206612
186	0	1.85785124	1.85785124
188	0	1.795867769	1.795867769
191	0	1.833057851	1.833057851
193	0	1.830991736	1.830991736
195	0	1.802066116	1.802066116
196	0	1.773140496	1.773140496
199	0	1.866115702	1.866115702
203	0	1.824793388	1.824793388
206	0	1.793801653	1.793801653
210	0	1.818595041	1.818595041
213	0	1.845454545	1.845454545
217	0	1.81446281	1.81446281
220	0	1.731818182	1.731818182
227	0	1.818595041	1.818595041
231	0	1.740082645	1.740082645
255	0.040283952	1.769008264	1.809292217
262	0.073846373	1.723553719	1.797400092
266	0.001173132	1.892975207	1.894148338
269	0.051182918	1.859917355	1.911100273
273	0.088566913	1.766942149	1.855509061
276	0	1.841322314	1.841322314
280	0	1.87231405	1.87231405
284	0.090842004	1.793801653	1.884643657
287	0	1.944628099	1.944628099
290	0	1.899173554	1.899173554
294	0.074166647	1.917768595	1.991935242
301	0	1.804132231	1.804132231
305	0.123229484	1.888842975	2.012072459
308	0.084333385	1.917768595	2.00210198
311	0.119698026	1.851652893	1.971350918
318	0	1.932231405	1.932231405
325	0	2.054132231	2.054132231
332	0	1.996280992	1.996280992
339	0	2.010743802	2.010743802
346	0	2.052066116	2.052066116

BV1 pulsed with Glut in the absence of nitrate

Days	sulfate (mM)	sulfide (mM)	sulfate and sulfide (mM)
0	0.041332511	1.782729805	1.824062316
2	0.035163479	1.660167131	1.69533061
7	0.189389266	1.65088208	1.840271346
9	0.059839605	1.73816156	1.798001165
11	0.066008637	1.523209664	1.5892183
13	0.047501542	1.623295945	1.670797487
15	0.053670574	1.638826575	1.692497148
17	0.028994448	1.692320966	1.721315414
20	0.16471314	1.585332183	1.750045323
22	0.0783467	1.623295945	1.701642644
24	0.041332511	1.549094047	1.590426557
27	0.035163479	1.757894737	1.793058216
29	0.140037014	1.749266609	1.889303623
31	0.041332511	1.726833477	1.768165988
34	0.047501542	1.716479724	1.763981266
36	0.022825416	1.730284728	1.753110145
38	0.053670574	1.555996549	1.609667122
41	0.103022825	1.645729077	1.748751902
43	0.084515731	1.764797239	1.84931297
45	0.022825416	1.728559103	1.751384519
48	0.047501542	1.804486626	1.851988169
50	0.849475632	0.996893874	1.846369506
52	1.439851943	0.577566868	2.017418811
55	1.538556447	0.586194996	2.124751442
57	0.719925972	1.336842105	2.056768077
59	0.004318322	1.628472821	1.632791143
62	0.022825416	1.792407248	1.815232664
64	0.066008637	1.845901639	1.911910276
66	1.884022209	0.273856773	2.157878982
69	1.884022209	0.277308024	2.161330233
71	1.93337446	0.325625539	2.258999999
74	1.217766811	0.78435196	2.002118771
77	0.022825416	1.780327869	1.803153285
79	0.016656385	1.826919758	1.843576143
83	0	1.771699741	1.771699741
85	1.492362734	0.080586713	1.572949447
87	1.587926311	0.065056083	1.652982394
90	1.884022209	0.061604832	1.94562704

91	1.921036397	0.082312338	2.003348736
94	1.63726095	0.311820535	1.949081485
96	1.575570635	0.537877481	2.113448116
98	1.304133251	0.874374461	2.178507712
101	0.251079581	1.723553719	1.9746333
103	0.047501542	1.932231405	1.979732947
105	0.066008637	1.897107438	1.963116075

BV1 pulsed with Glut with continuous injection of nitrate

days	**Sulfate (mM)**	**sulfide (mM)**	**nitrate (mM)**	**nitrite (mM)**	**Sulfate and sulfide (mM)**
0	0.035163479	1.874380165	0	0	1.909543645
3	0.022825416	1.921900826	0	0	1.944726243
7	0	1.950826446	0	0	1.950826446
9	0.657404203	1.566528926	0	0	2.223933129
11	0	1.830991736	0	0	1.830991736
13	0	1.946694215	0	0	1.946694215
21	0	1.851652893	0	0	1.851652893
24	0	2.054132231	0	0	2.054132231
26	0.36554951	1.769008264	0	0	2.134557774
28	0.035163479	1.845454545	0	0	1.880618025
31	0.091759335	1.878512397	0	0	1.970271731
33	0.010487353	2.002479339	0	0	2.012966692
35	0.011823512	1.888842975	0	0	1.900666487
37	0.096945016	2.07892562	0	0	2.175870635
39	0.011823512	2.037603306	0	0	2.049426818
41	0.077343416	1.868181818	0	0	1.945525234
44	0.056967666	2.070661157	0	0	2.127628823
46	0.851813854	1.126446281	0	0	1.978260135
47	0.748690531	1.248347107	0	0	1.997037639
48	0.998749487	0.907438017	0	0	1.906187504
49	0.011823512	1.680165289	0	0	1.691988801
50	1.785328835	0.171900826	0	0	1.957229662
51	1.033876752	1.02107438	0	0	2.054951132
52	1.211589404	0.8	0	0	2.011589404
53	0.775085927	1.130578512	0	0	1.905664439
54	0.713777274	1.258677686	0	0	1.97245496
56	0.445400269	1.502479339	0	0	1.947879608
59	0.449152619	1.508677686	0	0	1.957830305

61	0.707128846	1.163636364	0	0	1.870765209
62	0.738470911	1.200826446	0	0	1.939297357
63	0.011823512	1.913636364	0	0	1.925459876
66	0.011823512	1.797933884	0	0	1.809757396
68	0.011823512	1.940495868	0	0	1.95231938
70	1.516734392	0.35785124	1.160019971	0	1.874585631
71	1.76128874	0.246280992	1.211382916	0	2.007569732
73	1.440633532	0.35785124	0.936621095	0	1.798484771
75	1.224166776	0.895041322	0	0	2.119208098
77	0.592471465	1.37231405	0	0	1.964785514
78	0.011823512	1.773140496	0	0	1.784964008
80	0.011823512	1.979752066	0	0	1.991575578
84	0.011823512	1.899173554	0	0	1.910997066
108	0	1.859917355			1.859917355
122	0	1.897107438	0	0	1.897107438
123	0	2.027272727	0	0	2.027272727
124	0	1.942561983	0	0	1.942561983
125	2.086948842	0	2.120989821	0	2.086948842
126	1.94558	0.085123967	2.307034219	0	2.030703967
127	1.75244138	0.008677686	2.100945894	0	1.761119066
129	1.965807793	0.054132231	2.237539799	0	2.019940024
132	1.935187037	0.211157025	0	0	2.146344061
133	1.859541348	0.297933884	0	0	2.157475232
134	1.799083677	0.281404959	0	0	2.080488636
135	1.922084358	0.337190083	0	0	2.259274441
137	1.676769932	0.502479339	0	0	2.179249271
139	1.177342787	0.899173554	0	0	2.076516341
140	0.68511681	1.426033058	0	0	2.111149868
142	0.755811044	1.374380165	0	0	2.130191209
144	0.220005152	1.60785124	0	0	1.827856392
146	0.38626436	1.59338843	0	0	1.979652789
147	0.017852959	1.762809917	0	0	1.780662876
149	0.065177285	1.849586777	0	0	1.914764061
150	0.05346933	1.940495868	0	0	1.993965198
151	0.014425029	1.897107438	0	0	1.911532467
152	0	1.917768595	0	0	1.917768595
153	2.099251731	0	1.777774075	0.042887243	2.099251731
154	1.552880158	0.128512397	1.565827596	0	1.681392555
155	1.81472411	0	1.764768534	0	1.81472411

156	1.674986824	0	1.760077306	0	1.674986824
157	1.84800674	0	1.818769624	0	1.84800674
159	1.805006162	0.004545455	1.816435939	0	1.809551617
161	1.824247498	0.029338843	0	0	1.853586341
168	1.777952381	0.056198347	0	0	1.834150728
170	1.729826047	0	0	0	1.729826047
172	1.826575136	0	0	0	1.826575136
174	1.884616336	0	0	0	1.884616336
175	1.739414878	0.00661157	0	0	1.746026449
177	1.678218869	0.025206612	0	0	1.70342548
179	1.495232205	0.281404959	0	0	1.776637163
180	1.300336736	0.56446281	0	0	1.864799546
181	1.025680163	0.78553719	0	0	1.811217353
182	0.690792123	1.124380165	0.074213186	0	1.815172288
184	0.639217961	1.200826446	0.036321257	0	1.840044407
186	0.707023095	1.182231405	0.020519675	0	1.8892545
188	0.552424418	1.395041322	0	0	1.94746574
189	0.431751596	1.401239669	0	0	1.832991265
192	0.346701423	1.53553719	0	0	1.882238613
194	0.355151197	1.628512397	0	0	1.983663594
197	0.041853946	1.911570248	0	0	1.953424194
199	0	1.909504132	0	0	1.909504132
201	0	1.954958678	0	0	1.954958678
203	0	1.954958678	0	0	1.954958678
206	0	1.82892562	0	0	1.82892562
208	0.174411942	1.928099174	0	0	2.102511116
209	1.305587073	0.124380165	0.551040841	0.084912573	1.429967238
210	1.285556949	0.180165289	0.71511958	0.078111248	1.465722239
212	1.819687616	0.10785124	0.807383769	0.053990854	1.927538856
215	1.633857085	0.159504132	0	0	1.793361218
217	1.378663677	0.442561983	0	0	1.82122566
219	0.891509505	0.990082645	0	0	1.88159215
221	0.792300882	0.870247934	0	0	1.662548816
223	0.261693756	1.516942149	0	0	1.778635904
226	0	1.822727273	0	0	1.822727273
228	0	1.853719008	0	0	1.853719008
230	0	1.77107438	0	0	1.77107438
231	0.290262449	1.833057851	0	0	2.1233203
233	0	1.944628099	0	0	1.944628099

234	0	1.833057851	0	0	1.833057851
236	0	1.959090909	0	0	1.959090909
238	0	1.824793388	0	0	1.824793388
241	0	1.957024793	0	0	1.957024793
243	0	1.560330579	0	0	1.560330579
244	0	1.552066116	0	0	1.552066116
245	0	1.680165289	0	0	1.680165289
246	0	1.694628099	0	0	1.694628099
248	0	1.82892562	0	0	1.82892562
250	0	1.78553719	0	0	1.78553719
252	0	1.973553719	0	0	1.973553719
259	0	1.87231405	0	0	1.87231405
262	0	2.109917355	0	0	2.109917355

BV2 pulsed with BAC in the absence of nitrate

days	**sulfate (mM)**	**sulfide (mM)**	**sulfate and sulfide (mM)**
0	0.035163479	1.786443825	1.821607305
2	0.0783467	1.689879294	1.768225994
6	0.312769895	1.543175487	1.855945383
11	0	1.799442897	1.799442897
18	0.035163479	1.662024141	1.69718762
20	0.053670574	1.760445682	1.814116256
22	0.022825416	1.621169916	1.643995333
24	0.028994448	1.792014856	1.821009304
27	0.238741518	1.660167131	1.898908649
38	0.146206046	1.792014856	1.938220902
40	0.010487353	1.845868152	1.856355506
42	1.478716841	0.486108714	1.964825556
44	1.694632943	0.337704918	2.032337861
46	1.688463911	0.260051769	1.94851568
48	1.793337446	0.197929249	1.991266695
51	1.700801974	0.2013805	1.902182475
53	1.207279457	0.6811044	1.888383857
55	0.590376311	1.195340811	1.785717122
58	0.041332511	1.699223469	1.740555979
60	0.0783467	1.79930975	1.877656449
62	0.028994448	1.642277826	1.671272274
65	0.053670574	1.690595341	1.744265915

67	0.028994448	1.62502157	1.654016018
69	1.614435534	0.289387403	1.903822937
72	1.7254781	0.063330457	1.788808557
74	1.756323257	0.059879206	1.816202463
76	2.007402838	0.05470233	2.062105167
79	2.05675509	0.296289905	2.353044995
81	1.071560765	0.926143227	1.997703992
83	0.096853794	1.716479724	1.813333518
88	0.041332511	1.673339085	1.714671596
90	0.059839605	1.756169111	1.816008716
93	2.192473782	0.125452977	2.317926758
95	2.056755089	0.066781708	2.123536798
97	2.217149907	0.058153581	2.275303488
100	2.315854411	0.040897325	2.356751736
102	2.204811845	0.075409836	2.280221681
105	1.809993831	0.2438692	2.053863031
108	1.016039482	0.881276963	1.897316445
110	0.787785318	1.361000863	2.148786181
116	0.036268812	1.60949094	1.645759752
118	0.084392888	1.554270923	1.638663811
121	0.201727329	1.452459016	1.654186345
122	0.066008637	1.633649698	1.699658335
125	0.127698951	1.604314064	1.732013015
127	0.084515731	1.616393443	1.700909174
129	0.214065392	1.537014668	1.75108006
132	1.365823566	0.673966942	2.039790508
134	1.600246761	0.306198347	1.906445108
136	1.624922887	0.281404959	1.906327846
138	1.538556447	0.281404959	1.819961405
141	1.180752622	0.874380165	2.055132787
145	0	1.746280992	1.746280992
147	0	1.731818182	1.731818182
151	0	1.725619835	1.725619835
162	0	1.942561983	1.942561983
164	0	1.884710744	1.884710744
166	0.041332511	1.936363636	1.977696147
169	0	1.870247934	1.870247934
171	1.42751388	0.207024793	1.634538674
173	1.64134748	0.140909091	1.782256575
175	1.628936087	0.099586777	1.728522864
182	1.619439457	0.05	1.669439457

186	1.768496871	0.056198347	1.824695218
187	1.6980181	0.068595041	1.766613137
190	1.806001947	0.027272727	1.833274674
192	1.807776329	0.041735537	1.849511866
194	1.743105045	0.120247934	1.863352979
197	0.703581965	1.074793388	1.778375353
199	0	1.746280992	1.746280992
201	0	1.901239669	1.901239669
204	0	1.913636364	1.913636364
206	0	1.940495868	1.940495868
211	1.490324851	0.126446281	1.616771132
215	1.610453274	0.080991736	1.69144501
218	1.537133978	0.221487603	1.758621582
221	1.414757269	0.562396694	1.977153963
222	0.799121936	1.076859504	1.875981441
225	0	1.919834711	1.919834711
246	0	1.928099174	1.928099174

BV2 pulsed with BAC with continuous injection of nitrate

days	**sulfate (mM)**	**sulfide (mM)**	**nitrate (mM)**	**nitrite (mM)**	**sulfate and sulfdie (mM)**
0	0	1.928099174	0	0	1.928099174
1	0.882867688	1.283471074	0	0	2.166338762
2	0.67999758	1.417768595	0	0	2.09776618
3	0.480242947	1.50661157	0	0	1.986854517
4	0.31132393	1.473553719	0	0	1.784877653
5	0.29195074	1.469421488	0	0	1.761372227
9	0.37845696	1.514876033	0	0	1.893332997
12	0.22717041	1.570661157	0	0	1.797831563
13	0	2.163636364	0	0	2.163636364
14	0.01536458	2.07892562	0	0	2.094290204
15	0.71161802	1.012809917	0.069613325	0	1.724427938
16	0.19249831	1.483884298	0.044344499	0	1.676382607
17	0.02679511	1.793801653	0.082098631	0	1.820596764
19	0	1.899173554	0.121421637	0	1.899173554
20	0.75302453	1.142975207	0.239940412	0.113713044	1.89599974
21	0.58335957	1.38677686	0	0	1.970136432
22	0.71679326	1.167768595	0	0	1.88456185
23	0.46773932	1.32892562	0	0	1.796664942
24	0.479326463	1.250413223	0	0.02429988	1.729739686

25	0.611597139	1.275206612	0	0	1.886803751
26	0.233903662	1.568595041	0	0	1.802498704
27	0.365406928	1.614049587	0	0	1.979456515
30	0	1.682231405	0	0	1.682231405
31	0.17970988	1.725619835	0	0	1.905329713
32	0.06727818	1.890909091	0	0	1.95818727
33	0	2.000413223	0	0	2.000413223
34	0	2.000413223	0	0	2.000413223
35	0	1.911570248	0	0	1.911570248
36	0.02835313	1.702892562	0	0	1.731245696
37	0.050724623	1.628512397	0.015567098	0	1.67923702
41	0.281991227	1.304132231	0.118659034	0	1.586123459
45	0.666203341	0.996280992	0.216754775	0.078766425	1.662484333
46	0.416076889	1.275206612	0.102712696	0	1.691283501
48	0.31979387	1.907438017	0	0	2.227231886
50	0.143438131	1.905371901	0	0	2.048810032
51	0.214854406	1.667768595	0	0	1.882623001
52	0.620832096	1.13677686	0.052209744	0.028902748	1.757608956
53	0.278458236	1.657438017	0	0	1.935896252
55	0	1.954958678	0	0	1.954958678
58	0.196792218	1.909504132	0	0	2.10629635
59	0	1.824793388	0	0	1.824793388
61	0	1.983884298	0	0	1.983884298
63	0	1.921900826	0	0	1.921900826
65	0.447972945	2.00661157	0	0	2.454584516
66	0	1.936363636	0	0	1.936363636
67	1.585773705	0.291735537	0.719204077	0.225019887	1.877509242
68	1.750854297	0.145041322	1.198117022	0.209710058	1.895895619
70	1.958181354	0.012809917	1.629487591	0.092908473	1.970991271
71	1.758970903	0.116115702	1.530148243	0.082807707	1.875086605
73	1.751344986	0.111983471	1.622922602	0.040650299	1.863328457
75	1.797989249	0	0.820656567	0.526161258	1.797989249
78	1.695530362	0.089256198	0	0.218804775	1.78478656
79	1.638813484	0.153305785	0	0.041831944	1.792119269
81	1.541300126	0.155371901	0	0	1.696672027
87	1.607562741	0.287603306	0	0	1.895166047
89	1.262514673	0.452892562	0	0	1.715407235
94	1.157385825	0.634710744	0	0	1.792096569
95	1.487287557	0.601652893	0	0	2.088940449
96	0.996065136	0.775206612	0	0	1.771271747
99	0.564656453	1.326859504	0	0	1.891515957

104	0	1.764876033	0.007253108	0	1.764876033
106	0	1.938429752	0.085438236	0	1.938429752
111	0	1.826859504	0.007253108	0.013932889	1.826859504
113	0	1.909504132	0.007253108	0	1.909504132
114	1.948381532	0	1.228084752	0.182767494	1.948381532
115	1.972357912	0.00661157	1.390780654	0.063249393	1.978969483
116	2.178441991	0.002479339	1.547006715	0.046982784	2.18092133
117	1.881030379	0	1.748372961	0.056223909	1.881030379
118	1.925856507	0.033471074	1.78818695	0.04894799	1.959327581
119	1.832861989	0.008677686	1.694252144	0.04823579	1.841539675
120	1.811133135	0	1.652898876	0.07400727	1.811133135
122	1.855254319	0	0.500605728	0.636138378	1.855254319
123	1.807873807	0	0.234244574	0.106695242	1.807873807
125	1.786534745	0	0.028444338	0.104011181	1.786534745
127	1.484263168	0.376446281	0	0	1.860709449
129	1.461671795	0.47768595	0	0	1.939357745
132	1.206448991	0.903305785	0	0	2.109754776
136	1.123846173	1.116115702	0	0	2.239961875
138	0.797871899	1.442561983	0	0	2.240433883
140	0.514990421	1.547933884	0	0	2.062924305
142	0	1.913636364	0	0	1.913636364
146	0	1.905371901	0	0	1.905371901
150	0	1.85785124	0	0	1.85785124
153	1.62274729	0.173966942	0.982597599	0.206637833	1.796714232
155	1.716463339	0.014876033	1.475752851	0.146580924	1.731339372
157	2.013833484	0.140909091	1.575080543	0.094458053	2.154742575
160	2.01874321	0.000413223	0.044516651	0.01255009	2.019156433
162	1.859475688	0.080991736	0	0	1.940467423
164	1.838990852	0.171900826	0	0	2.010891679
167	1.761712433	0.151239669	0	0	1.912952102
170	2.038348939	0.080991736	0	0	2.119340674
171	1.871849524	0	0	0	1.871849524
174	1.655630842	0.178099174	0	0	1.833730015
176	1.576934241	0.200826446	0	0	1.777760687
178	1.362647935	0.364049587	0	0	1.726697521
184	0	1.77107438	0	0	1.77107438
187	0	1.750413223	0	0	1.750413223
189	0	1.769008264	0	0	1.769008264

BV-3 pulsed with cocodiamine in the absence of nitrate

days	sulfate (mM)	sulfide (mM)	sulfate and sulfide (mM)
0	1.355336212	0.662952646	2.018288858
2	1.231955583	0.750232126	1.982187709
4	0.904996915	0.913649025	1.818645941
11	0.047501542	1.610027855	1.657529397
13	0	1.600742804	1.600742804
20	0.066008637	1.788300836	1.854309472
22	0.090684762	1.775301764	1.865986527
24	0.189389266	1.64902507	1.838414336
26	1.818013572	0.319405757	2.137419329
29	1.959901295	0.183844011	2.143745307
33	1.76866132	0.118848654	1.887509974
35	1.645280691	0.237697307	1.882977998
40	1.139420111	0.813370474	1.952790585
42	0.954349167	1.030640669	1.984989836
44	1.00987045	0.988265746	1.998136197
48	0.954349167	0.955478861	1.909828028
50	1.349167181	0.701811907	2.050979088
53	0.584207279	1.354098361	1.93830564
57	0.355953115	1.443830889	1.799784004
60	0.035163479	1.788955997	1.824119476
62	0.6273905	1.43175151	2.05914201
64	0.103022825	1.647454702	1.750477528
67	0.158544109	1.690595341	1.849139449
69	0.022825416	1.775150992	1.797976409
71	1.417026527	0.527523727	1.944550254
74	1.552745219	0.13580673	1.688551949
76	1.713140037	0.108196721	1.821336758
78	2.007402838	0.16686799	2.174270827
81	0.800123381	1.134943917	1.935067298
83	0.010487353	1.738912856	1.749400209
85	0.066008637	1.833822261	1.899830897
90	0.066008637	1.818291631	1.884300267
92	0	1.704400345	1.704400345
95	0.096853794	1.795858499	1.892712293
97	0.090684762	1.878688525	1.969373287
99	0.047501542	1.882139776	1.929641318
102	0.066008637	1.776876618	1.842885254
104	0.047501542	1.787230371	1.834731913

112	0	1.832096635	1.832096635
127	0.035163479	1.707851596	1.743015076
129	0.041332511	1.680241588	1.721574098
131	1.600246761	0.396376186	1.996622948
134	1.834669957	0.062396694	1.897066651
136	1.871684146	0.031404959	1.903089104
138	1.822331894	0.02107438	1.843406274
140	1.748303516	0.118181818	1.866485335
143	1.316471314	0.667768595	1.984239909
145	0.296288367	1.580991736	1.877280103
147	0	1.820661157	1.820661157
149	0	1.758677686	1.758677686
164	0	1.903305785	1.903305785
166	0	1.752479339	1.752479339
168	0.004318322	1.729752066	1.734070388
171	0	1.82892562	1.82892562
173	0.884639112	0.634710744	1.519349855
175	1.616759019	0.080991736	1.697750755
177	1.664356072	0.019008264	1.683364337
179	1.632736249	0.02107438	1.653810629
181	1.600591507	0.000413223	1.60100473
184	1.539431853	0.076859504	1.616291357
186	1.208955817	0.436363636	1.645319454
187	0.966489055	0.566528926	1.533017981
188	0.67743571	0.936363636	1.613799347
192	0	1.421900826	1.421900826
194	0	1.512809917	1.512809917
196	0	1.52107438	1.52107438
199	0	1.512809917	1.512809917
201	0	1.55	1.55
203	0	1.63677686	1.63677686
206	0	1.537603306	1.537603306
208	0	1.504545455	1.504545455
210	0	1.473553719	1.473553719
211	0	1.496280992	1.496280992
213	0	1.419834711	1.419834711
217	0	1.502479339	1.502479339
220	0	1.510743802	1.510743802
224	0	1.566528926	1.566528926
248	0	1.580991736	1.580991736

BV-3 pulsed with cocodiamine with continuous injection of nitrate

days	sulfate (mM)	sulfide (mM)	nitrate (mM)	nitrite (mM)	sulfate and sulfide (mM)
0	0.411673654	1.519008264	0	0	1.930681919
1	0.66275847	1.200826446	0	0	1.863584917
2	0.312159463	1.589256198	0	0	1.901415662
3	0.327792607	1.57892562	0	0	1.906718226
4	0.279150985	1.572727273	0	0	1.851878258
5	0.241407049	1.572727273	0	0	1.814134322
7	1.289447895	0.351652893	0	0.211563684	1.641100787
9	1.285162871	0.399173554	0.016568854	0.068596847	1.684336425
10	1.276241438	0.494214876	0	0	1.770456314
11	1.012480191	0.63677686	0	0	1.649257051
12	0.922949846	0.777272727	0.034137527	0	1.700222573
13	0.887307741	0.878512397	0.045802546	0	1.765820138
14	0.661378867	1.101652893	0.034133267	0	1.76303176
15	0.656851529	1.3	0.03977535	0	1.956851529
18	0.503573497	1.576859504	0	0	2.080433002
20	0.217635426	1.585123967	0	0	1.802759393
21	0.310063528	1.411570248	0	0	1.721633776
23	0.649088608	1.105785124	0	0	1.754873732
24	0.496571717	1.289669421	0	0	1.786241138
25	0.269823335	1.490082645	0	0	1.75990598
26	0.566690929	1.378512397	0	0	1.945203325
31	0.403623611	1.523140496	0	0	1.926764107
33	0.444711593	1.421900826	0.032219346	0	1.866612419
35	1.144081874	0.415702479	0.036283688	0.226014417	1.559784354
37	1.420487909	0.231818182	0.105441512	0.024276594	1.652306091
38	1.568493108	0.153305785	0	0	1.721798894
40	1.655509988	0.047933884	0	0.047397467	1.703443872
44	2.101592795	0.21322314	0	0	2.314815936
45	1.530178214	0.450826446	0	0	1.98100466
48	1.270503341	0.721487603	0	0.029044488	1.991990944
49	0.980049421	0.833057851	0	0	1.813107272
51	0.709174937	1.248347107	0	0	1.957522045
53	0.512476656	1.337190083	0	0	1.849666739
55	0.465999162	1.423966942	0	0	1.889966104
57	0.599418159	1.337190083	0	0	1.936608242
59	0.438896935	1.318595041	0	0	1.757491977
61	0.329392994	1.465289256	0	0	1.794682251
63	0.707547006	1.53553719	0	0	2.243084196

64	1.785477812	0.074793388	0.504093645	0.413358544	1.8602712
66	1.606372067	0.215289256	0.073254419	0.105672623	1.821661324
68	1.819160457	0.142975207	0	0.097229048	1.962135664
70	1.962085615	0.012809917	1.200704748	0.266583565	1.974895532
72	2.074748916	0.068595041	0	0.054621567	2.143343957
74	1.921389076	0.22768595	0	0	2.149075026
76	1.725845036	0.434297521	0	0	2.160142557
78	1.244483868	1.087190083	0	0	2.331673951
80	0.806322925	1.38677686	0	0	2.193099785
82	0.819976724	1.308264463	0	0	2.128241186
84	0.703193159	1.382644628	0	0	2.085837787
86	0.820117767	1.384710744	0	0	2.204828511
88	0.669652195	1.266942149	0	0	1.936594344
89	0.824254624	1.240082645	0	0	2.064337269
91	0.979074423	1.428099174	0	0	2.407173596
94	0.824937418	1.562396694	0	0	2.387334112
98	0.110190303	1.59338843	0	0	1.703578733
99	1.064984217	0.118181818	0.481502579	0.193196031	1.183166036
100	1.519424606	0.248347107	0	0.061967426	1.767771713
103	1.882519595	0.016942149	0	0.070822914	1.899461744
104	1.168793726	0.008677686	0.827706663	0.255263385	1.177471412
105	1.889046983	0	0	0.235807731	1.889046983
106	1.907151559	0.012809917	0	0	1.919961477
108	1.928205789	0	0	0	1.928205789
110	1.794252041	0.039669421	0	0	1.833921462
112	1.578675278	0.190495868	0	0	1.769171146
116	0.54997259	1.345454545	0	0	1.895427136
117	0.328478734	1.304132231	0	0	1.632610966
124	0.622173153	1.413636364	0	0	2.035809517
126	0	1.423966942	0	0	1.423966942
129	0	1.405371901	0	0	1.405371901
131	0	1.57892562	0	0	1.57892562
136	0	1.659504132	0	0	1.659504132
138	0	1.859917355	0	0	1.859917355
150	0	1.994214876	0	0	1.994214876
151	0	1.740082645	0	0	1.740082645
152	0	1.651239669	0	0	1.651239669
153	0	1.682231405	0	0	1.682231405
154	0	1.459090909	0	0	1.459090909
157	0.826493092	0.874380165	0	0	1.700873257
159	0	1.659504132	0	0	1.659504132

161	0	1.59338843	0	0	1.59338843
163	0	1.572727273	0	0	1.572727273
165	0	1.523140496	0	0	1.523140496
167	0	1.529338843	0	0	1.529338843
169	0	1.580991736	0	0	1.580991736
171	0	1.707024793	0	0	1.707024793
175	0	1.926033058	0	0	1.926033058

BV4 pulsed with Glut in the absence of ntirate

days	**sulfate (mM)**	**sulfide (mM)**	**sulfate and sulfide(mM)**
0		1.816528926	
2		1.779338843	
4		1.81446281	
5		0.004545455	
6		0.010743802	
7		0.016942149	
8		0	
9		0.169834711	
10		0.169834711	
11		0.281404959	
12		1.031404959	
13		0.849586777	
14		1.302066116	
15		1.488016529	
17		1.430165289	
19		1.438429752	
20		1.525206612	
21		1.589256198	
24		1.72768595	
26		1.731818182	
28		1.773140496	
29	0.05793032	1.81446281	1.87239313
30	0.016719454	1.678099174	1.694818628
32	0.02162197	1.938429752	1.960051722
33	2.119238183	0.165702479	2.284940662
34	1.959283079	0.047933884	2.007216964
35	1.981023878	0.120247934	2.101271812
36	1.801526558	0.337190083	2.13871664
37	1.407448863	0.826859504	2.234308367
38	0.898957567	1.310330579	2.209288145

39	0.461629402	1.597520661	2.059150063
41	0.176140892	1.783471074	1.959611966
43	0.031232346	1.959090909	1.990323255
46	0.02586656	1.967355372	1.993221932
47	1.974682522	0.060330579	2.035013101
48	1.841395314	0.364049587	2.2054449
49	1.672938642	0.56446281	2.237401452
51	0.451206907	1.572727273	2.02393418
53	0.074542637	1.859917355	1.934459992
55	0.074422088	1.961157025	2.035579113
57	0	1.967355372	1.967355372
60	0.029891504	1.932231405	1.962122909
64	0.124488858	1.905371901	2.029860759
83	0.108080914	1.940495868	2.048576781
85	0.029111108	1.866115702	1.895226811
88	0.199961273	1.835123967	2.03508524
89	0.359108131	1.802066116	2.161174247
90	0.031150772	1.698760331	1.729911102
92	0.111412777	1.998347107	2.109759884
95	0.0344778	1.876446281	1.910924081
97	0.136832281	1.818595041	1.955427322
102	0.111537035	1.959090909	2.070627944
103	1.927614236	0.000413223	1.928027459
104	1.888438061	0	1.888438061
105	1.996024707	0.03553719	2.031561897
106	1.795545437	0.151239669	1.946785107
107	1.979959895	0	1.979959895
109	1.953956584	0.095454545	2.049411129
110	1.796465679	0.132644628	1.929110307
111	1.427857531	0.711157025	2.139014556
112	1.058725059	1.062396694	2.121121753
113	0.45022324	1.423966942	1.874190182
114	0.538474668	1.450826446	1.989301114
115	0.608806721	1.376446281	1.985253002
117	0.574964457	1.655371901	2.230336358
118	0.537660868	1.494214876	2.031875744
119	0.493317925	1.49214876	1.985466685
120	0.605325605	1.525206612	2.130532216
122	0.626074685	1.514876033	2.140950718
124	0.568730633	1.620247934	2.188978566
126	0.592121275	1.448760331	2.040881606
127	0.462709681	1.60785124	2.070560921

BV4 pulsed with Glut with continuous injection of nitrate

days	sulfate (mM)	sulfide (mM)	nitrate(mM)	nitrite (mM)	sulfate and sulfide(mM)
0	0.44785336	1.498347107			1.946200468
2	0.357704803	1.498347107	0	0	1.85605191
3	0	1.576859504	0	0	1.576859504
4	0.153067084	1.496280992	0	0	1.649348076
6	0	1.917768595	0	0	1.917768595
8	0	1.88677686	0	0	1.88677686
10	0	1.704958678	0	0	1.704958678
11	0	1.99214876	0	0	1.99214876
14	0	1.940495868	0	0	1.940495868
15	0.215002652	1.378512397	0	0	1.593515049
16	0	1.53553719	0	0	1.53553719
18	0	2.060330579	0	0	2.060330579
23		2.10785124	0	0	2.10785124
24	0	2.074793388	0	0	2.074793388
25	0.511533562	0.866115702	0	0	1.377649265
26	0	1.74214876	0	0	1.74214876
27	0	2.178099174	0	0	2.178099174
28		1.882644628			1.882644628
30		1.880578512			1.880578512
31	0	1.901239669	0	0	1.901239669
32	0.98076876	0.444628099	1.018795057	0.062395972	1.425396859
33	1.005816241	0.938429752	0	0	1.944245993
34	1.17062145	0.692561983	0	0	1.863183433
35	0.783183769	1.07892562	0	0	1.862109389
36	0.74244793	0.921900826	0	0	1.664348757
37	0.569677763	1.176033058	0	0	1.74571082
38	0.399659126	1.289669421	0	0	1.689328548
39	0.392263347	1.465289256	0	0	1.857552603
41	0	1.721487603	0	0	1.721487603
42	0	1.72768595	0	0	1.72768595
43	0	1.754545455	0	0	1.754545455
45	0	2.008677686	0	0	2.008677686
47		1.948760331			1.948760331
49		2.029338843			2.029338843
51	0	2.019008264	0	0	2.019008264
52	1.3759407	0.109917355	0.638343626	0.179058733	1.485858083
53	1.264585008	0.740082645	0	0	2.004667653
57	0.572666795	1.481818182	0	0	2.054484977
58	0.428747596	1.566528926	0	0	1.995276522
60	0.352706781	1.580991736	0.124286251	0.012779956	1.933698517
63	0	1.892975207	0	0	1.892975207
65	0.171543256	1.702892562	0	0	1.874435818
66	0.171720013	1.824793388	0	0	1.996513401

69	0.089771753	1.936363636	0	0	2.026135389
70	0	2.008677686	0	0	2.008677686
73	0	1.938429752	0	0	1.938429752
74	1.693006103	0.35785124	0.049684452	0.018627116	2.050857343
75	1.518424233	0.502479339	0	0	2.020903572
76	1.434234356	0.82892562	0	0	2.263159976
77	0.550836695	1.34338843	0	0	1.894225125
78	0.406248914	1.723553719	0	0	2.129802633
79	0.239784503	1.888842975	0	0	2.128627478
80	0	2.014876033	0	0	2.014876033
84	0.075964481	2.060330579	0	0	2.13629506
86	0.226363776	1.905371901	0.024641891	0	2.131735677
88	0.063007378	2.00661157	0	0	2.069618948
91	0.139355072	2.000413223	0.023924935	0	2.139768295

BV5 pulsed with BAC in the absence of nitrate

days	**sulfate (mM)**	**sulfide (mM)**	**sulfate and sulfide (mM)**
0	0.332077425	1.719884078	2.051961503
2	0.201445274	1.741511311	1.942956585
4	0.044440974	1.843159306	1.887600281
5	0.091509484	1.877762879	1.969272364
6	0.035644021	1.966434534	2.002078555
19	0.167844821	1.821532073	1.989376894
24	0.033026164	1.975085428	2.008111591
25	0	1.845322029	1.845322029
26	0	2.02266534	2.02266534
29	0.093176215	1.83234569	1.925521904
30	0	2.072407976	2.072407976
32	0.210658426	1.944807301	2.155465728
34	0.23028106	1.828020243	2.058301303
35	0	1.931830962	1.931830962
39		2.046455297	2.046455297
40	0.100518732	1.918854622	2.019373354
41	0.105620229	1.905878282	2.011498511
43	0.083520408	1.955620918	2.039141325
46	0	2.011851724	2.011851724
47	0	1.782603054	1.782603054
48	0.726797804	1.408451923	2.135249727
49	1.113702658	0.606081578	1.719784236
51	1.476585896	0.227605	1.704190896
53	1.352947728	0.25355768	1.606505408

54	1.566898252	0.078377092	1.645275345
56	0.763529028	0.960768199	1.724297228
57	0	1.791253947	1.791253947
60	0	2.087547039	2.087547039
64	0	1.994549937	1.994549937
83	0	2.102686102	2.102686102
85	0	2.070245253	2.070245253
88	0	1.910203729	1.910203729
90	0	1.938319131	1.938319131
104	0	1.946970025	1.946970025
106	0	1.944807301	1.944807301
109	0.014653461	1.815043903	1.829697365
110	1.203935905	0.716380466	1.920316372
111	1.431698916	0.288161253	1.719860169
112	1.850357663	0.100004325	1.950361989
113	1.93312716	0.106492495	2.039619656
114	1.781234069	0.095678879	1.876912948
115	1.973626092	0.100004325	2.073630417
116	2.073762176	0.028634457	2.102396632
117	1.957682026	0.097841602	2.055523628
118	2.063618843	0.035122626	2.098741469
119	1.938902457	0.039448073	1.97835053
120	1.986592211	0.130282452	2.116874663
121	2.121572752	0.071888923	2.193461675
122	1.853515572	0.216791384	2.070306955
123	1.664566978	0.381158355	2.045725333
124	1.31651285	0.781262165	2.097775015
125	1.158777692	0.932652796	2.091430488
126	0.438831871	1.501449025	1.940280896
127	0.355722165	1.626886976	1.982609141
129	0.213603581	1.778277607	1.991881188
130	0.014653461	1.888576496	1.903229957
131	0.014653461	1.972922704	1.987576166
132	0.014653461	1.946970025	1.961623486
133	0	1.951295471	1.951295471
137	0.157650231	1.899390112	2.057040343
138	0.215522745	1.676629612	1.892152357
139	0.465066164	1.520913534	1.985979699
140	0.495208734	1.34789567	1.843104404
141	0.943914666	1.190016869	2.133931535
142	1.485591295	0.843981141	2.329572436

143	1.170686935	0.988883602	2.159570538
144	0.996893249	1.086206151	2.083099399
145	0.564861403	1.401963753	1.966825155
146	0.13625836	1.797742117	1.934000477

BV5 pulsed with BAC with continuous injection of nitrate

days	**sulfate (mM)**	**sulfide (mM)**	**nitrate(mM)**	**nitrite (mM)**	**sulfate and sulfide (mM)**
0		2.01617717			
2	0	1.977248151	0	0	1.977248151
3	0	2.050780743	0	0	2.050780743
5	0	2.085384316	0	0	2.085384316
7	0	1.83234569	0	0	1.83234569
10	0	1.972922704	0	0	1.972922704
12	0	1.923180068	0	0	1.923180068
14	0	1.823694796	0.01754318	0	1.823694796
17	0	1.915702479	0	0	1.915702479
18	0	1.868181818	0	0	1.868181818
19	0.495416094	0.766942149	0.47504232	0.032447278	1.262358243
20	0.753879605	0.787603306	0.818927279	0.081505126	1.541482911
21	1.011429697	0.599586777	0	0.42963271	1.611016474
23	0.352664088	1.419834711	0	0	1.772498799
24	0.311061719	1.587190083	0	0	1.898251801
25	0.335403512	1.71322314	0	0	2.048626652
27	0	1.913636364	0	0	1.913636364
29		1.961157025			
31		1.954958678			
33	0	2.041735537	0	0	2.041735537
34	0.748176702	0.53553719	1.174248409	0.010416836	1.283713892
35	1.253158725	0.519008264	0.852901056	0.101953189	1.77216699
36	1.113658159	0.529338843	0	0	1.642997002
37	1.148848178	0.711157025	0	0	1.860005202
38	1.053537305	0.682231405	0	0	1.73576871
39	0.873661968	0.969421488	0	0	1.843083455
40	0.777397365	1.066528926	0	0	1.843926291
42	0.522650554	1.378512397	0.008476434	0	1.901162951
44	0.532339626	1.504545455	0	0	2.03688508
46	0.362171281	1.583057851	0	0	1.945229132
48	0.244849006	1.845454545	0	0	2.090303552
49	0	1.783471074	0	0	1.783471074

50	0	1.915702479	0	0	1.915702479
52	0	1.99214876	0	0	1.99214876
55	0	1.921900826	0	0	1.921900826
56	0.933631712	0.620247934	1.070804797	0	1.553879646
57	1.312450232	0.459090909	0	0	1.771541141
58	0.919826153	0.760743802	0	0	1.680569955
59	1.019274011	0.849586777	0	0	1.868860787
60	1.127877367	0.996280992	0	0	2.124158359
61	1.204547722	1.033471074	0	0	2.238018796
62	0.724584851	1.149173554	0	0	1.873758405
63	0.707358495	1.161570248	0	0	1.868928743
65	0	1.510743802	0.111728552	0	1.510743802
67	0	1.469421488	0.112475715	0	1.469421488
69	0	1.936363636	0	0	1.936363636
71	0	1.849586777	0.070687222	0	1.849586777
73	0	1.940495868	0	0	1.940495868
75	0	1.985950413	0	0	1.985950413
77	0	1.895041322	0	0	1.895041322
82	0	1.861983471	0	0	1.861983471
84	0	1.983884298	0	0	1.983884298
91	0	2.016942149	0	0	2.016942149
94	0	2.039669421	0	0	2.039669421
95	0	1.952892562	0	0	1.952892562
96	0	1.027272727	0.420205009	0	1.027272727
97	0	1.911570248	0	0	1.911570248
98	0	1.876446281	0	0	1.876446281
101	0	1.859917355	0	0	1.859917355
103	0	2.016942149	0	0	2.016942149

BV6 pulsed with Glut/BAC in the absence of nitrate

days	**sulfate (mM)**	**sulfide (mM)**	**sulfate and sulfide (mM)**
0	0	1.942644578	1.942644578
2	0	1.933993685	1.933993685
4	0	1.957783641	1.957783641
5	0	1.722046801	1.722046801
7	0	1.972922704	1.972922704
10	0	2.014014447	2.014014447
13	0.436599688	1.85785124	2.294450928
14	0	1.752479339	1.752479339

15	0.278701864	1.864049587	2.142751451
20	0	1.953458195	1.953458195
24	0.09220909	1.879925602	1.972134692
25	0	1.840996583	1.840996583
26	1.212233727	0.575803452	1.788037179
27	1.180188032	0.943466413	2.123654445
28	0.980848121	1.326268437	2.307116559
29	0.571244291	1.518750811	2.089995102
30	0.099135376	1.745836758	1.844972134
31	0.234715104	1.888576496	2.1232916
32	0	1.845322029	1.845322029
33	0.159908889	1.988061767	2.147970656
34	0.229584307	1.955620918	2.185205224
35	1.807557322	0.314113932	2.121671254
36	1.534463455	0.415761927	1.950225382
37	1.572430895	0.67961417	2.252045065
38	1.177761928	1.090531597	2.268293525
39	0.308234572	1.693931398	2.002165971
41	0.134312514	1.728534971	1.862847485
42	0	1.79341667	1.79341667
43	0	1.923180068	1.923180068
46	0	2.06808253	2.06808253
88	0	1.985899044	1.985899044
90	1.871501198	0	1.871501198
91	1.789619911	0.032959903	1.822579814
92	2.249433972	0.015658117	2.265092088
94	2.227240684	0.01782084	2.245061524
99	0.725121706	1.345732947	2.070854653
100	0.725121706	1.345732947	2.070854653
101	0.808180665	1.279338843	2.087519508
102	0.671620624	1.335123967	2.006744591
103	0	1.732860418	1.732860418
105	0	1.81288118	1.81288118
106	0	1.899390112	1.899390112
109	0	1.918854622	1.918854622
110	2.090232797	0.03079718	2.121029977
111	1.992370021	0	1.992370021
112	2.049611452	0	2.049611452
113	2.128872007	0	2.128872007
114	2.118015874	0	2.118015874
115	2.06545195	0.008677686	2.074129638

116	2.166358427	0.02430901	2.190667436
117	2.1098717	0.03728535	2.14715705
118	2.029416887	0.000519054	2.029935941
119	2.061686723	0.067563476	2.129250199
120	2.11282417	0.026471733	2.139295904
121	1.915058427	0.02430901	1.939367437
122	1.917123498	0.093516156	2.010639653
123	1.98979076	0.114049587	2.103840343
124	1.314562479	0.513084476	1.827646955
125	0.84834587	1.280851248	2.129197118
126	0.767227581	1.352221117	2.119448698
127	0.651948547	1.449543665	2.101492213
129	0.60295095	1.60958519	2.21253614
130	0.650605008	1.568493447	2.219098455
131	0.694682329	1.618236083	2.312918412
132	0.700315981	1.540378044	2.240694025
133	0.782835864	1.540378044	2.323213908
134	0.665864966	1.456031835	2.121896801
135	0.835482721	1.486309962	2.321792682
136	0.674199889	1.507937195	2.182137083

BV6 pulsed with Glut/BAC with continuous injection of nitrate

days	**sulfate (mM)**	**sulfide (mM)**	**nitrate(mM)**	**nitrite (mM)**	**sulfate and sulfide (mM)**
1	0.91595066	1.021324452	0	0	1.937275111
2	0.428079117	1.520913534	0	0	1.948992651
3	0.571365071	1.529564428	0	0	2.100929499
4	0.412915038	1.477659068	0	0	1.890574106
10	0	1.670141442	0	0	1.670141442
13	0	1.650676932	0	0	1.650676932
16	0.553974478	1.064578918	0.028714693	0.023097363	1.618553395
17	0	1.624724253	0	0	1.624724253
20	0	1.646351486	0	0	1.646351486
21	1.2538513	0.10785124	0	0.080389204	1.361702539
22	0.996143021	0.669834711	0	0	1.665977732
23	0.816757264	1.060330579	0	0	1.877087843
24	0.725545816	1.161570248	0	0	1.887116064
25	0.594262506	1.52107438	0	0	2.115336886
27	0	1.729752066	0	0	1.729752066

29	0	1.671900826	0	0	1.671900826
31	0	1.855785124	0.015634823	0	1.855785124
37	0	1.921900826	0	0	1.921900826
39	0	1.981818182	0	0	1.981818182
51	0	1.952892562	0.049126099	0	1.952892562
55	0	2.031404959	0	0	2.031404959
56	0	2.00661157	0	0	2.00661157
57	1.524513355	0	1.705349707	0.019071403	1.524513355
58	1.709741011	0	1.709908273	0.007505517	1.709741011
59	1.325737464	0.126446281	0	0	1.452183745
60	1.860841383	0	0.019938034	0	1.860841383
61	1.424833584	0.215289256	0	0	1.640122841
62	1.512905214	0.192561983	0	0	1.705467198
63	1.716987992	0.111983471	0	0	1.828971463
64	1.658525766	0.151239669	0	0	1.809765436
65	1.461392741	0.225619835	0	0	1.687012576
66	1.554960404	0.147107438	0.008167097	0	1.702067842
68	1.295836235	0.397107438	0	0	1.692943673
70	0.763050701	1.060330579	0	0	1.82338128
72	0.624925722	1.446694215	0	0	2.071619937
76	0	1.609917355	0	0	1.609917355
78	0	1.692561983	0	0	1.692561983
80	0	1.709090909	0	0	1.709090909
83	0	1.783471074	0	0	1.783471074
85	0.04114111	1.789669421	0	0	1.830810532
87	0	1.802066116	0	0	1.802066116
92	0	1.895041322	0	0	1.895041322
94	0	1.923966942	0	0	1.923966942
101	0	1.932231405	0	0	1.932231405
104	0	1.909504132	0	0	1.909504132
105	0	1.845454545	0	0	1.845454545
106	1.314545444	0.066528926	0.053134233	0.874103921	1.38107437
107	1.077486469	0.793801653	0	0	1.871288122
108	0.502997016	1.202892562	0	0	1.705889578
110	0	1.8	0	0	1.8
111	0	1.8	0	0	1.8
113	0	1.84338843	0	0	1.84338843
115	0	1.676033058	0.096712992	0	1.676033058
118	0	1.897107438	0	0	1.897107438
135	0	2.09338843	0	0	2.09338843
136	0.904477623	1.671900826	0	0	2.57637845
139	0	1.99214876	0.008125484	0	1.99214876

143	0	1.97768595	0	0	1.97768595
144	1.845536796	0.060330579	0	0	1.905867375
146	1.319333532	0.46322314	0	0	1.782556672
147	1.14745956	0.729752066	0	0	1.877211626
148	0.866713339	1.041735537	0	0	1.908448876
151	0.29274809	1.731818182	0.004049255	0	2.024566271
153	0	1.8	0	0	1.8
155	0	1.973553719	0	0	1.973553719

BV7 pulsed with THPS in the absence of nitrate

days	**sulfate (mM)**	**sulfide (mM)**	**sulfate and sulfide (mM)**
0	0.150693706	1.752324928	1.903018633
2	0	1.860461093	1.860461093
4	0.085144855	1.966434534	2.05157939
5	0.043905593	1.940481855	1.984387448
6	0.069753686	1.955620918	2.025374604
7	0	1.964271811	1.964271811
9	0	2.00320083	2.00320083
11	0.072168708	2.024828063	2.096996772
24	0.178509367	1.895064665	2.073574033
25	0.369700666	1.929668238	2.299368904
26	0.820690733	1.237596782	2.058287515
27	0.668360451	1.795579394	2.463939845
28	0.138060811	2.020502617	2.158563428
29	0.189052104	2.001038107	2.190090211
30	0.130337643	1.977248151	2.107585794
32	0.084697402	2.052943466	2.137640868
33	0.175971755	1.985899044	2.161870799
34	0.087265662	1.80639301	1.893658672
35	0	2.100523379	2.100523379
36	0.053571812	2.001038107	2.054609919
37	1.315335084	0.943466413	2.258801497
41	0.747798744	1.365197457	2.112996201
42	0.421629772	1.574981617	1.996611389
43	0.308938953	2.098360656	2.407299609
46	0.091470497	2.215147714	2.306618211
88	0	2.06159436	2.06159436
90	1.833995747	0.158397855	1.992393601
91	1.763111715	0.257883126	2.020994842

92	1.711867657	0.4503655	2.162233157
93	1.648855298	0.71205502	2.360910318
94	0.555475731	1.410614646	1.966090377
95	0.84250537	1.600934296	2.443439666
96	0.625228171	1.693931398	2.319159569
99	0.283419912	1.732860418	2.01628033
102	0.137967902	1.879925602	2.017893504
104	0.153220839	1.938319131	2.091539971
106	0.050994201	1.962109088	2.013103289
109	0	2.087547039	2.087547039
110	1.87245058	0.035122626	1.907573207
111	1.848783832	0.054587136	1.903370969
112	1.991917396	0.035122626	2.027040022
113	1.899812897	0.045936243	1.94574914
114	1.947314902	0.067563476	2.014878378
115	1.791113203	0.091353432	1.882466635
117	1.804349847	0.104329772	1.908679619
118	1.968959546	0.095678879	2.064638425
119	1.794680938	0.216791384	2.011472321
120	1.555433532	0.346554782	1.901988314
121	1.237138142	0.528223539	1.765361681
122	1.228003812	0.794238505	2.022242317
123	0.970702739	0.88291016	1.8536129
124	0.716888052	1.233271335	1.950159387
125	0.705167986	1.414940093	2.120108078
126	0.609449267	1.345732947	1.955182214
127	0.398505503	1.55767983	1.956185333
129	0.567688178	1.574981617	2.142669795
130	0.381149113	1.661490549	2.042639662
131	0.385983568	1.732860418	2.118843985
133	0.328405908	1.665815996	1.994221904
134	0.379394299	1.685280505	2.064674804
135	0.404688027	1.724209525	2.128897551
136	0.164417948	1.735023141	1.899441089
138	0.05930342	1.892901942	1.952205362
139	0.165876419	1.938319131	2.104195551

BV7 pulsed with THPS with continuous injection of nitrate

days	sulfate (mM)	sulfide (mM)	nitrate (mM)	nitrite (mM)	sulfate and sulfide (mM)
0	0	2.00320083	0	0	2.00320083
2	0	1.808555733	0	0	1.808555733
3	0	1.732860418	0	0	1.732860418
5	0	1.849647476	0	0	1.849647476
6	0	1.879925602	0	0	1.879925602
8	0	1.946970025	0	0	1.946970025
11	0	1.942644578	0	0	1.942644578
13	0	1.985899044	0	0	1.985899044
14	0	1.908041005	0	0	1.908041005
15	1.140674992	0.043801653	0.411662992	0.238560075	1.184476645
16	0.581787177	0.766942149	0	0	1.348729326
17	0	1.411570248	0	0	1.411570248
18	0	1.587190083	0	0	1.587190083
19	0	1.46322314	0	0	1.46322314
20	0	1.260743802	0	0	1.260743802
21	0	1.556198347	0	0	1.556198347
22	0	1.725619835	0	0	1.725619835
23	0	1.932231405	0	0	1.932231405
25	0	1.938429752	0	0	1.938429752
29	0	1.959090909	0	0	1.959090909
30	1.260605723	0.072727273	0.202425697	0.057733754	1.333332995
31	1.105936878	0.841322314	0	0	1.947259192
35	0	1.884710744	0	0	1.884710744
37	0	1.994214876	0	0	1.994214876
39	0	1.702892562	0	0	1.702892562
41	0	1.983884298	0	0	1.983884298
42	0.571731254	0.686363636	0.114566438	0.031283735	1.25809489
43	0	1.907438017	0.18064942	0	1.907438017
44	0	1.820661157	0	0	1.820661157
45	0	1.748347107	0	0	1.748347107
46	0	1.820661157	0	0	1.820661157
48	0	2.072727273	0	0	2.072727273
49	0	1.926033058	0	0	1.926033058
50	0	2.012809917	0	0	2.012809917
53	0	1.950826446	0	0	1.950826446
54	0	1.923966942	0	0	1.923966942
55	0	1.62231405	0.229644315	0	1.62231405

56	0	1.738016529	0.164185906	0.000634276	1.738016529
57	0	1.812396694	0	0	1.812396694
58	0	1.84338843	0	0	1.84338843
59	0	1.849586777	0	0	1.849586777
60	0	1.851652893	0	0	1.851652893
62	0	1.85785124	0	0	1.85785124
63	0	1.818595041	0	0	1.818595041
64	0	1.818595041	0	0	1.818595041
66	0	1.8	0.011517862	0	1.8
68	0	1.892975207	0	0	1.892975207
69	0	1.911570248	0	0	1.911570248

BV8 pulsed with cocodiamine in the absence of nitrate

days	**sulfate (mM)**	**sulfide (mM)**	**sulfate and sulfide (mM)**
0	0	1.929668238	1.929668238
2	0	1.853972923	1.853972923
4	0	1.910203729	1.910203729
5	0	1.946970025	1.946970025
6	0	1.840996583	1.840996583
7	0.288120428	1.741511311	2.029631739
11	0	1.983736321	1.983736321
12	0	1.979410874	1.979410874
13	0.399341021	1.908041005	2.307382027
14	0.23369067	2.014014447	2.247705117
15	0	2.01617717	2.01617717
17	0	2.128638782	2.128638782
20	0	1.866949263	1.866949263
26	0	1.979410874	1.979410874
27	0	1.897227389	1.897227389
28	0	2.037804403	2.037804403
29	0	1.970759981	1.970759981
30	0	2.059431636	2.059431636
32	0	2.135126952	2.135126952
34	0	2.119987889	2.119987889
35	0	2.113499719	2.113499719
39	0	2.057268913	2.057268913
41	0	2.107011549	2.107011549
43	0	1.972922704	1.972922704
46	0	1.760975821	1.760975821
47	0	1.849647476	1.849647476
48	0	2.081058869	2.081058869
49	0	2.076733423	2.076733423
50	0	2.176218695	2.176218695
51	0	2.104848826	2.104848826

53	0	2.145940568	2.145940568
56	0	2.00320083	2.00320083
57	0	1.802067563	1.802067563
60	0	2.091872486	2.091872486
64	0	2.044292573	2.044292573
85	0	1.892901942	1.892901942
88	0.110964277	1.952892562	2.063856839
89	1.643091929	0.510743802	2.15383573
91	0.765207011	1.628512397	2.393719408
94	0.765119881	1.597520661	2.362640542
95	0.696806384	1.583057851	2.279864235
97	0.440594517	1.764876033	2.20547055
99	0.103805522	1.888842975	1.992648497
102	0.067879045	1.946970025	2.01484907
103	1.573345305	0.357368398	1.930713703
104	1.469552263	0.562827112	2.032379375
105	1.081430931	1.190016869	2.2714478
106	0.568619631	1.644188762	2.212808394
107	0.436893248	1.549028937	1.985922186
109	0.381245644	1.771789437	2.153035081
110	0.439539085	1.717721355	2.157260439
111	0.437384344	1.665815996	2.10320034
112	0.453138278	1.696094122	2.1492324
113	0.339495156	1.613910636	1.953405793
114	0.377049363	1.724209525	2.101258887
116	0.349821826	1.637700593	1.987522419
118	0.260612844	1.7869285	2.047541345
119	0.130843397	1.871274709	2.002118106
120	0.011021632	1.817206627	1.828228259
121	0.126716265	1.81288118	1.939597445
122	0	1.834508413	1.834508413
124	0	1.992387214	1.992387214
125	0	1.834508413	1.834508413
127	0	1.992387214	1.992387214
130	0	1.959946364	1.959946364
133	0	2.02915351	2.02915351
134	1.253449542	0.091353432	1.344802974
135	1.144425433	0.24706951	1.391494943
136	1.039195112	0.467667287	1.506862399
137	0.795668025	0.738007699	1.533675724
138	0.48186135	1.142436957	1.624298307
140	0	1.39331286	1.39331286
141	0	1.540378044	1.540378044
142	0	1.709070462	1.709070462
143	0	1.57065617	1.57065617
144	0	1.559842554	1.559842554
146	0	1.592283403	1.592283403

BV8 pulsed with coocdiamine with continuous injection of nitrate

days	sulfate (mM)	sulfide (mM)	nitrite (mM)	nitrate (mM)	Sulfate and sulfide (mM)
0	0.31406987	1.57714434	0	0	1.891214211
2	0.423938235	1.546866214	0	0	1.970804449
3	0.418579477	1.564168	0	0	1.982747477
5	0.742613811	1.538215321	0	0	2.280829132
6	0.567958743	1.471170898	0	0	2.039129642
12	0.320093432	1.700419568	0	0	2.020513001
17	0	1.965289256	0	0	1.965289256
19	0	1.913636364	0	0	1.913636364
21	0	2.000413223	0	0	2.000413223
22	1.530785601	0	0.210332159	0.559551656	1.5307856
23	1.647864841	0.099586777	0	0.150409705	1.747451618
24	1.367322168	0.8	0	0	2.167322168
25	0.837571901	1.465289256	0	0	2.302861157
26	0.465687234	1.531404959	0	0	1.997092192
29	0.2638403	1.859917355	0	0	2.123757655
31	0	1.911570248	0	0	1.911570248
33	0	1.878512397	0	0	1.878512397
34	1.403070648	0.105785124	0.642705343	0.047384075	1.5088558
35	0.828159222	0.789669421	0	0	1.617828643
36	0.523291289	1.729752066	0	0	2.253043356
37	0.542723268	1.63677686	0	0	2.179500127
38	0.174374044	1.669834711	0	0	1.844208755
39	0.359667128	1.783471074	0	0	2.143138203
40	0.143945148	1.715289256	0	0	1.859234404
43	0	1.864049587	0	0	1.864049587
46	0	1.870247934	0	0	1.870247934
47	0	1.903305785	0	0	1.903305785
48	1.607818178	0.171900826	0.112082017	1.264224229	1.779719005
49	1.721739312	0.126446281	0.352685864	0.746101635	1.848185593
50	1.357488369	0.217355372	0.439714515	0.058173858	1.574843741
51	1.41526525	0.28553719	0	0	1.70080244
52	1.266698842	1.031404959	0	0	2.298103801
53	1.105941864	1.128512397	0	0	2.234454261
54	1.023698583	1.068595041	0	0	2.092293624
55	0.690790209	1.469421488	0	0	2.160211697
56	0.778090955	1.364049587	0	0	2.142140542
57	0.616492315	1.626446281	0	0	2.242938596
59	0	1.707024793	0	0	1.707024793
61	0	1.723553719	0	0	1.723553719
63	0	1.779338843	0	0	1.779338843
66	0	1.892975207	0	0	1.892975207

68	0	1.905371901	0	0	1.905371901
71	0	1.923966942	0	0	1.923966942
74	0	1.789669421	0	0	1.789669421
76	0	1.996280992	0	0	1.996280992
77	0	1.32892562	0	0	1.3289256
78	0	1.969421488	0	0	1.969421488
86	0	1.996280992	0	0	1.996280992
87	0	2.023140496	0	0	2.023140496
90	0	2.066528926	0	0	2.066528926
93	0	1.948760331	0	0	1.948760331
94	0	1.971487603	0	0	1.971487603
95	1.323669335	0	0.585945244	0	1.3236693
96	1.012610409	0.909504132	0	0	1.922114541
97	0.972879102	0.921900826	0	0	1.894779928
98	0.593710158	1.233884298	0	0	1.827594455
100	0	1.632644628	0	0	1.632644628
102	0	1.700826446	0	0	1.700826446
104	0	1.676033058	0	0	1.676033058
106	0	1.754545455	0	0	1.754545455
108	0	1.779338843	0	0	1.779338843
111	0	1.880578512	0	0	1.880578512