DENITRIFICATION AND DESULFURIZATION WITH ELEMENTAL SULFUR AND HYDROGEN SULFIDE

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ABSTRACT

Compared with conventional heterotrophic denitrification, sulfur-based autotrophic denitrification offers several advantages for the treatment of waters contaminated with nitrite or nitrate. First, it eliminates the needs for adding of organic carbons in the case of organic deficient wastewaters. Furthermore, the quantity of sludge produced under autotrophic conditions is substantially lower than that in a heterotrophic process which in turn reduces the cost associated with the treatment and digestion of the sludge.

Desulfurization under denitrifying conditions is a suitable alternative for removal of H_2S from contaminated gaseous streams because it eliminates the need of light energy input required for photoautotrophic desulfurization, and the supply of oxygen in aerobic chemolithotrophic desulfurization, in which simultaneous presence of both hydrocarbon gas and oxygen imposes a serious safety issue.

In this work sulfur-based autotrophic denitrification and denitritation were studied in batch system using freely suspended cells and in a continuous biofilm reactor. Desulfurization of a H_2S-containing gaseous stream under denitrifying conditions was studied in a semi-continuous packed bed reactor. Coleville enrichment, a mixed culture originated from a Canadian oil reservoir, which has the ability to function under both heterotrophic and autotrophic conditions, was used as the bacterial culture.

The order of preference for electron donors used by the Coleville enrichment during the denitrification was established as: sulfide > biologically produced sulfur > acetate > elemental sulfur. Sulfate productions closely matched with theoretical values expected from stoichiometry

in the batch experiments. Sodium bicarbonate functioned as an effective buffering agent and an inorganic carbon source during sulfur-based autotrophic denitrification. Strong inhibitory effect of nitrite on bacterial activity was observed.

In the continuous biofilm reactor, sulfur-based nitrate removal rate increased linearly with the increase of nitrate loading rate through either an increase of feed flow rate or a variation of feed concentration. Similar trends were observed in the nitrite removal experiment. The highest nitrate removal rate (17.3 mM h^{-1}) was obtained at a nitrate loading rate of 24.2 mM h^{-1} (corresponding residence time: 0.4 h) with a nitrate removal efficiency of 71.3% and a total nitrogen removal efficiency of 9.5%. The highest nitrite removal rate (13.2 mM h^{-1}) was achieved at a nitrite loading rate of 18.0 mM h^{-1} (corresponding residence time: 0.6 h) with a nitrite removal percentage of 73.6%. The removal rates obtained in the present work were much higher than those reported in the literature.

In the semi-continuous desulfurization experiments, the removal efficiency of H_2S remained greater than 98.6% and 99.4% with nitrate and nitrite as the electron acceptor, respectively. The reduction rates of nitrate and nitrite increased with the increase of H_2S loading rate through a variation of feed gas flow rate. The observed denitrification and denitritation rates were much higher than those obtained in the batch denitrification experiments with elemental sulfur and acetate.

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DEDICATIONS

I dedicate this thesis to my mother and father. Thanks for always being there for me.

And it is also dedicated to my lovely family, Amanda, Melinda and Shijie. Every moment of joy and happiness gives me endless power to keep moving forward.

In memory of my dear brother, Yijiang Sun.

NOMENCLATURE

a-NR-SOB	Autotrophic-nitrate reducing, sulfide oxidizing bacteria acclimated to elemental sulfur
BOD	Biological oxygen demand (mg L^{-1})
COD	Chemical oxygen demand (mg L^{-1})
Conc.	Concentration (mM)
CSB	Coleville synthetic brine
GC	Gas chromatography
h-NRB	Heterotrophic nitrate reducing bacteria
HRT	Hydraulic retention time (h)
IC	Ion chromatography
M	Moles per liter (mol L^{-1})
mM	Milimoles per liter (mmol L^{-1})
NR-SOB	Nitrate reducing, sulfide oxidizing bacteria
rpm	Revolution per minute
TN	Total nitrogen
VLR	Volumetric loading rate (mM h^{-1})
VRR	Volumetric removal rate (mM h^{-1})
VSS	Volatile suspended solids

GLOSSARY

Nitrification	Biological process of ammonium oxidation to nitrate
Denitrification	Biological process of nitrate reduction to nitrite and/or gaseous nitrogen
Denitritation	Biological process of nitrite reduction to gaseous nitrogen
Desulfurization	Process of sulfide oxidation to elemental sulfur or sulfate

Chapter 1 INTRODUCTION

Nitrate is one of the main contributors to eutrophication of surface water bodies which can cause severe ecological and environmental problems. High concentration of nitrate in the ground water is believed to be the cause of "blue baby syndrome" in rural areas (Moon *et al*., 2004; Kimura, *et al*., 2002). Nitrate can be converted to nitrous oxide (N_2O), which is a greenhouse gas contributing to global warming, acidic deposition and the formation of other secondary pollutants. Extensive utilization of synthetic fertilizer and release of improperly treated wastewater from industrial or municipal facilities are the causes of nitrate contamination in natural water systems. Biological removal of nitrate has been studied for several decades (Gulf south research institute, 1970; Klapwijk, *et al*., 1981; Henze, 1990; Ra *et al*., 2000) and heterotrophic denitrification has been applied in numerous municipal wastewater plants worldwide. However, for high nitrate strength wastewaters with low BOD contents (organic-deficient waste waters), addition of external organic materials is essential which increases the operation costs. Residual organics and high yield of cells in heterotrophic denitrification may cause biofouling (Sierra-Alvarez *et al.*, 2007) and make it unsuitable for the treatment of nitrate contaminated groundwater. In these specific situations, sulfur based autotrophic denitrification is a promising alternative.

Sulfur based autotrophic denitrification is carried out by a group of microorganisms referred to as nitrate-reducing, sulfide-oxidizing bacteria (NR-SOB). Under anaerobic conditions, NR-SOB use reduced sulfur compounds like sulfide, elemental sulfur, thiosulfate or sulfite as electron

donors as well as energy sources, while using inorganic compounds such as bicarbonate as a carbon source to accomplish the reduction of nitrate or nitrite.

Hydrogen sulfide is a highly toxic and odorous gas which can be produced from other sulfur compounds by sulfate reducing bacteria (SRB) under anaerobic conditions (Nemati *et al*., 2001). Biogenic production of sulfide is common in oil reservoirs, landfills, wastewater collection and treatment plants. H_2S is also a minor component of biogas, a by-product of anaerobic digestion of biodegradable substances (Syed *et al*., 2006). The corrosive, toxic and flammable natures of H_2S cause problems in the transportation of H_2S-containing fuels like natural gas. Upon combustion, H_2S is oxidized to sulfur dioxide (SO_2), a primary air pollutant which is the main cause of acid rain, as well as smog and haze. Biological removal of sulfide is a cost effective method when compared with physicochemical desulfurization approaches and has been studied extensively (Henshaw and Zhu, 2001; Ng *et al*., 2004; Duan *et al*., 2005; Vaiopoulou *et al*., 2005; Gadekar *et al*., 2006; Ma *et al*., 2006; Datta *et al*., 2007; Vannini *et al*., 2008; Rattanapan *et al*., 2009; Tang *et al*., 2010; An *et al*., 2010). Desulfurization under denitrifying conditions is a suitable alternative for the removal of H_2S from fuels. Because it eliminates the need of light energy input required for photoautotrophic desulfurization. And in the case of aerobic chemolithotrophic desulfurization, simultaneous presence of hydrocarbon gas and oxygen imposes a serious safety issue.

Coleville enrichment is a mixed nitrate-reducing, sulfide-oxidizing (NR-SOB) microbial culture which has been enriched from the produced water of a Canadian oil reservoir. The main microbial component of Coleville enrichment, *Thiomicrospira* sp. CVO, tolerates sulfide at concentrations as high as 16 mM, a level much higher than that reported for other NR-SOBs (An

et al., 2010). *Thiomicrospira* sp. CVO also has the ability to function autotrophically using CO_2 and reduced sulfur compounds as carbon and energy sources, respectively, or heterotrophically where organic compounds serve as both carbon and energy sources (Gevertz *et al*., 2000). Nitrate and/or nitrite are used by *Thiomicrospira* sp. CVO as the terminal electron acceptor during either modes of activity. These characteristics of *Thiomicrospira* sp. CVO together with the presence of other heterotrophic species make Coleville enrichment a suitable biocatalyst for denitrification, desulphurization and removal of BOD. Using sulfide and acetate as energy sources (electron donor), kinetic aspects of autotrophic and heterotrophic denitrification by Coleville enrichment have been investigated by others in the same laboratory (An *et al*., 2010; Tang *et al*., 2010; An *et al*., 2011; An *et al*., 2012). Sulfur-based autotrophic denitrification offers several advantages when compared with its heterotrophic counterpart, especially in the case of organic-deficient waters. The objectives of this thesis were (1) to better understand the principles of autotrophic denitrification and denitritation with elemental sulfur as the electron donor; (2) to evaluate the capability of Coleville enrichment culture in sulfur-based autotrophic denitrification and denitritation process; (3) to study the biodesulfurization of gaseous hydrogen sulfide under denitrifying conditions. The thesis presented here consists of literature review, specific research objectives, materials and methods, results and discussions, conclusions and recommendations for future work.

Chapter 2 LITERATURE REVIEW

2.1 Biological Removal of Nitrate

Biological removal of nitrogen compounds through nitrification-denitrification processes in wastewater treatment systems has already been applied worldwide for several decades (Gulf South Research Institute, 1970; Klapwijk, *et al*., 1981; Henze, 1990; Ra *et al*., 2000). Biological processes of converting ammonium to nitrate (nitrification) and then reducing nitrate to nitrogen gas (denitrification) using different groups of bacteria are feasible and popular because of their relative low cost and comparable high efficiency over physicochemical counterparts (Hansen *et al*., 1998; Zhao *et al*., 1999; Ergas and Rheinheimer, 2004; Dhamole *et al*., 2007; Molinuevo *et al*., 2009).

Depending on the available types of electron donors and carbon sources and the constraints of each particular treatment situation, there are several different approaches to choose from as far as biological nitrate removal is considered.

2.1.1 Heterotrophic denitrification

Heterotrophic denitrification is a broadly applied biological approach for the treatment of nitrate- and BOD- containing wastewater because of its high removal efficiency and the ability to remove nitrate and organic compounds simultaneously (Klapwijk, *et al*., 1981; Ra *et al*., 2000; Tang *et al*., 2010; An *et al*., 2011).

In municipal wastewater treatment plants, ammonium is oxidized to nitrate through the aerobic nitrification process and then under anaerobic conditions nitrate is reduced to nitrite and subsequently to nitrogen gas through the heterotrophic denitrification process. The main reactions during the nitrification process can be summarized as Equation 2.1 and 2.2 (McHarness and McCarty, 1973):

$$2NH_4^+ + 3O_2 \longrightarrow 2NO_2^- + 2H_2O + 4H^+ \quad (2.1)$$

$$2NO_2^- + O_2 \longrightarrow 2NO_3^- \quad (2.2)$$

Under anaerobic conditions, the produced nitrate is reduced to nitrogen gas by a group of bacteria referred to as heterotrophic nitrate reducing bacteria (h-NRB). Organic compounds such as acetate, methanol and many others function as the electron donors as well as carbon sources in the heterotrophic denitrification process. Nitrate reduction in the presence of acetate and methanol can be described by Equation 2.3 and 2.4 (Tang *et al.*, 2010; Gulf South Research Institute, 1970):

$$8NO_3^- + 5CH_3COO^- \longrightarrow 4N_2 + 10CO_2 + H_2O + 13OH^- \quad (2.3)$$

$$6NO_3^- + 5CH_3OH \longrightarrow 3N_2 + 5CO_2 + 7H_2O + 6OH^- \quad (2.4)$$

An advantage of heterotrophic denitrification is that the removal of nitrate and organic compounds (COD or BOD) is accomplished efficiently at the same time. Since most municipal wastewater contains certain amount of biodegradable organics which can serve as carbon sources and electron donors, heterotrophic denitrification is widely applied in the treatment of municipal wastewater or a mixture of a high nitrate concentration wastewater and a high organic-containing wastewater. Tang *et al*. (2010) achieved high nitrate and acetate removal rates of 183.2 and 88.0 mM h^{-1}, respectively, in a biofilm reactor with a nitrate removal efficiency of 100%

(corresponding residence time: 0.8 h). Ficara and Canziani (2007) established a lab-scale sequencing batch reactor (SBR) to study heterotrophic denitrification and reported a maximum denitrification rate of 16.3 ± 1.2 mg N (g VSS h)$^{-1}$ with acetate as the carbon source. Sun *et al*. (2012) suggested that rapid in situ denitrification of 35.7 mM nitrate (500 mg NO_3^--N L^{-1}) could be obtained at initial biodegradable COD to NO_3^--N ratios higher than 6.0 in the feed which was a leachate.

In many situations the level of readily biodegradable substrates is often the limiting factor for complete denitrification when dealing with high nitrate strength wastewater which is deficient in organics. Typical examples are contaminated ground water, landfill leachate and leather and fertilizer-processing wastewaters (Shao *et al*., 2010, Sun *et al*., 2012). In these situations addition of external organic carbon sources is required which increases the operation costs considerably. Furthermore, the dosage of external organics has to be carefully monitored otherwise the excess organics residing in the effluent may cause biofouling and other environmental problems.

Some researchers tried to supply the denitrification process with an inexpensive external carbon source to lower the operation cost. Thalasso *et al*. (1997) supplied methane as the sole carbon source to study the denitrification process and reported a denitrification rate of 0.6 kg NO_3^--N (kg VSS day)$^{-1}$ in batch reactors. In this process methane was oxidized by methanotrophic bacteria to intermediate organic compounds which were later consumed as electron donors in the denitrification process. However, the utilization efficiency of methane was low, in some experiments 90% of the total methane oxidation was not related to denitrification. Warneke *et al*. (2011) used maize cobs, wheat straw, green waste, sawdust, pine woodchips or eucalyptus woodchips as alternative carbon sources. Nitrate removal rates obtained by these researchers

ranged from 3.9×10^{-3} mM h^{-1} (1.3 g N m^{-3} d^{-1} with pine woodchips) to 1.9×10^{-2} mM h^{-1} (6.2 g N m^{-3} d^{-1} with maize cobs), and were predominantly limited by the bioavailability of carbon source and temperature when nitrate concentrations remained above 1 mg L^{-1}. Sage *et al*. (2006) reported that the denitrification rate with a readily biodegradable moiety of the mixtures from dairy effluent (lactose, lactate, proteins, and fat) was similar to the highest rate obtained with individual components such as lactose or lactate.

2.1.2 Autotrophic denitrification

Autotrophic denitrification is another alternative for biological nitrate removal in which either hydrogen gas or reduced sulfur compounds such as sulfide or H_2S, sulfur and thiosulfate serve as electron donors while inorganic materials such as bicarbonate serves as a carbon source.

2.1.2.1 Hydrogen based autotrophic denitrification

H_2-based autotrophic denitrification in which hydrogen gas is used as the electron donor can be described by Equation 2.5 (Lee and Rittmann, 2002; Chang *et al.*, 1999). The end products are comprised of nitrogen gas and water implying that there is no concern regarding treated effluent since the produced nitrogen and residual hydrogen can be easily stripped from the effluent. This feature makes it a unique clean option for drinking water denitrification.

$$5H_2 + 2NO_3^- + 2H^+ \longrightarrow 6H_2O + N_2 \qquad (2.5)$$

Chang *et al.* (1999) employed a fluidized bed reactor to study the hydrogenotrophic denitrification process and obtained a maximum nitrogen removal rate of 1.8 – 2.1 mM h^{-1} (0.6-0.7 kg N $m^{-3}d^{-1}$). A novel hollow-fiber membrane biofilm reactor was used by Lee and Rittmann

(2002) to study the hydrogenotrophic denitrification of drinking water. The system achieved partial nitrate removals between 39% and 92% with effluent nitrate concentration in the range of 0.03 – 0.65 mM (0.4 - 9.1 mg NO_3^--N L^{-1}) and effluent hydrogen concentration below 0.1mg H_2 L^{-1}. Lee *et al*. (2010) reported a maximum nitrate removal rate of 7.2 mM h^{-1} (1.68 mg NO_3^--N L^{-1} min^{-1}) when using a packed bed reactor to study the hydrogenotrophic denitrification. Sunger and Bose (2009) obtained 95% nitrate removal at a nitrate loading of 8.6×10^{-5} mM h^{-1} (28.9 mg $m^{-3}d^{-1}$) and a HRT of 15.6 days in a continuous experiment.

The nitrate removal ability of hydrogenotrophic denitrification process is significantly lower than that of heterotrophic denitrification. Furthermore, the utilization efficiency of hydrogen gas is relatively low in general. Significant amount of hydrogen gas escapes with the effluent stream when a traditional bioreactor configuration is used which results in a high operation cost and a potential of explosion (Lee and Rittmann, 2002). To circumvent this problem, one option is to employ a membrane bioreactor system which results in high operation and maintenance costs (Sierra-Alvarez *et al.*, 2007).

2.1.2.2 Sulfur based autotrophic denitrification

Sulfur based autotrophic denitrification process is carried out by a group of microorganisms referred to as nitrate reducing sulfide oxidizing bacteria (NR-SOB). Under anaerobic conditions, NR-SOB use reduced sulfur compounds like sulfide, elemental sulfur, thiosulfate or sulfite as electron donors as well as energy sources, while using inorganic carbon compounds like bicarbonate as a carbon source to convert nitrate to nitrite, and subsequently to nitrogen gas.

When sulfide serves as the electron donor, this process could also be referred to as anaerobic chemolithotrophic desulfurization, or simultaneous denitrification and desulfurization. The ability of simultaneous nitrate and sulfide removal has made this process a worthy topic of detailed studies. The process of biodesulfurization can be carried out through a variety of pathways as described by Equation 2.6-2.10 (An *et al*., 2010; Tang *et al*., 2010) where elemental sulfur or sulfate and nitrite or nitrogen gas are the end products of sulfide oxidation and nitrate reduction.

$$5S^{2-} + 2NO_3^- + 6H_2O \longrightarrow 5S + N_2 + 12OH^- \quad (2.6)$$

$$S^{2-} + NO_3^- + H_2O \longrightarrow S + NO_2^- + 2OH^- \quad (2.7)$$

$$3S^{2-} + 2NO_2^- + 4H_2O \longrightarrow 3S + N_2 + 8OH^- \quad (2.8)$$

$$S^{2-} + 4NO_3^- \longrightarrow SO_4^{2-} + 4NO_2^- \quad (2.9)$$

$$5S^{2-} + 8NO_3^- + 4H_2O \longrightarrow 5SO_4^{2-} + 4N_2 + 8OH^- \quad (2.10)$$

Within this category, sulfur-based autotrophic denitrification where elemental sulfur serves as the electron donor (described by Equation 2.11-2.13) (An *et al*., 2010; Tang *et al*., 2010) shows several unique advantages and has drawn more attentions recently. For instance, in treatment of nitrate-contaminated groundwater, sulfur-based autotrophic denitrification eliminates the potential problems associated with residual organics in heterotrophic denitrification (Sierra-Alvarez *et al.*, 2007; Reyes *et al*., 2007) and it produces less sludge which in turn reduces the cost associated with the processing of sludge. Since sulfur is insoluble in water, a biological treatment system with sulfur particles as packing material makes it unnecessary to closely control the dosage of electron donors (Kim *et al*., 2004). Finally, elemental sulfur is cheaper than acetate, ethanol or methanol, the common external organics added in wastewater treatment plants

where a heterotrophic denitrification process is used, especially in the case of organic deficient wastewaters.

$$S + 3NO_3^- + H_2O \longrightarrow SO_4^{2-} + 3NO_2^- + 2H^+ \quad (2.11)$$

$$5S + 6NO_3^- + 2H_2O \longrightarrow 5SO_4^{2-} + 3N_2 + 4H^+ \quad (2.12)$$

$$S + 2NO_2^- \longrightarrow SO_4^{2-} + N_2 \quad (2.13)$$

Studies focusing on the use of elemental sulfur as the energy source in autotrophic denitrification for the treatment of nitrate-contaminated groundwater, landfill leachate and surface water have been reported recently. Soares (2002) applied an upflow reactor packed with granular elemental sulfur and obtained a nitrate removal rate of 0.6 mM h^{-1} (0.20 kg NO_3^--N $m^{-3}d^{-1}$) at a hydraulic retention time of 1 h with a nitrate loading of 0.7 mM h^{-1} (0.24 kg NO_3^--N m^{-3} d^{-1}). Using a fluidized bed bioreactor with sulfur and limestone granules, Kim *et al.* (2004) reported nitrate removal efficiencies greater than 91.7% up to a loading rate of 7.5 mM h^{-1} (2.53 kg NO_3^--N m^{-3} day^{-1}) at a retention time of 0.2 h. A bioreactor packed with elemental sulfur and limestone granules was established by Sierra-Alvarez *et al*. (2007) to study the autotrophic denitrification. Complete removal of nitrate was obtained at a nitrate loading rate up to 0.9 mM h^{-1} (21.6 mM d^{-1}).

Moon *et al*. (2004) proposed in situ treatment of nitrate-contaminated bank filtrate through sulfur-based autotrophic denitrification and obtained a nitrate removal efficiency higher than 90% at a nitrate loading of 0.36 mM h^{-1}. Similarly, Read-Daily *et al*. (2011) proposed that addition of elemental sulfur to the sediment layer can stimulate sulfur-based autotrophic denitrification to treat nitrate-contaminated aquatic environment such as agricultural ditches and streams.

Denitrification rates in the mesocosm experiments were reported up to 100 times higher than those for agricultural streams (Read-Daily *et al*., 2011).

Considerable amount of protons produced during sulfur-based autotrophic denitrification require alkaline addition in this process. Furthermore, high concentrations of sulfate in the treated effluent may raise environmental concerns and may require further treatment. Limestone as a low cost alkaline source is used in a number of studies (Kim *et al.*, 2004, Darbi and Viraraghavan, 2003, Zhang and Lampe, 1999, Moon *et al.*, 2006, Koenig and Liu, 2002, Moon *et al.*, 2004, Sierra-Alvarez *et al.*, 2007, Zhou *et al.*, 2011). However, the increase of effluent hardness limits the application of sulfur-limestone autotrophic denitrification. Wan *et al.* (2009) employed a bioelectrochemical process to consume the protons generated during sulfur-based autotrophic denitrification and obtained nitrate removal efficiencies greater than 95% at nitrate loading rates up to 0.7 mM h^{-1} (0.24 kg N $m^{-3}d^{-1}$).

Another option to circumvent the problems of acid generation and sulfate accumulation is to combine the heterotrophic process with sulfur-based autotrophic denitrification by adding small amount of organics. The effects of combined heterotrophic and autotrophic denitrification process were studied by several researchers (Oh *et al*., 2001, Kim *et al*., 2002, Liu *et al*., 2009, Aminzadeh *et al*., 2010, Sahinkaya *et al*., 2011). Addition of biodegradable organics at a level of less than stoichiometric requirement for heterotrophic denitrification not only helped to lower the consumption of alkaline but also increased the nitrate removal efficiency. As shown in Equation 2.3 and 2.4, considerable amount of hydroxide ions are produced in a heterotrophic denitrification process which can be used to neutralize the protons produced in the process of sulfur-based autotrophic denitrification. Furthermore, partial reduction of nitrate through the

heterotrophic pathway decreases the production of sulfate as well. Therefore a combined heterotrophic and sulfur-based autotrophic denitrification system may not need an external alkaline source to maintain the pH and also decreases the extent of produced sulfate.

2.2 Biological Removal of Hydrogen Sulfide

Physicochemical processes like Amine absorption, Claus, Lo-Cat and Holmes-Stretford have been established to treat H_2S-containing gaseous streams. However, the excessive costs associated with the energy consumption (high pressure and temperature), catalysts and chemicals make these processes only feasible to treat large volumes of contaminated streams. Biological desulphurization processes by contrast can be operated at ambient temperatures and pressures, and is capable of treating small volumes of contaminated streams which makes it worthy of more attention and further studies (Tang *et al*., 2009; An *et al*., 2010; Tang *et al*., 2010).

Biological desulfurization is an autotrophic process carried out by different groups of bacteria (phototrophic or chemolithotrophic) in which dissolved sulfide or gaseous hydrogen sulfide is oxidize to elemental sulfur or sulfate. Elemental sulfur is the preferred end product due to the facts that the insolubility of sulfur in water makes its separation from the effluent easier and that sulfur is also a necessary raw material in the manufacturing of fertilizers. When sulfate is the end product, it has to be removed to avoid the production of sulfide through the activities of sulfate reducing bacteria after the discharge of the effluent. Carbon dioxide, oxygen and nitrate or nitrite can serve as the terminal electron acceptor in the process of biological removal of sulfide.

2.2.1 Phototrophic desulfurization

Phototrophic oxidation of sulfide is an anaerobic process carried out by green sulfur bacteria and purple sulfur bacteria (Tang *et al*., 2009; Busca and Pistarino, 2003). These bacteria use light energy to convert sulfide to elemental sulfur or sulfate, while using CO_2 as the carbon source and electron accepter. The photosynthetic reaction involved in the oxidation of sulfide is referred to as van Niel's reaction and shown below (Madigan *et al*., 2003; Tang *et al*., 2009).

$$2H_2S + CO_2 \xrightarrow{\text{light}} 2S + H_2O + CH_2O \qquad (2.14)$$

Most purple sulfur bacteria store the produced elemental sulfur inside the cells, while green sulfur bacteria tend to store sulfur extracellularly which makes the separation of the produced elemental sulfur much easier (Syed *et al.*, 2006).

Henshaw *et al*. (1998) studied the conversion of hydrogen sulfide to elemental sulfur using *Chlorobium limicola*, a green sulfur bacterium. In a continuously stirred tank reactor, sulfide was converted to elemental sulfur completely at a sulfide loading rate of 105.6 g S m^{-3} day^{-1} (4.4 mg L^{-1} h^{-1}), while at a sulfide loading rate of 50.4 g S m^{-3} day^{-1} (2.1 mg L^{-1} h^{-1}), nearly all sulfide was converted to sulfate. In a fixed-film continuous-flow photobioreactor, sulfide was completely removed at a loading rate of 2.7 - 6.9 kg S m^{-3} day^{-1} (111-286 mg S L^{-1} h^{-1}), and 92 - 95% of sulfide was converted to elemental sulfur (Henshaw and Zhu, 2001). Syed and Henshaw (2003) further increased the maximum sustainable sulfide loading to 34.8 kg S m^{-3} day^{-1} (1451 mg L^{-1} h^{-1}) by applying smaller diameter tubes in the fixed-film tubular bioreactor at a retention time of 6.74 min.

The advantage of phototrophic desulfurization is the relatively high conversion of sulfide to elemental sulfur which is non-toxic, non-soluble solid relatively easy to be removed from the treated water. However, the light energy required for phototrophs increases the operation cost and the utilization efficiency of light energy is the main constraint limiting large scale applications of phototrophic desulfurization.

2.2.2 Chemolithotrophic desulfurization

Some chemolithotrophic bacteria are able to use oxygen or nitrate, nitrite as the terminal electron acceptor to oxidize sulfide to elemental sulfur or sulfate. The energy obtained from the redox reaction is used for biomass growth and maintenance. Inorganic material such as bicarbonate serves as the carbon source in the process of chemolithotrophic desulfurization.

2.2.2.1 Aerobic chemolithotrophic desulfurization

Under aerobic conditions, oxygen is used as the electron acceptor in the biological desulfurization process. This process can be described by the following reactions (Kim *et al*., 2008; Lohwacharin and Annachhatre, 2010):

$$2H_2S + O_2 \longrightarrow 2S + 2H_2O \quad (2.15)$$

$$2S + 3O_2 + 2H_2O \longrightarrow 2SO_4^{2-} + 4H^+ \quad (2.16)$$

$$H_2S + 2O_2 \longrightarrow SO_4^{2-} + 2H^+ \quad (2.17)$$

Kim *et al.* (2008) obtained almost complete removal of sulfide at a loading rate up to 144 g S m^{-3} day^{-1} (6 g m^{-3} h^{-1}) in an immobilized cell biofilter. In an airlift reactor with limited oxygen supply (0.2-1.0 mg L^{-1}), a sulfide removal rate of 93% was achieved at a sulfide loading rate of 4.0 kg S

m^{-3} day^{-1} and 90% of removed sulfide was converted to elemental sulfur (Lohwacharin and Annachhatre, 2010). Mojarrad Moghanloo *et al*. (2010) studied the sulfide oxidation in a biofilm airlift suspension reactor and complete removal of sulfide was observed at a loading rate of 3.7 kg S m^{-3} day^{-1} (4.8 mol S m^{-3} h^{-1}). The major end product was sulfate because of the high oxygen concentration in the reactor. Vannini *et al*. (2008) reported up to 79% conversion of sulfide to elemental sulfur in a membrane bioreactor. Datta *et al*., (2007) reported a removal rate of 960 g S m^{-3} day^{-1} (40 g m^{-3} h^{-1}) of H_2S at a high temperature (70 ºC) in a biotrickling filtration with addition of glucose and monosodium glutamate.

Activated carbon has been used as the support matrix for the growth of sulfide-oxidizing bacteria in several studies (Ng *et al*., 2004, Duan *et al*., 2005, Ma *et al*., 2006,). The mechanisms of H_2S removal by the bio-activated carbon were possibly composed of physical adsorption, chemisorptions, and biodegradation. Physical adsorption by activated carbon provides a high initial removal efficiency of H_2S and later slowly released H_2S is oxidized by sulfide-oxidizing bacteria. This characteristic provides the bioreactor with a good capacity to cope with shock loading conditions and also prolongs the life span of the activated carbon. A maximum elimination capacity of 627.5 g S m^{-3} day^{-1} (666.7 mg H_2S L^{-1} d^{-1}) was reported by Ma *et al*. (2006). Similarly, a higher maximum elimination capacity of 1.4 kg S m^{-3} day^{-1} (56.7 g S m^{-3} h^{-1}) was reported by Ramirez *et al*. (2009) in a biotrickling filter packed with polyurethane foams. Ng *et al*. (2004) reported a removal capacity of 0.084 g H_2S (g immobilized activated carbon)$^{-1}$. A maximum removal capacity of 2.6 kg S m^{-3} day^{-1} (113 g H_2S m^{-3} h^{-1}) with a removal efficiency of 96% was obtained at a H_2S volumetric loading of 900 m^3 m^{-3} h^{-1} by Duan *et al*. (2005).

The disadvantages of aerobic chemolithotrophic denitrification process include aeration cost and the inhibitory effect of end products like sulfuric acid. Furthermore, it is hard to control the end product of sulfide oxidation in the form of elemental sulfur. Finally, treating H_2S-containing hydrocarbon gases such as biogas and natural gas in an oxygen-rich environment raise a serious safety concern.

2.2.2.2 Anaerobic chemolithotrophic desulfurization

Under anaerobic conditions, some chemolithotrophic bacteria, like *Thiomicrospira denitrificans* CVO, can carry out the biological desulfurization process described by Equation 2.6-2.10 where nitrate or nitrite serves as the electron acceptor during the biooxidation of sulfide to elemental sulfur or sulfate. The simultaneous removal of sulfide and nitrate or nitrite makes the anaerobic chemolithotrophic desulfurization process a suitable candidate for the combination of H_2S removal from biogas and denitrification treatment of nitrate-contaminated water like swine wastewater.

In an up-flow anoxic sulfide oxidizing reactor, the effect of sulfide to nitrate loading ratios was studied by Jing *et al*. (2008). Removal rates of 4.86 kg S m^{-3} day^{-1} and 2.9 × 10^{-3} mM h^{-1} (0.99 kg NO_3^--N m^{-3} d^{-1}) were obtained at a sulfide to nitrate molar ratio of 5 to 2. An *et al*. (2010) reported maximum sulfide and nitrate removal rates of 1.5 kg S m^{-3} day^{-1} (2.0 mM h^{-1}) and 0.92 mM h^{-1} at loading rates of 2.1 and 0.93 mM h^{-1}, respectively, in a continuous stirred tank bioreactor; while Tang *et al*. (2010) obtained maximum sulfide and nitrate removal rates of 23.0 kg S m^{-3} day^{-1} (30.0 mM h^{-1}) and 24.4 mM h^{-1}, respectively, in biofilm reactors.

Some bacteria strains such as *Thiomicrospira denitrificans* CVO can carry out denitrification processes under both autotrophic and heterotrophic conditions. A mixed culture of heterotrophic and autotrophic denitrifyers can also function through both denitrification pathways. Therefore, the removal of sulfide, nitrate and biodegradable COD in one system can be accomplished under proper conditions (Reyes-Avila *et al*., 2004, Chen *et al*., 2009, Tang *et al*., 2010). Chen *et al*. (2009) employed an expended granular sludge bed reactor to study the anaerobic desulfurization process and obtained a desulfurization rate of 4.8 kg S m^{-3} day^{-1} of sulfide with a removal efficiency of 97%, a nitrate removal rate of 7.7 mM h^{-1} (2.6 kg NO_3^--N m^{-3} d^{-1}) with a removal efficiency of 92% and an acetate removal rate of 2.7 kg C m^{-3} d^{-1} with a removal efficiency of 95%. Reyes-Avila *et al*. (2004) reported removal efficiencies of both carbon and nitrogen higher than 90% at loading rates of 0.29 kg C m^{-3} d^{-1} and 0.2 kg N m^{-3} d^{-1} (the ratio of acetate to nitrate: 1.45). After adding sulfide at loading rates up to 0.294 kg S^{2-} m^{-3} d^{-1}, the removal efficiency of sulfide increased to 99% with partial oxidation to elemental sulfur. During the removal of sulfide, the removal efficiency of nitrate remained higher than 90%. However, the removal efficiency of acetate decreased to around 65% probably due to the competition between two available electron donors (sulfide and acetate).

The kinetics of autotrophic denitrification using elemental sulfur and gaseous hydrogen sulfide have not been studied extensively. The lack of this information is especially clear in the case of novel microbial species such as NR-SOB *Thiomicrospira denitrificans* CVO (the main microbial component of Coleville enrichment) which has the ability of functioning both autotrophically and heterotrophically (Gevertz *et al*., 2000) and is able to tolerate sulfide at concentrations as

high as 16 mM (An *et al*., 2010). Therefore, more research is needed to investigate the capability of Coleville enrichment in sulfur-based autotrophic denitrification and desulfurization under denitrifying conditions. Autotrophic denitrification with sulfur is the appropriate approach for the treatment of nitrate-contaminated groundwater and drinking water. Biodesulphurization under denitrifying conditions eliminates the cost and risk associated with aerobic desulfurization (i.e. aeration cost), as well as the light energy input required during photoautotrophic desulfurization process.

Chapter 3 RESEARCH OBJECTIVES

In specific situations such as the treatment of organic-deficient wastewater and nitrate-contaminated groundwater, autotrophic denitrification with elemental sulfur offers several advantages over conventional heterotrophic denitrification process. The use of sulfur as the electron donor eliminates the requirement of adding external organics thus lowers the operation cost. Furthermore, there is no need to control the dosage of the electron donors. Finally, the extent of sludge produced in this process would be less than that of heterotrophic process, thus the cost associated with the treatment of sludge is reduced.

In wastewater treatment processes, ammonia/ammonium ion is first oxidized to nitrite and then to nitrate in the nitrification process. On the other hand, in denitrification processes nitrate is first reduced to nitrite and then further to nitrogen gas. Nitrite, as an intermediate product, is produced in both nitrification and denitrification processes. This offers a shortcut approach for complete removal of nitrogenous compounds in which ammonium is oxidized to nitrite and the produced nitrite is then reduced to nitrogen gas. The elimination of nitrite oxidation to nitrate and nitrate reduction to nitrite steps offers considerable cost saving. Therefore in this study both processes of denitrification and denitritation in the presence of sulfur were investigated.

The process of autotrophic denitrification with hydrogen sulfide makes it possible to remove nitrate, nitrite, and gaseous H_2S simultaneously. It can be used in odor control of wastewater treatment plant, treatment of landfill leachate, and desulfurization of natural gas or biogas.

The main goals of this research were (1) to better understand the principles of autotrophic denitrification and denitritation processes with elemental sulfur as the electron donor; (2) to evaluate the capability of Coleville enrichment culture in sulfur-driven autotrophic denitrification and denitritation processes, especially in a continuously operated biofilm reactor and to study the effects of nitrate and nitrite loadings on denitrification and denitritation rates; and (3) to study the biodesulfurization process under denitrifying conditions.

The overview of the experimental approach is summarized in Figure 3.1. In this study a novel microbial culture, Coleville enrichment originated from an oil reservoir, was used as the candidate microbial culture. *Thiomicrospira denitrificans* CVO, the dominant microbial species in Coleville enrichment, has the ability to function both autotrophically and heterotrophically (Gevertz *et al*., 2000) and is able to tolerate sulfide at concentrations as high as 16 mM (An *et al*., 2010).

The processes of denitrification and denitritation were investigated using elemental sulfur and gaseous H_2S as the electron donors separately. Sulfur-based autotrophic denitrification and denitritation processes were studied in batch systems as well as a continuously operated bioreactor using nitrate and nitrite as the electron acceptors, respectively. With sulfur, the effects of nitrate and nitrite concentrations on the extent of denitrification, as well as the composition of end products were investigated in the batch system. The use of biofilm reactor as a means to improve the removal efficiency and the extent of denitrification and denitritation were evaluated. The effects of nitrate and nitrite loading rates and residence times on the performance of continuous biofilm reactor were investigated. Simultaneous removal of gaseous H_2S and nitrate or nitrite was investigated in packed bed bioreactors continuously fed with H_2S, with the focus

being on the effects of H_2S loading rate on the extent of denitrification and desulphurization processes.

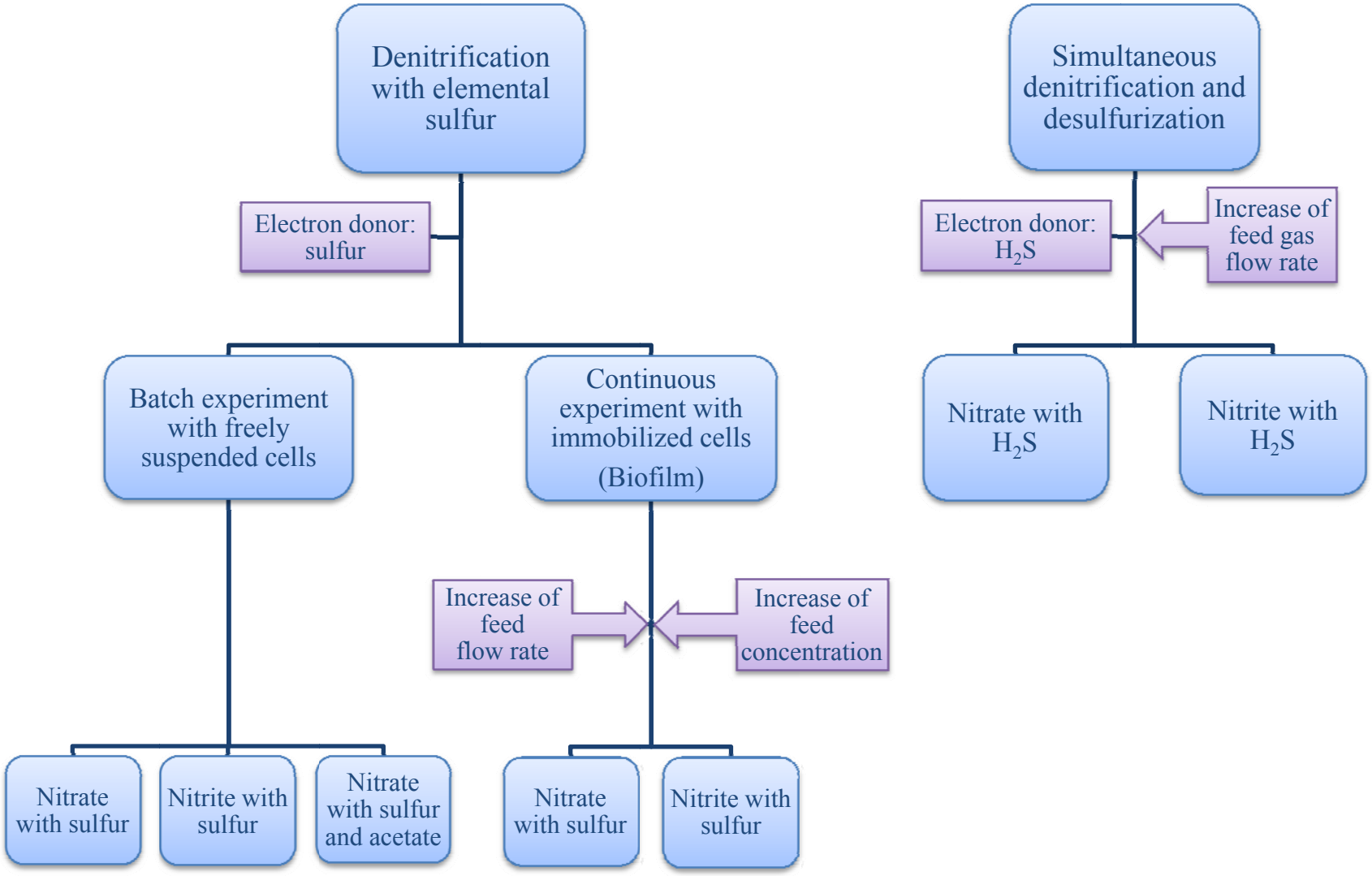

Figure 3.1 The overall experiment layout for the present study

Chapter 4 MATERIALS AND METHODS

4.1 Microbial Culture and Medium

4.1.1 Microbial culture

A mixed culture of nitrate reducing, sulfide oxidizing bacteria (NR-SOB) enriched from the produced water of the Coleville oil field in Saskatchewan, Canada was used in this study and was referred to as Coleville enrichment in the following sections. The dominant species in Coleville enrichment culture, *Thiomicrospira denitrificans* sp. CVO, has the ability to function both autotrophically and heterotrophically (Gevertz *et al.*, 2000). It can use organic compounds as well as reduced sulfur compounds (such as sulfide or elemental sulfur) as the electron donors to accomplish the reductions of nitrate and nitrite. It has the potential to remove nitrate, nitrite, sulfide and biodegradable organic compounds in one system. Furthermore, Coleville enrichment can tolerate sulfide at concentrations as high as 16 mM (An *et al*., 2010), which indicates its potential of dealing with higher sulfide concentrations and loading rates.

4.1.2 CSB medium and maintenance of the stock culture

Coleville Synthetic Brine (CSB) containing around 5 mM sulfide and 10 mM nitrate was used for the maintenance of Coleville enrichment. The composition of CSB medium is given in Table 4.1 (Gevertz *et al*., 2000; An *et al*., 2011). All medium components were dissolved in reverse osmosis water and the pH was adjusted to 6.9-7.1 using 2M HCl. Each serum bottle (125 mL) containing 100 mL of CSB medium was purged with nitrogen gas for 5 minutes to create

anaerobic conditions, and then sealed with a rubber septum and an aluminum cap and autoclaved at 121°C for 30 minutes.

Table 4.1. Composition of CSB medium

Component Name	**Amount (g L^{-1})**	**Concentration (mM)**
Sodium Chloride (NaCl)	7.0	119.78
Magnesium Sulfate ($MgSO_4 \cdot 7H_2O$)	0.68	2.76
Calcium Chloride ($CaCl_2 \cdot 2H_2O$)	0.24	1.63
Ammonium Chloride (NH_4Cl)	0.02	0.37
Potassium Phosphate (KH_2PO_4)	0.027	0.20
Sodium Acetate ($NaC_2H_3O_2 \cdot 3H_2O$)	0.68	8.29
Potassium Nitrate (KNO_3)	1.0	9.89
Sodium Bicarbonate ($NaHCO_3$)	1.9	22.62
Trace Element Solution*	0.5 ml L^{-1}	-
Resazurin	1.0 ml L^{-1}	-
Tris Base ($C_4H_{11}NO_3$)	6.057	50

*Trace element solution: 0.5 mL concentrated H_2SO_4, 2.28 g $MnSO_4 \cdot H_2O$, 0.5 g $ZnSO_4 \cdot 7H_2O$, 0.5 g H_3BO_3, 0.025 g $CuSO_4 \cdot 5H_2O$, 0.025 g $Na_2MoO_4 \cdot 2H_2O$, 0.045 g $CoCl_2 \cdot 6H_2O$ and 0.58 g $FeCl_3$ per liter of reverse osmosis water.

After serum bottles cooled down to room temperature, 0.5 mL filter-sterilized sodium sulfide solution (1 M, filtered by a 0.2 μm Supor® membrane syringe filter) was added to each bottle to achieve a sulfide concentration close to 5 mM. This was followed by an addition of 0.5 mL filter-sterilized HCl (2M) to readjust the pH to about 7.0. A stock culture of Coleville enrichment

(10 mL) was then added to each bottle as an inoculum. Microbial cultures were maintained at room temperatures (23-25 °C) and subcultured on a biweekly basis.

4.1.3 Modification of medium and acclimation of microbial culture

In order to study the processes of autotrophic denitrification (nitrate removal) and denitritation (nitrite removal) in the presence of elemental sulfur, CSB medium was modified to enable the Coleville enrichment to acclimate to the utilization of sulfur as the sole electron donor. Acetate and tris base were excluded from the medium because they both can serve as organic electron donors for the heterotrophic denitrification process. According to Equation 2.16 during the autotrophic denitrification with elemental sulfur, 0.7 mM hydrogen ion will be produced with the removal of every 1 mM nitrate. Therefore, the amount of sodium bicarbonate was increased from 22.6 to 50 mM to function as a buffering agent, as well as a carbon source. In the case of nitrite reduction with sulfur (Equation 2.17), the amount of sodium bicarbonate was kept as original level in the CSB medium.

To each serum bottle containing 100 mL of modified CSB medium, 25 mM sulfur powder (sublimed sulfur, Fisher Scientific) was added. The bottles were then purged with nitrogen gas and then sealed with rubber septa and aluminum caps. A stock culture of Coleville enrichment (10 mL) was added as an initial inoculum and subsequently subcultured 3 times (once a week) using modified CSB medium in order to flush out any residual acetate and tris base transferred from the original inoculum and to allow the acclimation of Coleville enrichment to the utilization of sulfur as the only electron donor. The acclimated culture, referred to as a-NR-SOB for the remaining of the thesis, was used for subsequent subculturing and eventual use as the inoculums

to study autotrophic denitrification and denitritation with elemental sulfur in the batch system, as well as in the continuous up-flow biofilm reactor.

4.2 Batch Experiments

The effects of initial concentrations of nitrate or nitrite on the extent of denitrification or denitritation in the presence of elemental sulfur were investigated in batch systems. The composition of end products, maximum nitrate and nitrite reduction rates were calculated and compared.

4.2.1 Nitrate removal in the presence of sulfur

A series of 125 mL serum bottles containing 100 mL modified CSB medium as described in section 4.1.3 with around 2.5, 5, 10, and 20 mM of nitrate were used to study the autotrophic denitrification process. Under all tested conditions the concentration of sulfur was kept constant at 25 mM which was in excess of the theoretical value required for complete reduction of 20 mM nitrate to ensure sulfur was not limiting. Bicarbonate concentration was kept at 50 mM to maintain pH. A 10-day-old a-NRSOB culture was used as the inoculums (10% v/v). The serum bottles were placed on a rotary shaker (JEIO TECH, Inc.) at a speed of 150 rpm to keep the sulfur particles in suspension. All the experiments were carried out in room temperatures (23-25ºC). During the course of the experiments, concentrations of nitrate, nitrite, sulfate, and thiosulfate were monitored by regular sampling.

4.2.2 Nitrite removal in the presence of sulfur

Similar batch experiments were carried out to study the autotrophic denitritation process with sulfur. The initial concentrations of nitrite were set as 5, 10, 20, 30, and 50 mM, while sulfur and bicarbonate concentrations were kept constant at 25 mM. A 10-day-old a-NRSOB culture was used as the inoculums (10% v/v). The serum bottles were kept on a shaker at a speed of 150 rpm. The concentrations of nitrite, sulfate, and thiosulfate were monitored during the course of the experiments. To assess the reproducibility of results some experiments were carried out in duplicates. The average value of the data and associated standard deviation were used in presenting the results.

4.2.3 Nitrate removal in the presence of sulfur and acetate

In order to better understand the interaction of heterotrophic and autotrophic denitrification processes, a third set of batch experiments were conducted in which both acetate and sulfur were supplied in the CSB medium. In the presence of acetate (as the electron donor), according to Equation 2.3 complete reduction of 1 mM nitrate will produce 1.6 mM hydroxide ion therefore tris base was also supplied in the medium to ensure sufficient buffering capacity. The initial concentration of acetate was kept constant at around 32 mM, while the concentrations of nitrate and elemental sulfur were set as 5, 10, 20, 30, and 50 mM of each (the ratio of nitrate to sulfur was 1:1). It should be pointed out that sulfur and acetate were both provided in excess of theoretical values required for complete reduction of nitrate to nitrogen gas. A 3-day-old Coleville enrichment was used as the inoculums (10% v/v) in this set of experiments. The concentrations of acetate, nitrate, nitrite, sulfate, and thiosulfate were monitored regularly. All

the experiments were carried out in duplicates and the average values were used in presenting the results. Standard deviations were also calculated and used as error bars.

4.3 Denitrification and Denitritation with Sulfur in a Up-flow Biofilm Reactor

4.3.1 Experimental set-up

An up-flow biofilm reactor was used to study the autotrophic denitrification and denitritation processes in the presence of sulfur. The experimental set-up is shown in Figure 4.1. The reactor was made of a glass column (D: 4 cm and H: 33.5 cm) with three sampling ports at 11.5 cm intervals. The bioreactor was packed with sulfur granules (Aqua-medic of North America, Loveland, Colorado, US) with diameters in the range of 3.0-4.5 mm (Figure 4.2). A small piece of a dual density microfiltration pad (Super MicroFiltration, Mars Fishcare) was placed at the bottom of the bioreactor to support the sulfur granules. Sulfur granules served as matrix for the establishment of biofilm (Figure 4.3) and also as electron donors for the denitrification processes. The three sampling ports were sealed with rubber septa. Modified CSB medium (with no acetate or tris-base) as described in section 4.1.3 containing either nitrate or nitrite at designated concentrations was prepared using bleach-sterilized utensils to prevent contamination. It was purged with nitrogen gas for 30 min and then transferred into bleach-sterilized collapsible medium bags by applying pressurized nitrogen gas (to ensure anaerobic condition). The modified CSB medium was then introduced into the bottom of the biofilm reactor using a multi-speed peristaltic pump (Amersham Biosciences).

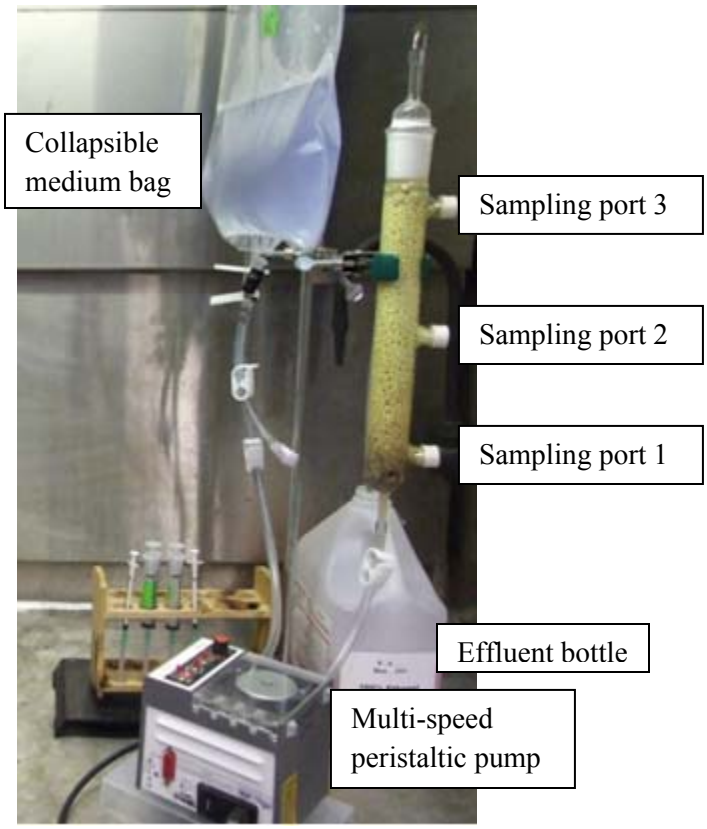

Figure 4.1 Experimental set-up in autotrophic denitrification with sulfur

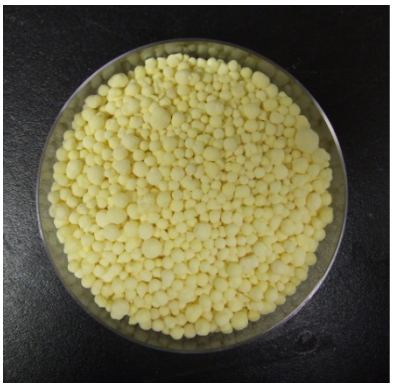

Figure 4.2 Sulfur granules

Figure 4.3 Established biofilm

The working volumes of the biofilm reactor were determined at the beginning and the end of all experiments as 95.5 and 92.5 mL, respectively. The average value (94 mL) was used in the calculation of residence time, loading and removal rates.

4.3.2 Experimental procedures

4.3.2.1 Nitrate removal (denitrification) with sulfur

Before starting the experimental runs approximately two pore volumes of modified CSB medium containing around 10 mM nitrate was pumped through the bioreactor to ensure that reactor voidages were filled. The peristaltic pump was then stopped and the bioreactor was inoculated with a 10-day-old a-NRSOB culture by injecting 20 mL inoculum into each port. Nitrate and nitrite concentrations were monitored daily. The bioreactor was operated batch-wise until 100% removal of nitrate (with no residual nitrite) was obtained in both top and bottom parts of the bioreactor. The reactor was then switched to continuous mode by pumping modified CSB medium containing around 10 mM nitrate into the reactor at a low flow rate (about 1.0 mL h^{-1}) to allow the establishment of the biofilm. The flow rate of the feed was increased stepwise to 3.0, 4.9, 9.7, 21.3, 34.8, 49.9, 76.1, 98.2, 130.2, 175.7 and 220.6 mL h^{-1} to assess the impacts of nitrate loading rate and hydraulic retention time on the performance of the system. At each flow rate sufficient time was given for the establishment of steady state conditions. Steady state was assumed when complete reduction of nitrate was observed or when residual concentrations of nitrate and nitrite (intermediate product) in the effluent changed less than 10% over a period of at least two-three days. Following the completion of this part of experiment (the variation of loading rate through the increase of flow rate), the feed flow rate was decreased to around 30 mL h^{-1} and the concentration of nitrate in the feed was increased stepwise to 21.1, 31.0, 42.4 and 53.4 mM (the variation of loading rate through the increase of feed concentration). The bioreactor was run with each feed concentration for sufficient time (at least three days) to allow the system to reach the steady state.

In both parts of the experiments, samples (0.3 mL) were taken from top and bottom ports on a daily basis using 1.0 mL syringe. Nitrate, nitrite, and sulfate concentrations were determined using an ion chromatograph. The average values of nitrate, nitrite and sulfate concentrations in the samples taken after the establishment of steady state were calculated and used in presenting the results. Standard deviation was also calculated and used as error bars in association with the average data. The effluent pH was monitored daily during the entire course of the experiments to ensure the pH within the range of Coleville enrichment tolerance.

4.3.2.2 Nitrite removal (denitritation) with sulfur

Following the completion of the experiments with nitrate as the substrate, denitritation experiments (removal of nitrite) with elemental sulfur was carried out in the same biofilm reactor. The bioreactor was first drained and then filled with modified CSB medium containing approximately 10 mM nitrite and operated batch-wise until the concentrations of nitrite in the top and bottom ports reached zero. The reactor was then switched to continuous mode by pumping modified CSB medium containing around 10 mM nitrite into the reactor. The flow rate of the feed was increased stepwise from 3.3 to 9.4, 20.7, 44.8, 85.0, 121.3, 162.0, and 197.9 mL h^{-1} (the variation of loading rate through the increase of flow rate). After the completion of this part, the flow rate was decreased and maintained constant at about 30 mL h^{-1} and the concentration of nitrite in the feed was increased stepwise from 10.3 to 21.4, 31.4, 42.0 and 51.4 mM. With each tested condition sufficient time was given to allow the establishment of steady state conditions. In both parts of the operational procedures, sampling and analysis were similar to those described for the experiments with nitrate.

4.3.3 Determination of biomass holdup in the up-flow biofilm reactor

Following the completion of all the experimental runs, the sulfur packing was taken out from the biofilm reactor carefully. The packing was divided into three parts, representing the top, middle and bottom parts of the bioreactor, respectively. From each part two samples of sulfur granules (around 16 g each) were taken. One sample was transferred into a petri dish and placed immediately in an oven until it dried completely (the difference between two consecutive weights was less than 1%). The other sample was washed with bleach and then rinsed with reverse osmosis water for several times to remove all the biomass. This sample was also dried completely in the oven. The weight differences between these two samples from the same region of the reactor were calculated as the biomass holdup in terms of g cell dry weight (g sulfur)$^{-1}$.

4.4 Denitrification and Denitritation with H_2S in a Packed Bed Reactor

4.4.1 Experimental set-up

The experimental system used to study simultaneous desulfurization and denitrification consisted of a pressurized tank containing 508 ppm H_2S, 5% CO_2, balanced with nitrogen as the feed gas (Praxair Technology Inc. Saskatoon Distributor. Saskatoon, Canada), a glass bioreactor (250 mL) with a central porous diffuser for introducing of feed gas and two outlet ports for both gaseous and liquid effluents, a Mass Flow Controller (0 -500 mL min^{-1}; Aalborg instruments & controls, Inc. Orangeburg, New York, USA) to adjust the flow rate of the H_2S gas, and a peristaltic pump for transferring of the liquid medium into the bioreactor. A schematic diagram of the

experimental set-up is shown in Figure 4.4. The bioreactor was packed with narrow strips of a spongy material with approximate dimensions of 0.5 cm× 0.5 cm× 10 cm (scotch-brite heavy duty scour pad) as the matrix to support the establishment of the biofilm. The bioreactor was filled with modified CSB medium containing designated concentrations of nitrate or nitrite (with no acetate but with tris base to maintain the pH in the range of 6.5-8.0). H_2S feed gas was introduced into the bottom of the bioreactor through the porous diffuser in the form of small bubbles. The concentration of H_2S in the feed was adjusted by the supplier at 508 ppm H_2S, 5% CO_2, balanced with nitrogen. However, the designed set-up has the option of diluting the incoming gas with nitrogen gas. The set-up also provides the option of continuous feeding the bioreactor with CSB medium from a collapsible medium bag using a peristaltic pump. The working volume of the bioreactor was measured as 230 mL.

The effluent gas was transferred into the bottom of a glass bottle filled with 1 M NaOH solution to absorb any remaining trace of H_2S. Phenol Red (pH indicator) was added in the NaOH solution as a means to monitor the saturation of H_2S absorption. Liquid and gas samples were taken from Ports 1 and 2, respectively. To ensure the safety and to eliminate any possible leakage of H_2S, the entire experimental systems were placed in a walk-in fume hood, equipped with H_2S alarms.

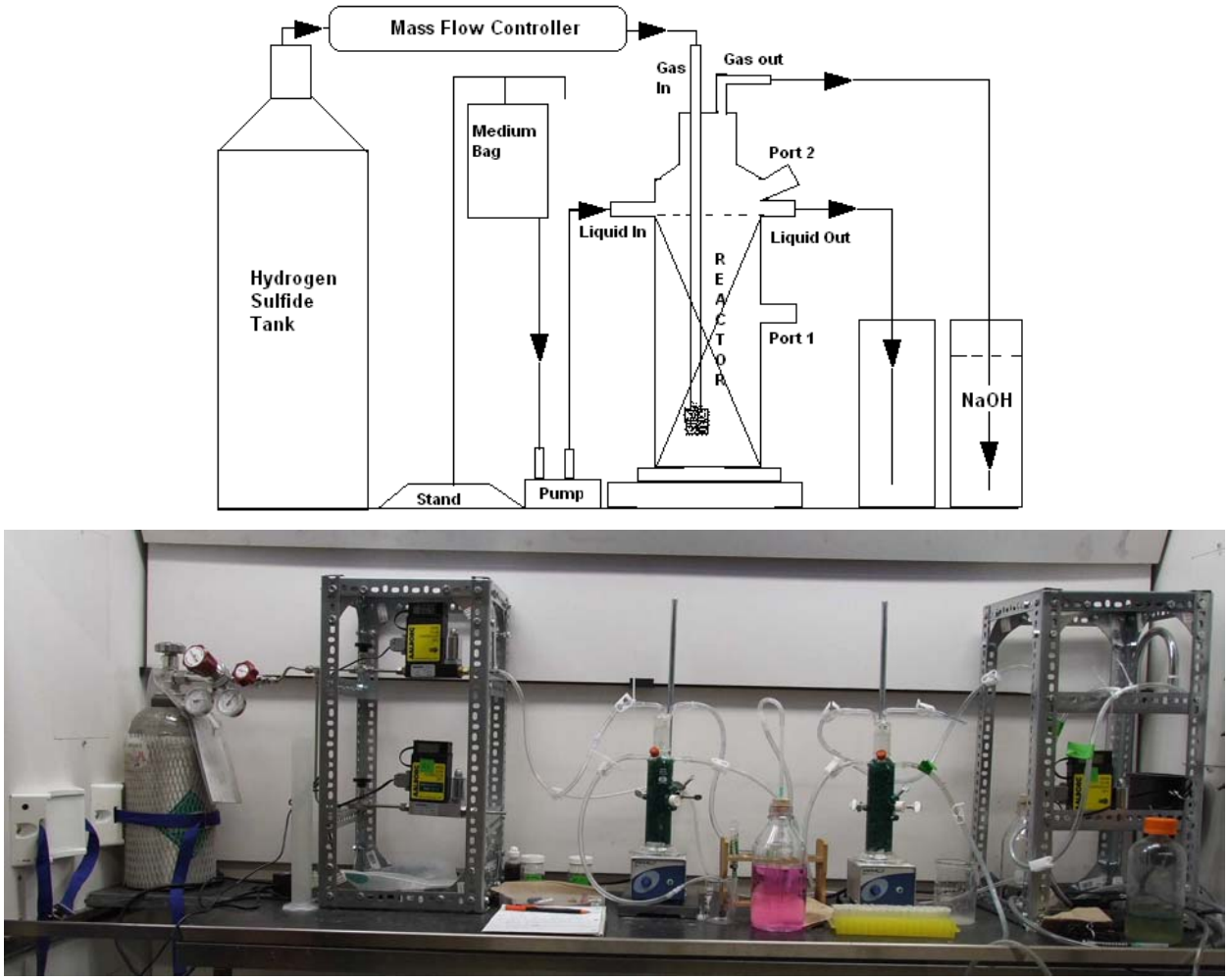

Figure 4.4 The schematic diagram and picture of the experimental set-up for simultaneous desulfurization and denitrification

4.4.2 Experiment procedures

CSB medium used in this series of semi-continuous experiments (continuous flow of gas; no flow of liquid medium) was modified to exclude acetate. All the other components were maintained at the same level as in Table 4.1 in section 4.1.2. Fresh CSB medium containing 10 mM nitrate was purged with nitrogen gas for 10 minutes. To obtain a sulfide concentration of around 10 mM, 2.3 mL sulfide solution (1 M) was added to the medium. The pH was adjusted to

around 7.0 using 2 M HCl and then the medium was transferred into the bioreactor by applying pressurized nitrogen gas. The reactor was inoculated by adding 30 mL of a 3-day-old Coleville enrichment through port1. The concentrations of nitrate, nitrite, sulfate, thiosulfate and sulfide in the liquid phase were monitored regularly. After the exhaustion of sulfide or nitrate, the liquid content of the bioreactor was replaced by fresh CSB medium containing 10 mM nitrate and 10 mM sulfide following the same procedure. This process was repeated to allow the formation of the biofilm in the sponge strips and to achieve a substantial biomass hold-up in the bioreactor.

After eight sequential batch runs, simultaneous desulfurization and denitrification experiments were carried out semi-continuously using the feed gas (508ppm H_2S, 5% CO_2, balance nitrogen) and CSB medium containing around 10 mM nitrate. Each experiment ran for around 8 hours with continuous supply of H_2S gas at designated flow rates. Gas flow rate was regulated by a Mass Flow Controller and the flow rates of 25, 50, 75, and 100 mL min^{-1} were tested. To start each experiment, around 230 mL fresh CSB medium containing 10 mM nitrate was transferred into the bioreactor by applying pressurized nitrogen gas and the liquid contents of the bioreactor were then purged with N_2 for 10 minutes. A sample (0.35 mL) was taken from port 1 to measure the initial concentration of nitrate and the background level of dissolved sulfide. H_2S feed gas was then introduced into the bioreactor from the porous diffuser continuously at designated flow rates. Liquid samples (0.35 mL) were taken from port 1 every hour and the concentrations of sulfide, sulfate, thiosulfate, nitrate, and nitrite were measured. The concentration of H_2S in the treated gas was measured by a gas chromatograph every 10 to 30 minutes (more frequent sampling was carried out for the first 2-3 h). Gas samples (1000 µL) were drawn from port 2 using a gas-tight syringe (Hamilton CO., RENO, Nevada, US). The needle of the syringe was

sealed with a rubber septum and then appropriate amount of gas samples were injected into the GC within 3 minutes.

Following the completion of H_2S removal experiments with nitrate, similar experiments with nitrite were carried out in the same reactor. The operational procedures, sampling and analysis were similar to those described for the experiments with nitrate.

4.5 Analytical Methods

4.5.1 Sulfide concentration measurement

The dissolved sulfide concentration was determined using a spectrophotometric method (Cord-Ruwisch 1985). An acidic copper sulfate solution containing 0.8 g L^{-1} of copper sulfate (5 mM) was prepared. A liquid sample (0.1 mL) was added into 0.9 mL of 5.0 mM acidic copper sulfate solution and mixed by shaking. The absorbance of the mixture was then measured at 480 nm using a spectrophotometer (SHIMADZU UVmini-1240 spectrophotometer). A calibration curve, generated previously using standard sodium sulfide solutions (0-10 mM), was used to determine the concentration of dissolved sulfide in the samples.

4.5.2 Nitrate, nitrite, acetate, sulfate, and thiosulfate concentrations

The concentrations of nitrate, nitrite, acetate, sulfate, and thiosulfate were determined using a Dionex ion chromatograph (ICS-2500) with a conductivity detector (CD25A) equipped with an IonPac CG5A guard column and an IonPac CS5A analytical column. The eluent was 1.0 mM

KOH and the flow rate of the eluent was set at 1.0 mL h^{-1}. The system was calibrated using standard solutions of acetate, nitrite, nitrate, sulfate, and thiosulfate with concentrations of 5, 10, 20, 30, and 50 ppm. To establish the calibration curves, standard solutions with each concentration were injected three times (injection volume = 25.0 uL). The calibration curves were quadratic for all the ions. The relative standard deviations associated with acetate, nitrite, nitrate, sulfate, and thiosulfate measurements were 3.41%, 1.49%, 0.90%, 0.73%, and 0.99% and the correlation coefficients for acetate, nitrite, nitrate, sulfate, and thiosulfate calibration curves were 99.46%, 99.75%, 99.98%, 99.99%, and 99.99%, respectively.

To prepare the samples for IC analysis, liquid samples (0.3 mL) were centrifuged (Microfuge® 18 Centrifuge, BECKMAN COULTER$_{TM}$) at 14000 rpm for 8 minutes. Following centrifugation, 0.1 mL supernatant was transferred into a 1.5 mL microcentrifuge tube filled with 0.9 mL Millipore water (10 times dilution). Samples were further diluted (overall dilution ratio of 40 folds) to ensure the concentrations of ions fell in the concentration ranges of the developed calibration curves. Diluted samples were then analyzed by IC.

4.5.3 H_2S concentration

The concentration of H_2S was determined by a Varian gas chromatograph (CP-3800) equipped with a pulsed flame photometric detector (PFPD). The GC was equipped with a GS-GasPro 30 m× 0.32 mm I.D. capillary column (Agilent Technologies, Canada). The carrier gas was ultra-high purity grade Helium at a flow rate of 3.0 mL min^{-1}. The injector, column oven, and PFPD were maintained at 200 °C, 60 °C, and 220 °C, respectively. The gas chromatograph was calibrated using calibration gases containing 1.24, 19.4, 98, 508, and 1949 ppm H_2S balanced

with nitrogen gas (Praxair Technology Inc. Saskatoon Distributor. Saskatoon, Canada). Two calibration curves (quadratic with the regression coefficient close to 1.0) were generated covering concentration ranges of 1.24-98 and 98-1949 ppm. Each point of the calibration curve represented the average value of at least three measurements of H_2S calibration gas sample. Two different methods with split ratios (gas sample: carrier gas) of 1: 10 and 1: 200 were used for the low and high concentration ranges, respectively. The injection volumes of samples were 700 and 300 μL for the low and high concentration ranges, respectively.

4.5.4 pH measurement

The pH values of liquid samples were determined using a pH meter (PerpHecT Meter, Models 330, Thermo Orion, USA).

Chapter 5 RESULTS AND DISCUSSION

This section presents the results obtained in the batch experiments with freely suspended cells (sulfur-based autotrophic denitrification) and the continuously operated bioreactor with biofilm (sulfur-based autotrophic denitrification and simultaneous denitrification and desulfurization).

5.1 Batch Denitrification and Denitritation

5.1.1 Nitrate removal in the presence of sulfur

The autotrophic denitrification in the presence of elemental sulfur were investigated in serum bottles with 100 mL modified CSB medium containing approximately 2.5, 5, 10 and 20 mM nitrate with 25 mM elemental sulfur and 50 mM sodium bicarbonate. To assess the reproducibility of the results, all the experiments were conducted more than once. However, because the reduction of nitrate took place at a slightly different time scale (especially the lag phase), the presentation of duplicate data in the form of average values was not possible. Figure 5.1 shows the representative results of the concentration profiles of various ions as a function of time during the reduction of nitrate.

As shown in Figure 5.1 with 2.8, 5.0, 9.9, and 20.4 mM nitrate, lag phases of 8.0, 4.0, 5.9, 10.7 days were observed, respectively, following the inoculation. After these periods, nitrate concentration started to decrease coupled with proportional formation of nitrite. Nitrite concentration kept increasing until nitrate was completely reduced which illustrated that nitrite was the intermediate product of nitrate reduction. Following the exhaustion of nitrate, the reduction of nitrite to other nitrogenous compounds, possibly nitrogen gas, started. With 2.8, 5.0

and 9.9 mM nitrate, complete reductions of both nitrate and nitrite were achieved in 2.7, 3.7 and 8.9 days, respectively. With 20.4 mM nitrate, the produced nitrite showed strong inhibitory effect on microbial activities. After nitrate was completely reduced in 4.1 days, the bacterial activities ceased and the produced nitrite (15-16 mM) was not subsequently reduced.

During the reduction of nitrate and nitrite, sulfate concentration increased significantly which indicated that elemental sulfur was utilized as the electron donor in the denitrification process. The experimental sulfate productions obtained at different initial nitrate concentrations were compared with corresponding theoretical values and summarized in Table 5.1. The discrepancies between the theoretical and experimental values were in the range of 1.2-13.3% and were attributed to errors associated with the measurement of nitrate, nitrite and sulfate concentrations. The experimental sulfate productions were calculated using average sulfate concentration after complete reduction of nitrate and nitrite minus average sulfate concentration during the lag phase. The theoretical values of sulfate production were calculated based on the stoichiometry of Equation 5.1 and 5.2. Assuming that nitrate was first reduced to nitrite and subsequently to nitrogen gas, according to Equation 5.1 and 5.2, every 3 mM nitrate reduced to nitrite should produce 1 mM sulfate and every 2 mM nitrite reduced to nitrogen gas should produce 1 mM sulfate. The concentrations of nitrate and nitrite[1] used in the calculation of theoretical sulfate productions were the average values of concentrations during the lag phase and after the complete reduction. Thiosulfate concentrations were detected to be below 0.1 mM throughout all the experiments, a value close to that observed in the modified CSB medium.

[1] Small amount of nitrite (0.3-0.5 mM) brought in with the inoculums was included in the calculation of theoretical sulfate productions (data in Table 5.1).

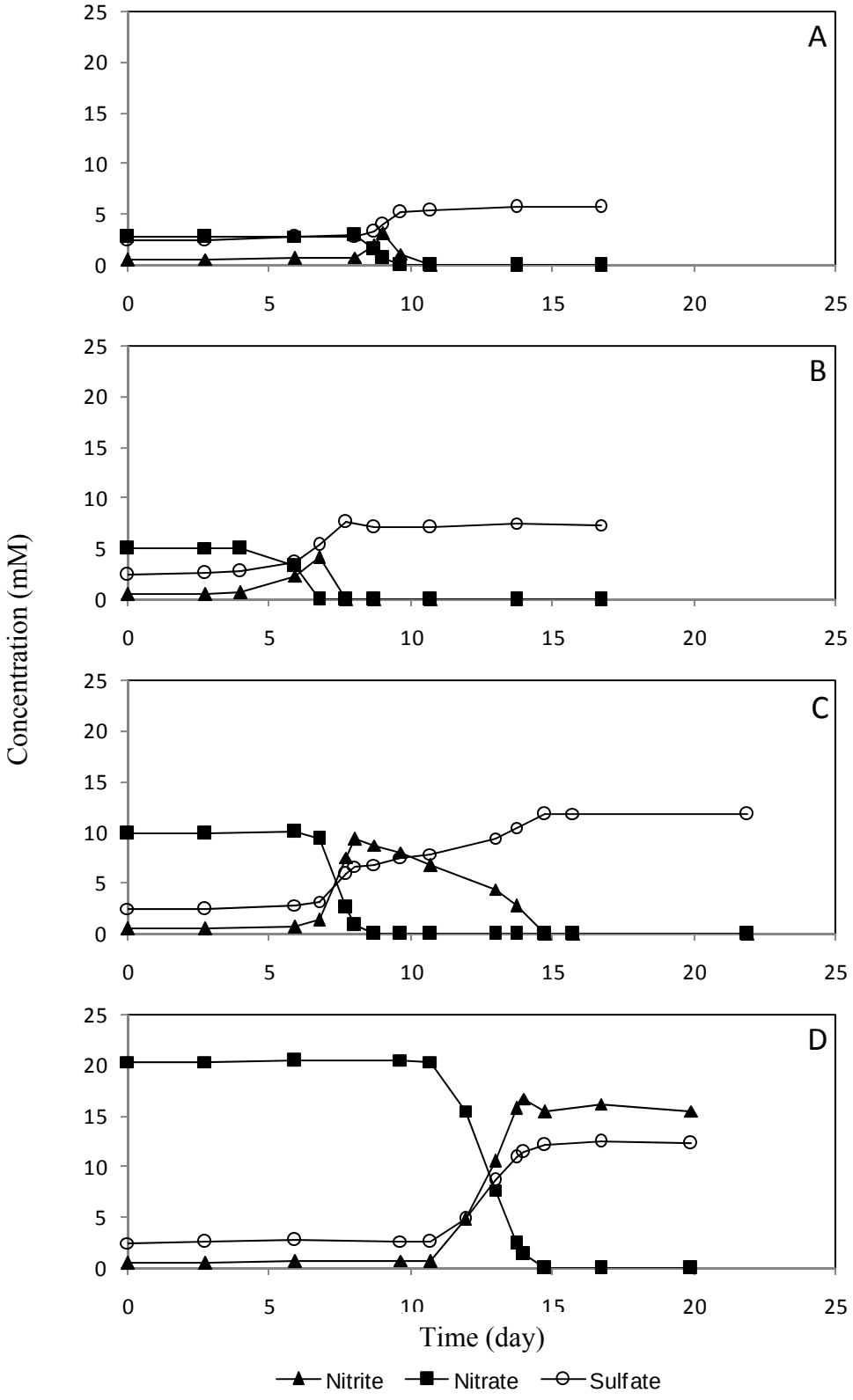

Figure 5.1 Autotrophic denitrification (nitrate reduction) of (A) 2.8 (B) 5.0 (C) 9.9 and (D) 20.4 mM nitrate with 25 mM elemental sulfur

$$3NO_3^- + S + H_2O \longrightarrow 3NO_2^- + SO_4^{2-} + 2H^+ \quad (5.1)$$

$$2NO_2^- + S \longrightarrow N_2 + SO_4^{2-} \quad (5.2)$$

$$6NO_3^- + 5S + 2H_2O \longrightarrow 3N_2 + 5SO_4^{2-} + 4H^+ \quad (5.3)$$

The maximum reduction rates of nitrate and nitrite were calculated as the slope of linear part in the concentration profiles and included in Table 5.1. With low initial nitrate concentrations (2.5 and 5 mM), the reduction rates of nitrate and nitrite were close. With initial nitrate concentrations increased from 10 to 20 mM, nitrate reduction rates became much faster than nitrite reduction rates. The maximum nitrate reduction rates increased with the increase of initial nitrate concentration with the highest value of 0.32±0.01 mM h^{-1} obtained with 20 mM nitrate.

Table 5.1 Highlights of the results obtained in the batch experiments of nitrate removal in the presence of sulfur

Designated nitrate conc. (mM)	Actual nitrate conc. (mM)	Residual nitrite conc. (mM)	Theoretical sulfate production[a] (mM)	Actual sulfate production (mM)	Discrepancy (%)	Maximum nitrate reduction rate (mM h^{-1})	Maximum nitrite reduction rate (mM h^{-1})
2.5	2.8	0	2.6	3.0	13.3%	0.13	0.13
	2.8	0	2.7	2.9	6.9%	-[b]	-[b]
5	5.0	0	4.4	4.8	8.3%	0.16	0.17
	5.3	0	4.8	5.0	4.0%	-[b]	-[b]
10	9.9	0	8.5	9.2	7.6%	0.28	0.10
	10.2	0	8.6	8.4	2.3%	0.29	0.06
20	20.4	15.6	9.5	9.7	2.1%	0.31	-[c]
	20.5	17.9	8.4	8.3	1.2%	0.32	-[c]

a The theoretical values are calculated based on the measurement of nitrate and nitrite concentration. Details of calculation are given in Appendix.

b The reduction of nitrate and nitrite completed overnight (i.e. not enough data to calculate the corresponding reduction rates).

c The produced nitrite was not reduced due to inhibition.

5.1.2 Nitrite removal in the presence of sulfur

The autotrophic denitritation with elemental sulfur as the electron donor was investigated in serum bottles with 100 mL modified CSB medium containing approximately 5, 10, 20, 30 and 50 mM nitrite with 25 mM elemental sulfur and 25 mM sodium bicarbonate. Figure 5.2 shows the concentration profiles of various ions as a function of time during the reduction of nitrite. Some of the experiments were conducted in duplicates and the average values of the data and standard deviations (error bars) were used in presenting the results.

As shown in Figure 5.2, lag phases of 7.0 and 3.7 days were observed in the experiments with 4.8 and 10.1 mM nitrite, respectively. With 32.7 and 52.5 mM nitrite, much longer lag phases of 22.9 and 41.0 days were observed, respectively. However, in both cases nitrite was reduced at a very slow rate during the lag phase which then proceeded with a faster reduction phase. With 18.8 mM nitrite, the reduction of nitrite proceeded with a lag phase of 2.0 days where no nitrite reduction happened, and then 5-6 days slow reduction followed by a fast reduction phase during the last stage. The complete reductions of nitrite were achieved in 3.0, 4.0, 9.0, and 28.7 days with 4.8, 10.1, 18.8, and 32.7 mM nitrite, respectively. In the case of 52.5 mM nitrite, after 98.7 days, 5.2±0.2 mM residual nitrite was detected in the system (Figure 5.2, panel E). According to Equation 5.2, 25 mM sulfur should be enough for complete reduction of 50 mM nitrite. Thus, the incomplete reduction of nitrite may be attributed to the deficiency of a carbon source (bicarbonate).

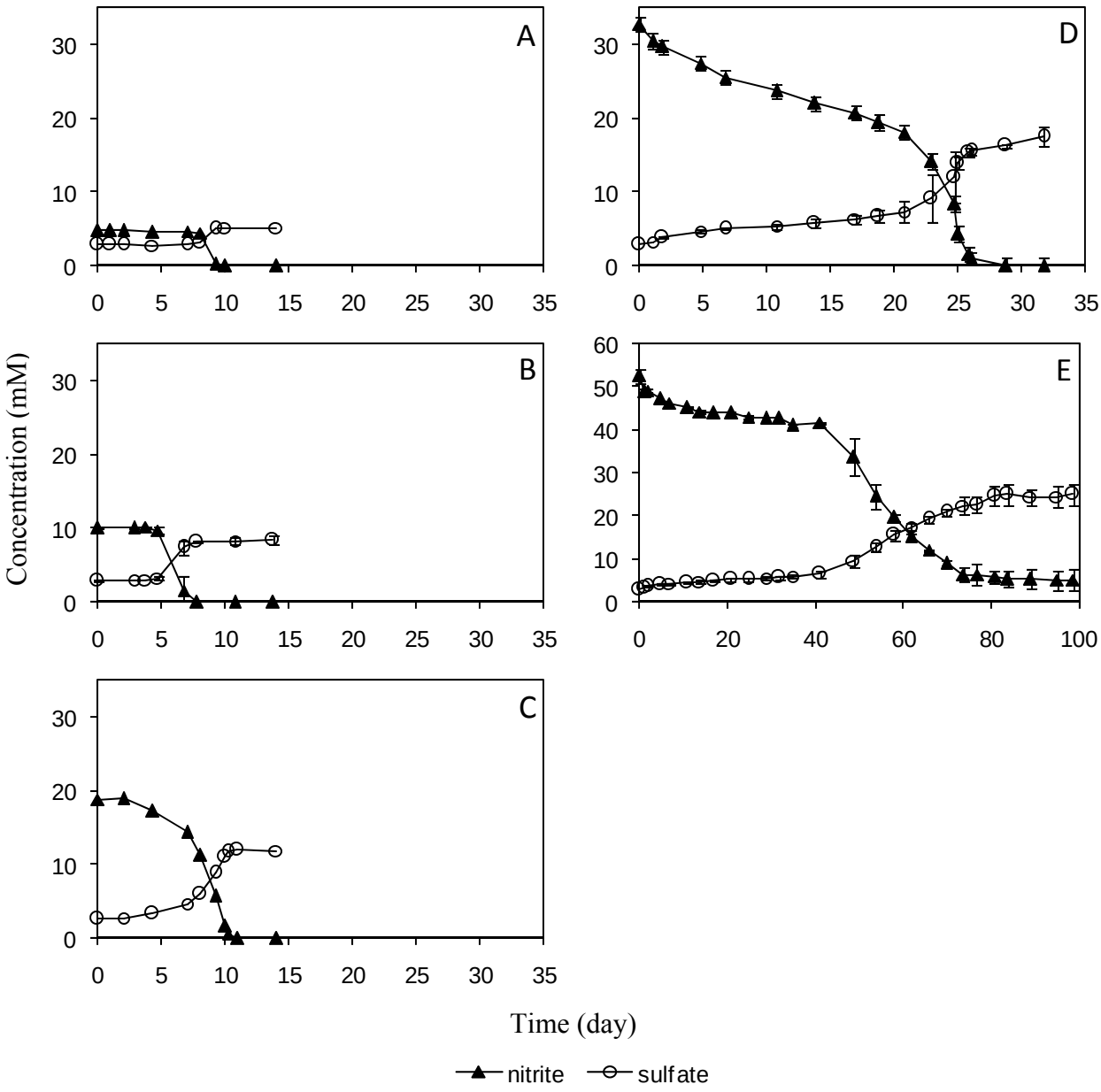

Figure 5.2 Autotrophic denitritation (nitrite reduction) of (A) 4.8 (B) 10.1 (C) 18.8 (D) 32.7 and (E) 52.5 mM nitrite with 25 mM elemental sulfur

During the reduction of nitrite, sulfate concentrations increased considerably indicating the utilization of elemental sulfur as the electron donor in the denitritation process. The experimental sulfate productions obtained at different initial nitrite concentrations were compared with corresponding theoretical values and summarized in Table 5.2. The discrepancies between the

theoretical and actual values were in the range of 0.04-18.9% and were possibly caused by errors associated with the measurement of nitrite and sulfate concentrations. The molar ratios of reduced nitrite to produced sulfate were in the range of 1.82-2.47, close to the stoichiometric value of 2 according to Equation 5.2. The calculation procedures of the theoretical sulfate productions were similar to those described in section 5.1.1. No thiosulfate was detected during the denitritation experiments, regardless of the initial nitrite concentrations.

The strong inhibitory effect of nitrite on the activities of bacteria observed during the denitrification (section 5.1.1) was not observed when nitrite was used as the original substrate. Following either a lag phase with no activity or a lag phase with a slow nitrite reduction rate, bacterial culture got acclimated to the nitrite as the only electron acceptor thus were able to reduce nitrite even at the highest concentration of 53.5 mM. It can also be speculated that the produced nitrite in the experiment with 20 mM nitrate (Figure 5.1, panel D) might be able to be reduced if given longer enough time.

The maximum nitrite reduction rates (the slopes of linear part in the concentration profiles) were also summarized in Table 5.2. The maximum nitrite reduction rates increased with the increase of initial nitrite concentration from 5 to 20 mM and decreased afterwards when initial nitrite concentration further increased to 30 and 50 mM. The highest nitrite reduction rate of 0.18±0.03 mM h^{-1} was obtained in the experiment with 20 mM nitrite.

Table 5.2 Highlights of the results obtained in the batch experiments of nitrite removal in the presence of sulfur

Designated nitrite conc. (mM)	Actual nitrite conc. (mM)	Residual nitrite conc. (mM)	Theoretical sulfate production[a] (mM)	Actual sulfate production (mM)	Discrepancy (%)	Maximum nitrite reduction rate (mM h^{-1})
5	4.7	0	2.361	2.362	0.04%	0.14
	4.7	0	2.37	2.43	2.5%	0.09
10	10.2	0	5.1	5.6	8.9%	0.15
	10.0	0	5.0	5.1	2.0%	0.20
20	18.8	0	9.4	9.2	2.1%	0.20
	19.9	0	9.9	8.9	10.1%	0.16
30	31.7	0	15.8	13.1	17.1%	0.13
	33.7	0	16.9	13.7	18.9%	0.14
50	53.5	3.5	25.0	23.8	4.8%	0.05
	51.4	6.8	22.3	20.2	9.4%	0.06

a The theoretical values are calculated based on the measurement of nitrite concentration. Details of calculation are given in Appendix.

5.1.3 Nitrate removal in the presence of sulfur and acetate

Earlier works done by others in the same laboratory have revealed that when both sulfide and acetate were present in the system, sulfide was used as the preferred electron donor during the denitrification process and the utilization of acetate (heterotrophic denitrification) was started only after complete exhaustion of sulfide and biologically produced sulfur, which is an intermediate product of sulfide oxidation (An *et al*., 2010).

The denitrification process with both acetate and elemental sulfur as the electron donors were investigated in serum bottles with 100 mL CSB medium containing 30 mM acetate and different concentrations of nitrate and sulfur (nitrate to sulfur ratio was kept constant at 1:1). Figure 5.3 shows the concentration profiles of various ions as a function of time during the reduction of nitrate at different initial concentrations. All the experiments were carried out in duplicates and the average values of the data were used in presenting the results and the associated standard deviations was used as error bars.

As shown in Figure 5.3, regardless of initial nitrate concentration, no lag phase was observed in any of the experiments. The reduction of nitrate started right after the inoculation and was associated with the production of nitrite. The reduction of nitrite started after nitrate was completely utilized. With 5.6, 9.8, 19.4, and 30.4 mM nitrate, complete reductions of both nitrate and nitrite were achieved in 4.0, 7.0, 7.0, and 7.9 days, respectively, while with 49.0 mM nitrate, 13.7 days were required.

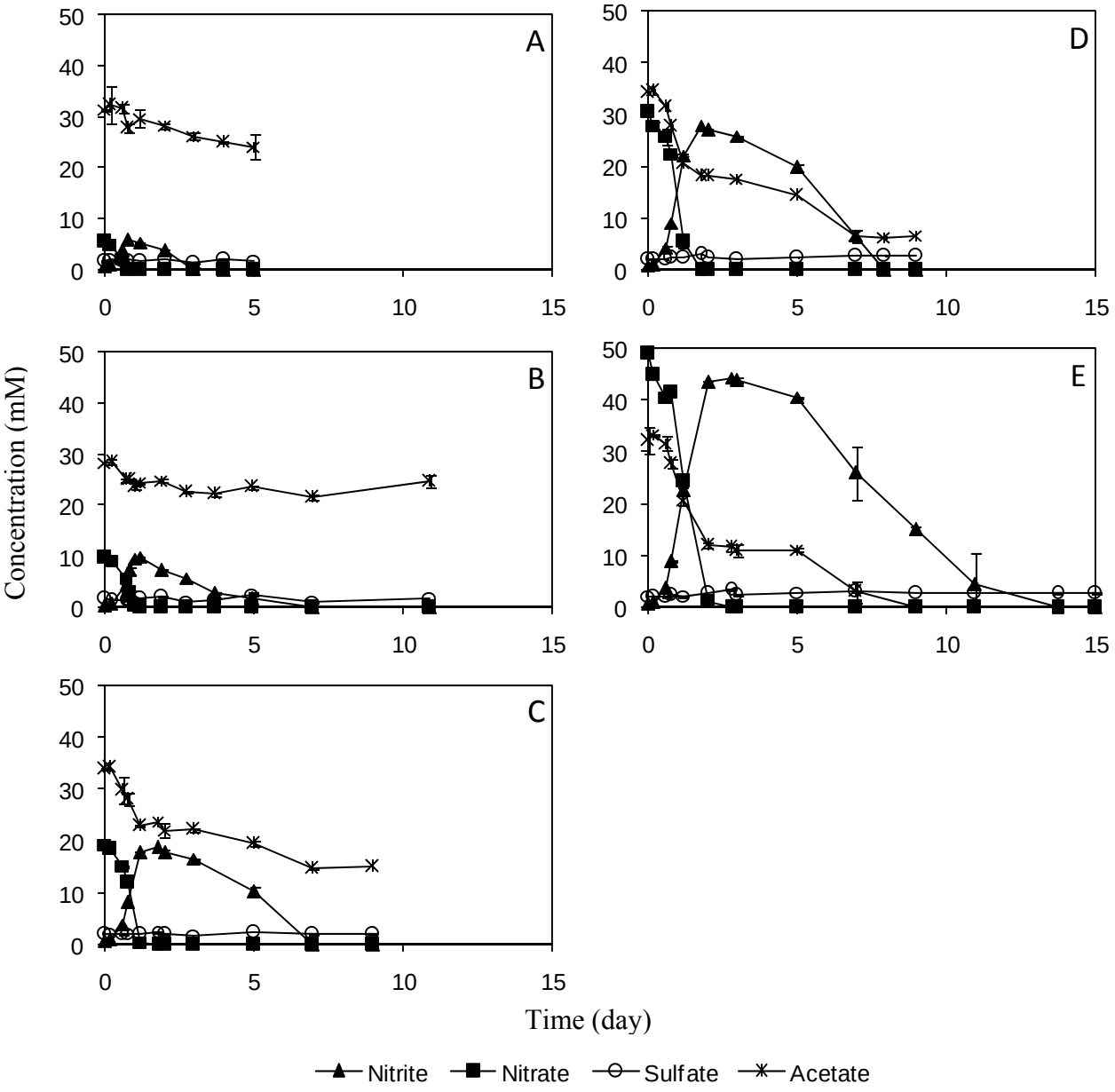

Figure 5.3 Denitrification with nitrate and sulfur at concentrations of around (A) 5 (B) 10 (C) 20 (D) 30 and (E) 50 mM of each nitrate and sulfur and around 30 mM acetate

During the reductions of nitrate and nitrite, sulfate and thiosulfate concentrations did not show any significant change but acetate concentration decreased as the denitrification progressed. This indicated that elemental sulfur was not used as the electron donor when sufficient acetate was present in the system for complete reduction of nitrate. The experimental acetate consumptions

obtained at different initial nitrate concentrations were compared with corresponding theoretical values in Table 5.3. The discrepancies between the theoretical and actual values were in the range of 1.6-50.6%. This may be attributed to errors associated with the measurement of nitrate, nitrite and acetate concentrations. Another possible reason is that acetate may also be consumed by other heterotrophic bacteria in the mixed culture as a carbon source for the growth. The theoretical values of acetate consumption were calculated based on the stoichiometry of nitrate and nitrite reduction in the presence of acetate (Equation 5.4 and 5.5). According to Equation 5.4 and 5.5 complete reduction of 1.6 mM nitrate require 1 mM acetate, while 1 mM acetate is sufficient for reduction of 2.7 mM nitrite to nitrogen gas.

$$8NO_3^- + 5CH_3COO^- \longrightarrow 4N_2 + 10CO_2 + H_2O + 13OH^- \quad (5.4)$$

$$8NO_2^- + 3CH_3COO^- + H_2O \longrightarrow 4N_2 + 6CO_2 + 11OH^- \quad (5.5)$$

The maximum nitrate reduction rates (Table 5.3) obtained in this series of batch experiments were about 2-4 times higher than those obtained in the presence of elemental sulfur (section 5.1.1). Moreover, nitrate reduction rate increased with the increase of initial nitrate concentration. The highest nitrate reduction rate (1.8 ± 0.1 mM h^{-1}) was observed in the experiment with 50 mM nitrate. In all cases the maximum nitrate reduction rates were much higher than the maximum nitrite reduction rates.

Contrary to what happened during the autotrophic denitrification (with sulfur as the sole electron donor), no inhibitory effect on the activities of bacteria was observed. The produced nitrite was reduced completely in all cases despite the fact that nitrite was produced at concentrations as high as 44 mM in the experiment with 50 mM nitrate (Figure 5.3, panel E). This indicated that

the presence of a suitable organic carbon source (electron donor) in addition to sulfur may somehow eliminate the inhibition of nitrite. The effects of adding small quantities of organics on the nitrate removal efficiency during the sulfur-based autotrophic denitrification process have been investigated by other researchers. Oh *et al*. (2001) established several up-flow packed bed bioreactors to study the effects of methanol addition on sulfur-based autotrophic denitrification and found that small amount of methanol increased the maximum nitrate loading rate from 1.4 to 1.92 kg m^{-3} d^{-1} with nitrate removal efficiency maintained greater than 97%.

Table 5.3 Highlights of the results obtained in the batch experiments of nitrate removal in the presence of sulfur and acetate

Designated nitrate conc. (mM)	Actual nitrate conc. (mM)	Theoretical acetate consumption[a] (mM)	Actual acetate consumption (mM)	Discrepancy (%)	Maximum nitrate reduction rate (mM h^{-1})	Maximum nitrite reduction rate (mM h^{-1})
5	5.6	3.8	7.7	50.6%	0.52	0.16
	5.6	3.8	5.6	32.1%	0.50	0.15
10	9.7	6.3	5.7	9.5%	0.67	0.11
	10.0	6.4	4.6	28.1%	0.68	0.10
20	19.3	12.4	19.1	35.1%	1.20	0.23
	19.4	12.4	19.2	35.4%	1.22	0.20
30	30.3	19.2	27.6	30.4%	1.86	0.28
	30.4	19.4	27.7	30.0%	1.71	0.29
50	48.6	30.7	34.0	9.7%	1.73	0.26
	49.4	31.1	30.6	1.6%	1.87	0.27

a The theoretical values are calculated based on the measurement of nitrate and nitrite concentration. Details of calculation are given in Appendix.

From the results of the batch experiments, a common pattern of the denitrification processes can be established. Nitrate is first reduced to nitrite and subsequently to other nitrogenous compounds, possibly nitrogen gas. The sulfur-based autotrophic denitrification process starts with a lag phase or slow reduction phase, which allows the acclimation of microbial culture, and then followed by a rapid reduction phase. The total reduction time required for complete removal of nitrate and/or nitrite increased with the increase of initial substrate concentration in both heterotrophic and autotrophic denitrification processes.

Previous results obtained in the denitrification experiments in the presence of acetate and dissolved sulfide conducted by others in our research laboratory, revealed that when sulfide and acetate co-exist in the system, Coleville enrichment preferred dissolved sulfide as the electron donor and acetate was used only after the complete oxidation of sulfide to sulfate (An *et al*., 2010). However, in the present work when acetate and elemental sulfur were both supplied as electron donors, acetate was the preferred electron donor and elemental sulfur was not consumed. Another important observation was that in earlier work, sulfide was first oxidized to elemental sulfur and then to sulfate during the reduction of nitrate and no acetate was used until all the produced sulfur was converted to sulfate. This means that the bacterial metabolic activity in the presence of biologically produced sulfur is quite different with that in the presence of non-biological elemental sulfur. Overall the order of preferred electron donors for the Coleville enrichment was determined as: sulfide > biologically produced sulfur > acetate > elemental sulfur.

Sulfate productions were closely matched with the theoretical values expected from the stoichiometry on the assumption of nitrite and nitrogen gas as the end products of nitrate

reduction. Sierra-Alvarez *et al.* (2007) reported similar trends in the sulfur-utilizing autotrophic denitrification process.

Nitrate reduction rates obtained in the heterotrophic denitrification process (in the presence of acetate) were much higher than those obtained in the autotrophic process (in the absence of acetate), while nitrite reduction rates showed no significant differences. The highest nitrate reduction rate (1.8 ± 0.1 mM h^{-1}) and nitrite reduction rate (0.29 ± 0.01 mM h^{-1}) were obtained in the heterotrophic batch experiments with initial nitrate concentrations of 50 mM and 30 mM, respectively. In the autotrophic denitrification process, the highest nitrate and nitrite reduction rates were 0.32 ± 0.01 mM h^{-1} and 0.18 ± 0.03 mM h^{-1} with 20 mM nitrate and 20 mM nitrite, respectively.

In the autotrophic denitrification experiment with 20 mM nitrate, the produced nitrite (15 - 16 mM) imposed strong inhibitory effect on microbial activities. While in the experiments supplied with both sulfur and acetate such inhibitory effect was not observed even when the concentration of produced nitrite (44 mM) was much higher than that observed during the autotrophic process. The organic compound in some way helped the bacteria to cope with this unfavorable condition.

5.2 Denitrification and Denitritation with Sulfur in a Up-flow Biofilm Reactor

5.2.1 Denitrification with sulfur

The continuous denitrification experiment with sulfur was studied over a period of 81 days. The steady state profiles of nitrate, nitrite and sulfate concentrations in port 1 (bottom of the

bioreactor) and 3 (top of the bioreactor) are shown in Figure 5.4. The effects of nitrate loading rates on the performance of the bioreactor were investigated in two different ways: (i) maintaining a constant nitrate concentration in the feed and increasing feed flow rate; (ii) maintaining a constant flow rate and increasing nitrate feed concentration.

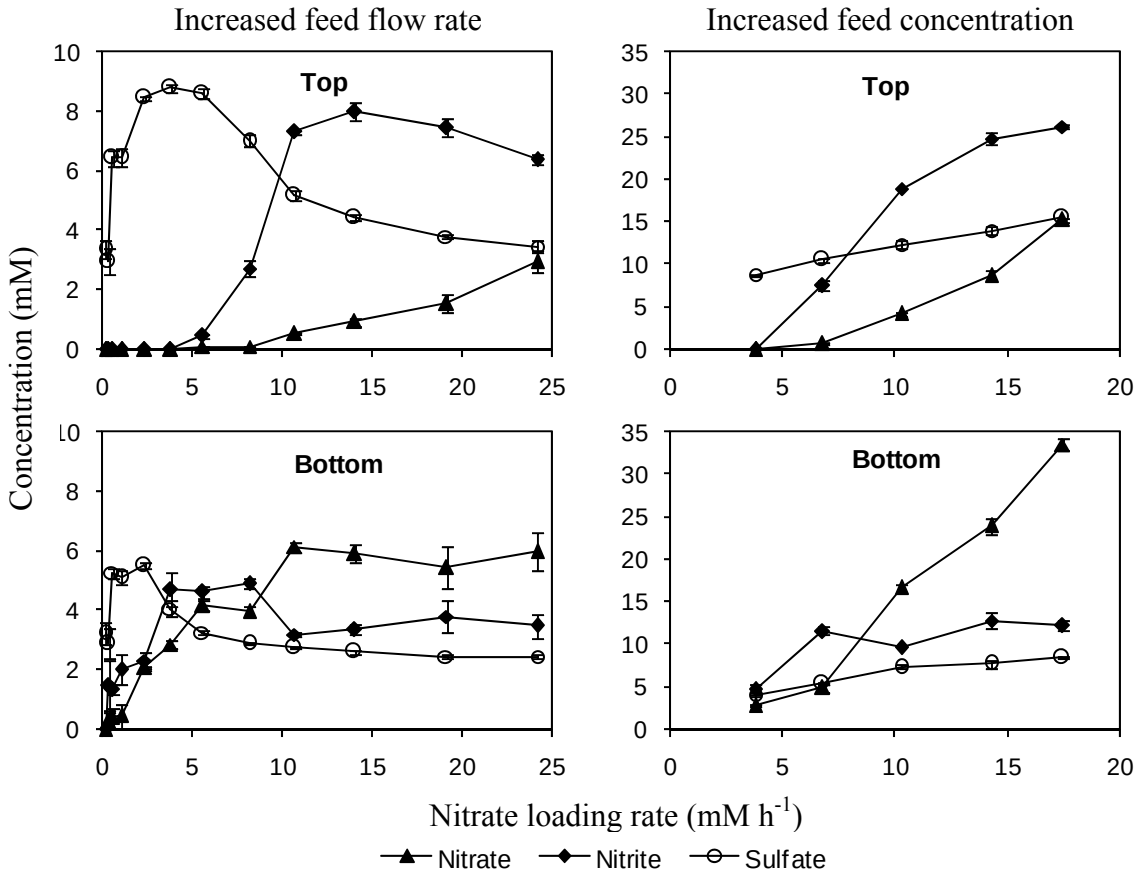

Figure 5.4 The steady state concentration profiles of various ions as a function of nitrate loading rate in the upper and lower regions of bioreactor. Left panels: increases in feed flow rate; Right panels: increases in feed concentration.

When nitrate concentration in the feed maintained constant (10.2±0.3 mM) and the flow rate was increased, nitrate and nitrite residual concentrations remained zero in the upper part of the bioreactor for nitrate loading rates up to 3.8 mM h^{-1} (Figure 5.4, top left panel). For the same

range of nitrate loading rates, nitrate and nitrite concentrations in the lower part of the bioreactor increased from 0 to 2.9±0.1 mM and from 0 to 4.7±0.6 mM, respectively (Figure 5.4, bottom left panel). Nitrate concentration in the upper region of the bioreactor kept increasing with further increases in nitrate loading rate, while in the lower part of the bioreactor nitrate concentration increased to 6.1 mM at a nitrate loading rate of 10.7 mM h^{-1} and then stabled at around 5.8±0.3 mM afterwards. During the entire course of the experiment, nitrate concentrations in the bottom part were considerably higher than those in the top part. The residual concentrations of nitrate decreased as the denitrification process proceeded along the length of the bioreactor. Nitrite concentration in the lower part of the bioreactor was higher than that in the upper part at low nitrate loading rates since the produced nitrite was further reduced along the bioreactor. However after the nitrate loading rate increased to 10.7 mM h^{-1}, only less than half of the nitrate was converted to nitrite in the lower part and incomplete reduction of nitrate to nitrite occurred as the reaction medium flowed along the length of the bioreactor resulting in a higher concentration of nitrite in the upper part. This was due to a shorter residence time which did not allow the bacteria to accomplish the reduction of nitrate to nitrite in the lower part. Instead, the reduction only proceeded half way resulting in the accumulation of nitrite in the upper part of the bioreactor.

Sulfate concentrations in both lower and upper parts of the bioreactor showed similar patterns throughout the experiment. Sulfate concentrations in the upper part increased significantly from 3.4 to 8.8 mM for nitrate loading rates up to 3.8 mM h^{-1}. The reason possibly could be that at starting stage during which a low loading rate (long residence time) was applied, Coleville enrichment accomplished the denitrification process partly through the heterotrophic pathway using organic materials from bacteria autolysis. With the increase of flow rate, the autolysis

potentially might not have been fast enough to supply organic materials for the heterotrophic denitrification therefore the Coleville enrichment used elemental sulfur as the electron donor to accomplish the denitrification through the autotrophic pathway. Considering the autotrophic denitrification reaction (Equation 5.3), the stoichiometric ratio of reduced nitrate to produced sulfate should be 1.2 when nitrate is reduced to nitrogen gas. This means that for complete reduction of 10.2 mM nitrate, 8.5 mM sulfate should be generated. The actual ratio of reduced nitrate to produced sulfate decreased with the increase of nitrate loading rate indicating more and more sulfur was used for nitrate reduction. At a nitrate loading rate of 3.8 mM h^{-1}, the actual ratio was 1.5 (lower than expected stoichiometric ratio) with 100% removal of nitrate without accumulation of nitrite and the highest amount of produced sulfate was 6.8 mM. With further increase of nitrate loading rate, part of nitrate was only reduced to nitrite (Equation 5.1), resulting in a decrease in sulfate productions in both upper and lower parts of the bioreactor. At high nitrate loadings, the low solubility of sulfur may become a rate-limiting factor resulted in the decrease of nitrate removal rate. No thiosulfate production was observed throughout the experiment.

The experimental sulfate productions at different loading rates were compared with the theoretical expectations calculated based on the assumption that nitrate is reduced to nitrite and subsequently to nitrogen gas (Equation 5.1 and 5.2) and presented in Table 5.4. The observed discrepancies were in the range of 20.0% - 48.6%. This could be attributed to possible precipitations of sulfate with trace element ions in the medium such as Ca^{2+} and Fe^{3+}, a potential of denitrification through the heterotrophic pathway, and errors in the measurement of nitrate, nitrite and sulfate concentrations. It should be pointed out that the reduction of nitrate could also

result in the formation of other end products such as N_2O and NO (Equation 5.6 and 5.7) with a different stoichiometry.

$$4S + 6NO_3^- + H_2O \longrightarrow 4SO_4^{2-} + 3N_2O + 2H^+ \quad (5.6)$$

$$S + 2NO_3^- \longrightarrow SO_4^{2-} + 2NO \quad (5.7)$$

Table 5.4 Summary of the results obtained in the biofilm reactor operated with nitrate (increase of flow rate)

Feed flow rate (mL h^{-1})	Nitrate loading rate (mM h^{-1})	Nitrate removal rate (mM h^{-1})	Theoretical sulfate production[a] (mM)	Actual sulfate production (mM)	Discrepancy (%)	Reduced nitrate /produced sulfate
34.8	3.8	3.76	8.5	6.8	20.0%	1.5
49.9	5.6	5.52	8.45	6.6	21.9%	1.6
76.1	8.1	8.08	7.0	5.1	27.1%	2.0
98.2	10.7	10.12	4.4	3.3	25.0%	3.0
130.2	14.0	12.73	3.7	2.5	32.4%	3.7
175.7	19.1	16.25	3.5	1.8	48.6%	4.9
220.6	24.2	17.26	2.9	1.5	48.3%	4.9

a The theoretical values are calculated based on the measurement of nitrate and nitrite concentration. Details of calculation are given in Appendix.

When the feed flow rate was maintained constant at 31.7 ± 1.8 mL h^{-1} and nitrate concentration in the feed was increased (the increase of loading rate through the increase of feed concentration), residual nitrate, nitrite, and sulfate concentrations increased proportionally in both lower and upper parts of the bioreactor with the increase of nitrate loading rate (Figure 5.4 right panels). Nitrate concentration increased from 0 to 15.2 ± 0.3 mM in the upper part and from 2.9 ± 0.1 to 33.6 ± 0.6 mM in the lower part of the bioreactor when nitrate loading rate increased from 3.8 to 17.5 mM h^{-1} (feed nitrate concentration: 10.2 to 53.4 mM). Residual nitrate concentration in the lower part was always higher than that in the upper part because of continuous microbial activities along the length of the bioreactor. For same range of nitrate loading rates, nitrite concentration increased from 0 to 26.2 ± 0.2 mM in the upper region and from 4.7 ± 0.6 to 12.2 ± 0.6 mM in the lower region of the bioreactor. Similar to previous experiment, at low loading rates nitrite concentration in the lower region was higher than that in the upper region. However at nitrate loading rates higher than 10.3 mM h^{-1}, in the lower part of the bioreactor less than half of nitrate was reduced to nitrite and as the reaction medium passed along the bioreactor nitrate reduction continued, and nitrite concentration in the upper region became higher than that in the lower region. For similar loading rates when feed concentration was increased, the residual concentrations were much higher than those obtained in the experiment with variable feed flow rate. For instance, at a nitrate loading rate of 19.1 mM h^{-1}, residual concentrations of nitrate and nitrite in the upper part of the reactor were 1.5 and 7.5 mM, respectively when feed flow rate was increased. With increases in feed concentration 15.2 mM nitrate and 26.2 mM nitrite were detected, respectively in the upper part of the reactor at a loading rate of 17.5 mM h^{-1}, which were 10 and 3.5 times higher than their counterparts.

The trend of sulfate concentration had a different pattern when feed concentration was increased. Sulfate concentration in both upper and lower parts of the bioreactor kept increasing with the increases of feed concentration. To be more quantitative sulfate concentration increased from 8.8 ± 0.1 to 15.5 ± 0.1 mM in the upper region and from 4.1 ± 0.3 to 8.5 ± 0.2 mM in the lower region, respectively. The amounts of produced sulfate at different nitrate loading rates were compared with theoretical values calculated according to Equation 5.2 and 5.3 in Table 5.5 based on the assumption that nitrate was reduced partially to nitrite and partially to nitrogen gas. The observed discrepancies might have been due to the various factors described previously. The stoichiometric ratio of reduced nitrate to produced sulfate should be in the range of 1.2 - 3 according to Equation 5.1 and 5.3. With the increase of loading rate, more nitrate was only reduced to nitrite instead of nitrogen gas and the actual ratio of reduced nitrate to produced sulfate correspondingly increased from 1.5 to 3.0, indicating N_2O (Equation 5.6) might be the main component of the end products in earlier stage and nitrite became the main end product later. Park *et al*. (2002) reported that N_2O composition in the gas phase increased from 7.8% to 20% as the nitrate loading rate increased from 4.9 to 5.7 mM h^{-1}.

Table 5.5 Summary of the results obtained in the biofilm reactor operated with nitrate (increase of feed concentration)

Feed nitrate concentration (mM)	Nitrate loading rate (mM h^{-1})	Nitrate removal rate (mM h^{-1})	Theoretical sulfate production[a] (mM)	Actual sulfate production (mM)	Discrepancy (%)	Reduced nitrate /produced sulfate
10.2	3.8	3.76	8.5	6.8	20.0%	1.5
21.1	6.8	6.53	13.2	8.4	36.4%	2.4
31.0	10.3	8.90	12.9	10.3	20.2%	2.6
42.4	14.3	11.33	15.7	11.5	26.8%	2.9
53.4	17.5	12.48	18.7	12.7	32.1%	3.0

a The theoretical values are calculated based on the measurement of nitrate and nitrite concentration. Details of calculation are given in Appendix.

Figure 5.5 shows volumetric removal rates and removal efficiencies of nitrate and total nitrogen as a function of nitrate loading rate. During the entire course of the run with increased flow rate, nitrate removal rate increased linearly with the increase of nitrate loading rate. The maximum nitrate removal rate (17.3 mM h^{-1}) was obtained at a nitrate loading rate of 24.2 mM h^{-1} (corresponding residence time: 0.4 h) with a nitrate removal efficiency of 71.3% and a total nitrogen removal efficiency of 9.5%. Complete removal of 10.2±0.3 mM nitrate was achieved for nitrate loading rates up to 3.8 mM h^{-1} without the accumulation of nitrite (corresponding residence time: 2.7 h). With further increase of loading rate, nitrate and nitrite concentrations in the effluent kept increasing resulting in a decrease in the removal efficiencies of both nitrate and total nitrogen. The removal rate of total nitrogen increased with the increase of nitrate loading rate up to 8.2 mM h^{-1} and decreased dramatically afterwards due to the incomplete reduction of nitrate to nitrite. The maximum removal rate of total nitrogen was 5.9 mM h^{-1} at a nitrate loading rate of 8.2 mM h^{-1} (corresponding residence time: 1.2 h) with the removal efficiencies of nitrate and total nitrogen being 99.1% and 72.0%, respectively. A significant decrease from 5.9 to 2.5 mM h^{-1} in the removal rate of total nitrogen with a drop of removal efficiency from 72.0% to 23.3% occurred when nitrate loading rate increased from 8.2 to 10.7 mM h^{-1}. After that the removal rate and removal efficiency of total nitrogen fluctuated in the range of 1.6 - 2.3 mM h^{-1} and 9.5 - 12.0%, respectively.

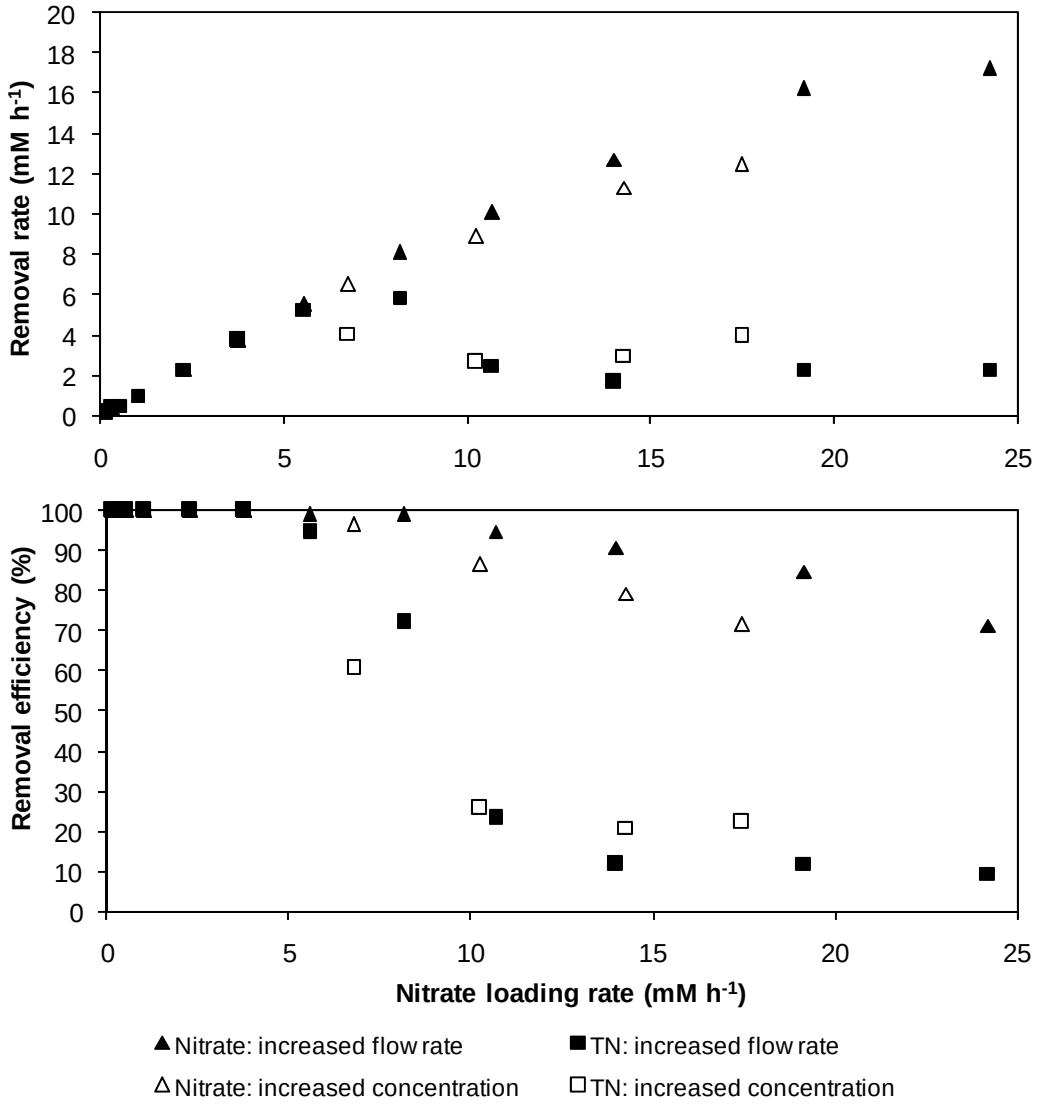

Figure 5.5 The removal rates (top) and removal efficiencies (bottom) of nitrate and total nitrogen (TN) as a function of nitrate loading rate (data for both modes of operations are included).

When the loading rate increased by the variation of feed concentration, a similar linear increase in nitrate removal rate was observed. The highest nitrate removal rate (12.5 mM h^{-1}) was achieved at a nitrate loading rate of 17.5 mM h^{-1} (corresponding residence time: 3.1 h) with the removal efficiencies of nitrate and total nitrogen being 71.5% and 22.4%, respectively. For similar nitrate loading rates, the removal rate and removal efficiency of nitrate were both lower

than those obtained in the experiment with variable flow rates. The removal rate of total nitrogen fluctuated in the range of 2.6 - 4.1 mM h^{-1}, while the removal efficiency decreased from 100% to 60.6% and then stabled at around 23.0%. However, it should be pointed out that the removal rate and removal efficiency of total nitrogen were higher than those observed in the experiment with variable flow rates for nitrate loading rates higher than 14 mM h^{-1}.

5.2.2 Denitritation with sulfur

The same biofilm reactor was subsequently used to study the autotrophic denitritation process over a period of 120 days (the biofilm reactor was not dismantled between the denitrification and denitritation experiments). The steady state profiles of nitrite and sulfate concentrations in port 1 (bottom port) and 3 (top port) of the bioreactor are shown in Figure 5.6. Similar to nitrate removal experiments, the effects of nitrite loading rate on the performance of the bioreactor were investigated in two ways: (i) increasing the feed flow rate; and (ii) increasing nitrite concentration in the feed.

When the feed concentration was kept constant (10.7 ± 0.6 mM nitrite) and the loading rate increased through the variation of flow rate, nitrite residual concentration remained zero in the upper region of the bioreactor for nitrite loading rates up to 5.0 mM h^{-1} (Figure 5.6, top left panel). Thereafter nitrite concentration in the upper region increased from 0 to 4.5 mM with further increase in nitrite loading rate from 5.0 to 22.0 mM h^{-1}. Nitrite concentration in the lower part of the bioreactor kept increasing from 0.1 to 8.0 mM (Figure 5.6, bottom left panel) during the increase of nitrite loading rate from 0.4 to 22.0 mM h^{-1} and it was always higher than that in the upper part.

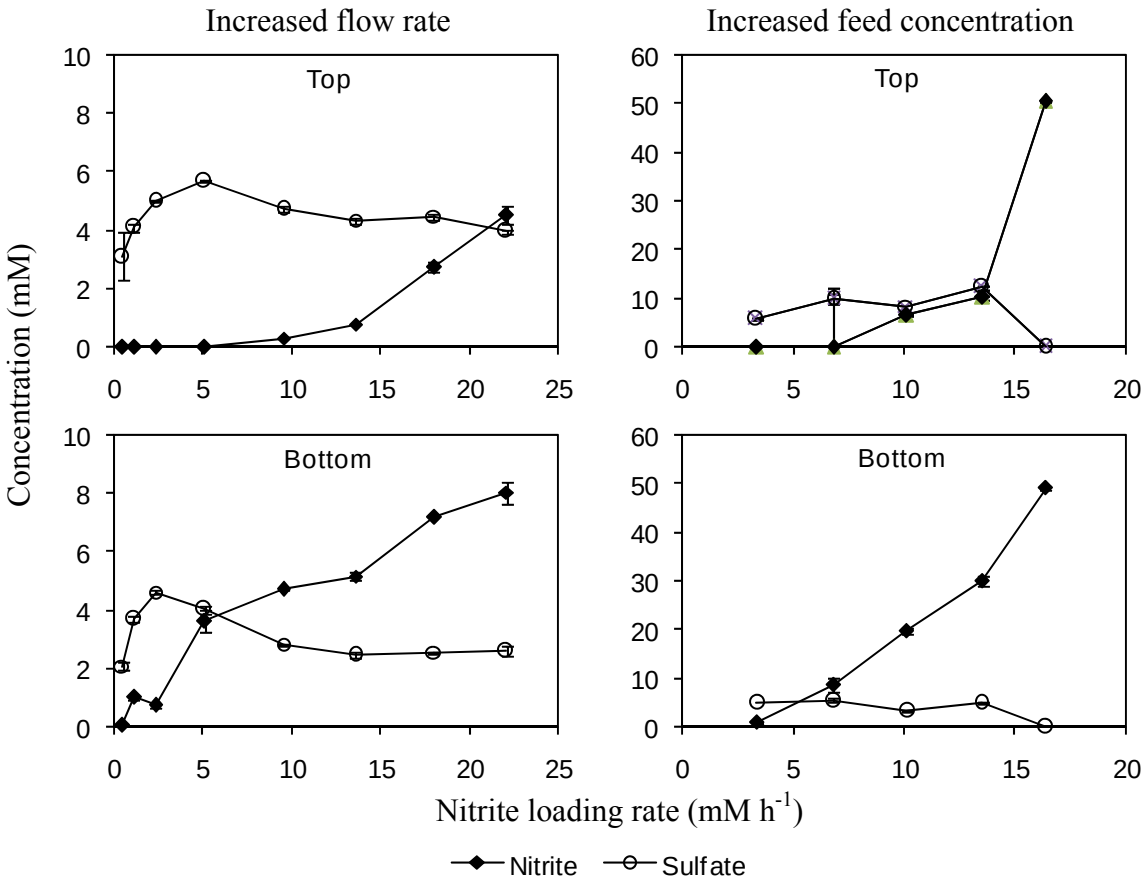

Figure 5.6 The steady state concentration profiles of various ions as a function of nitrite loading rate in the lower and upper regions of the bioreactor. Left panels: increases in feed flow rate; Right panels: increases in feed concentration.

Sulfate concentration in both lower and upper regions of the bioreactor followed similar pattern as in the nitrate removal experiment. Sulfate concentration in the upper part first kept increasing from 3.1 to 5.7 mM for nitrite loading rates up to 5.0 mM h^{-1} and gradually decreased to 4.0 mM afterwards (Figure 5.6, top left panel). In the lower part, sulfate concentration increased from 2.1 to 4.6 mM for nitrite loading rates up to 2.3 mM h^{-1} and decreased to 2.6 mM afterwards (Figure 5.6, bottom left panel). As speculated earlier, at low flow rates the denitritation could have been accomplished partly through the heterotrophic pathway using organic materials released from bacteria autolysis. At shorter residence times (higher loading rates) autolysis could not have

supplied enough organic materials, therefore the reduction of nitrite was increasingly carried out through the autotrophic pathway with sulfur as the electron donor, resulting in the increase of sulfate concentration. No thiosulfate was detected throughout the experiment.

With the increase of flow rate, residual nitrite concentration kept increasing indicating a decrease in the extent of denitritation resulting in less sulfur consumption and therefore less sulfate production. According to Equation 5.2, complete reduction of 10.7 mM nitrite should produce 5.35 mM sulfate. The discrepancies between actual sulfate productions and the theoretical expectations were summarized in Table 5.6. The theoretical values of sulfate production were calculated on the assumption of complete reduction of nitrite to nitrogen gas (Equation 5.2). The discrepancies were in the range of 32.1%-53.1% and were attributed to the reasons described previously. However, N_2O and NO could also be the end products of nitrite reduction (Equation 5.8 and 5.9).

$$2S + 6NO_2^- + H_2O \longrightarrow 2SO_4^{2-} + 3N_2O + 2OH^- \quad (5.8)$$

$$S + 6NO_2^- + 2H_2O \longrightarrow SO_4^{2-} + 6NO + 4OH^- \quad (5.9)$$

Table 5.6 Summary of the results obtained in the biofilm reactor operated with nitrite (increase of flow rate)

Feed flow rate (mL h^{-1})	**Nitrite loading rate (mM h^{-1})**	**Nitrite removal rate (mM h^{-1})**	**Theoretical sulfate production[a] (mM)**	**Actual sulfate production (mM)**	**Discrepancy (%)**	**Reduced nitrite /produced sulfate**
44.8	5.0	5.04	5.3	3.6	32.1%	3.0
85.0	9.5	9.21	5.1	2.7	47.1%	3.8
121.3	13.6	12.56	4.9	2.3	53.1%	4.2
162.0	18.0	13.23	3.8	2.5	34.2%	3.1
197.9	22.0	12.51	3	1.9	36.7%	3.1

a The theoretical values are calculated based on the measurement of nitrite concentration. Details of calculation are given in Appendix.

When the flow rate maintained constant at 30.1 ± 0.2 mL h^{-1} and nitrite concentration in the feed increased from 10.3 to 51.4 mM (the increase of loading rate through the increase of feed concentration), residual nitrite concentration in both upper and lower regions of the bioreactor increased dramatically (Figure 5.6, right panels). Complete removal of 10.3 mM nitrite was achieved at a nitrite loading rate of 3.3 mM h^{-1}. With further increase of nitrite loading rate from 6.8 to 13.5 mM h^{-1}, nitrite concentration in the upper region increased from 0.1 to 10.1 mM. With the highest tested concentration of 51.4 mM nitrite (nitrite loading rate: 16.3 mM h^{-1}), around 50.6 mM nitrite was detected in the upper part of the bioreactor. Nitrite concentration in the lower part of the bioreactor kept increase from 1.0 to 49.0 mM with the increase of nitrite loading rate through variable feed concentrations.

Similar to nitrate removal experiment, the residual concentration of nitrite with variable feed concentration was considerably higher than that obtained in the experiment with increased flow rate for similar loading rates. For instance, in the first run (increase in feed flow rate) at a nitrite loading rate of 13.6 mM h^{-1}, only 0.8 mM nitrite was detected in the top part, while in the second run (increase in feed concentration) residual nitrite concentration of 10.1 mM was observed in the top region of the bioreactor at a nitrite loading rate of 13.5 mM h^{-1}. At the nitrite loading rate of 16.3 mM h^{-1} with the highest feed concentration, the reduction of nitrite almost ceased (nitrite removal efficiency: 1.6%). This observation implicates that high feed nitrite concentration has a strong inhibitory effect on microbial activities and adversely influence the performance of the bioreactor.

With the increase of nitrite loading rate from 3.3 to 13.5 mM h^{-1}, sulfate concentration in the upper part increased from 5.7 to 12.2 mM and dramatically dropped to 0.05 mM when nitrite

loading rate further increased to 16.3 mM h^{-1} due to the inhibitory effect of high feed concentration (51.4 mM nitrite). The discrepancies between actual sulfate productions and the theoretical expectations were in the range of 26.2% - 48.0% (Table 5.7). The theoretical value of sulfate production was calculated on the assumption of complete reduction of nitrite to nitrogen gas (Equation 5.2). The discrepancies were considered coming from the possible reasons described previously. The actual ratio of reduced nitrite to produced sulfate was 2.7 - 3.9, considering possible reactions of Equation 5.2, 5.8 and 5.9, indicating that N_2O might be one of the end products of nitrite reduction.

Table 5.7 Summary of the results obtained in the biofilm reactor operated with nitrite (increase of feed concentration)

Feed nitrite concentration (mM)	Nitrite loading rate (mM h^{-1})	Nitrite removal rate (mM h^{-1})	Theoretical sulfate production[a] (mM)	Actual sulfate production (mM)	Discrepancy (%)	Reduced nitrite /produced sulfate
10.3	3.3	3.33	5.2	3.8	26.2%	2.7
21.4	6.8	6.77	10.7	6.4	40.2%	3.3
31.4	10.1	8.00	12.5	6.5	48.0%	3.9
42.0	13.5	10.24	16.0	10.1	36.7%	3.2
51.4	16.3	0.26	0.4	0	-	-

a The theoretical values are calculated based on the measurement of nitrite concentration. Details of calculation are given in Appendix.

Figure 5.7 shows the volumetric removal rates and removal efficiencies of nitrite as a function of nitrite loading rate. The removal rate of nitrite increased linearly with the increase of nitrite loading rate through the increase of either flow rate or feed concentration. The removal efficiencies in the case of increased feed concentration were lower than those observed with increased flow rate which implied that bacterial activities were influenced negatively by applying higher concentrations of nitrite.

With the increase of flow rate, complete reduction of 10.7 ± 0.6 mM nitrite was accomplished at nitrite loading rates up to 5.0 mM h^{-1} (corresponding residence time: 2.1 h). The highest nitrite removal rate (13.2 mM h^{-1}) was obtained at a nitrite loading rate of 18.0 mM h^{-1} (corresponding residence time: 0.6 h) with a nitrite removal efficiency of 73.6%. With the increase of feed concentration, the highest nitrite removal rate (10.2 mM h^{-1}) was obtained at a nitrite loading rate of 13.5 mM h^{-1} with 42.0 mM nitrite in the feed and when nitrite concentration increased to 51.4 mM, the removal rate of nitrite dropped to 0.3 mM h^{-1} almost lost all of the nitrite removal ability.

Biomass holdups in the bottom, middle and top parts of the up-flow biofilm reactor were 88, 36, and 53 mg cell dry weight (g sulfur)$^{-1}$, respectively, with the average value being 59 mg cell dry weight (g sulfur)$^{-1}$.

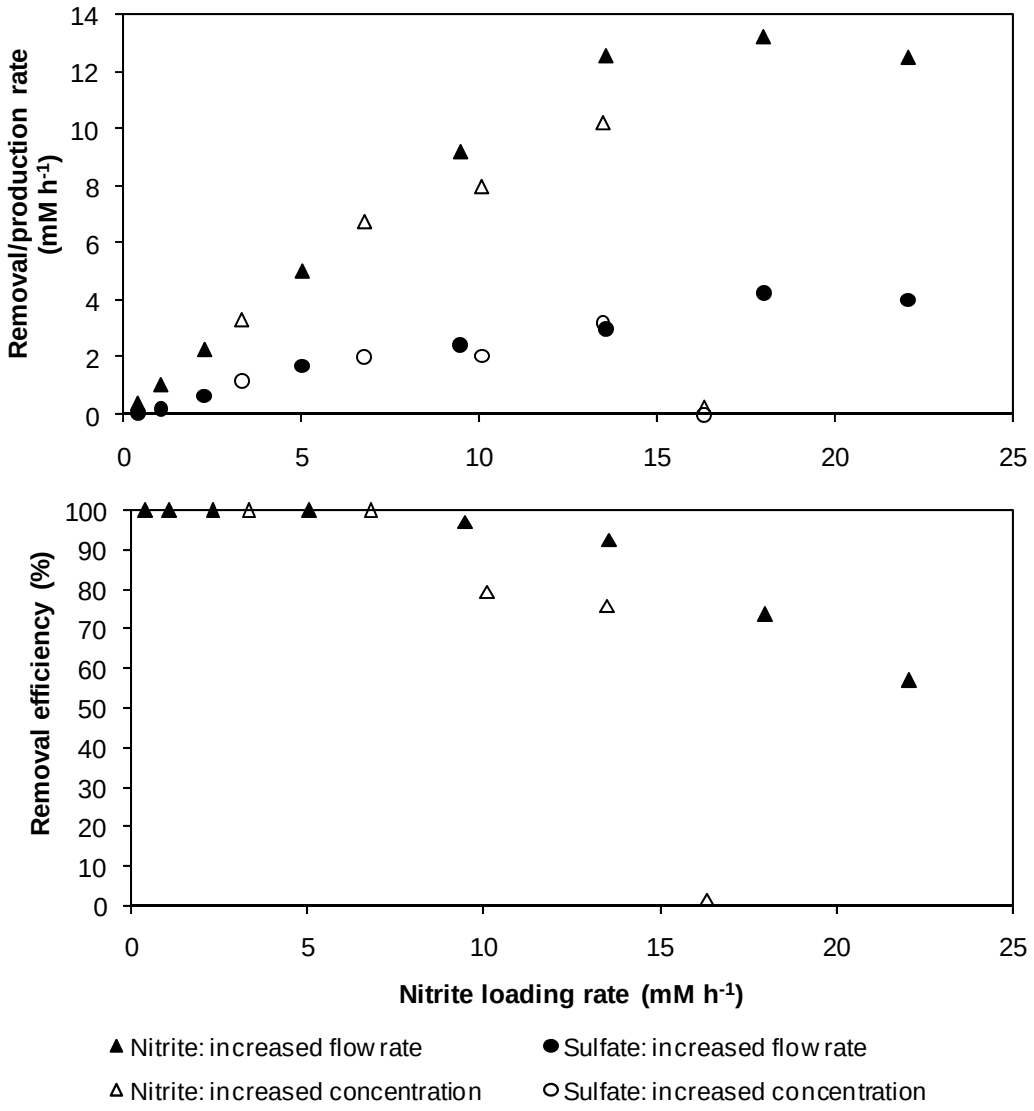

Figure 5.7 Nitrite removal and sulfate production rates (top panel) and nitrite removal efficiencies (bottom panel) as a function of nitrite loading rate (data for both modes of operations are included).

With either nitrate or nitrite as the terminal electron acceptor, the maximum loading rates and removal rates obtained in this study were significantly higher than those reported in the literature as compared in Table 5.8.

Table 5.8 Performance of bioreactors used for sulfur-based autotrophic denitrification as reported in various works

Reference	Microbial culture	Bioreactor	Alkaline added	Feed	Conc. (mM)	HRT (h)	Maximum Loading Rate (mM h^{-1})	Maximum Removal Rate (mM h^{-1})
Soares, 2002	Oxidation pond sediment	Packed-bed	-	Nitrate	1.6	1.0	0.71	0.60
Kimura *et al*., 2002	Activated sludge	Rotating disks with membrane	-	Nitrate	1.8	2.7	0.70	0.70
Koenig and Liu, 2002	*Thiobacillus denitrificans*	Packed-bed	Limestone	Nitrate	15.5	5.5	2.83	2.59
Park *et al*., 2002	Tidal flats sediments	Packed-bed	-	Nitrate	12.5	1.5	8.13	7.63
					50.0	5.1	9.77	7.03
Moon *et al*., 2004	*Thiobacillus denitrificans*	Packed-bed	Limestone	Nitrate	4.3	12.0	0.36	0.32
Kim *et al*., 2004	Tidal flats sediments	Fluidized-bed	Limestone	Nitrate	1.4	0.2	7.54	6.91
					50.0	6.1	7.99	7.84
Sierra-Alvarez *et al*., 2007	Anaerobic granular sludge	Packed-bed	Limestone	Nitrate	1.3	1.8	0.75	0.72
					7.3	1.8	0.90	0.90
Wan *et al*., 2009	Denitrifying reactor effluent	Packed-bed and bioelectro-chemical	-	Nitrate	1.6	2.1	0.71	0.71
Read-Daily *et al*., 2011	Activated sludge	Rectangular channel mesocosm	NaOH	Nitrate	0.4	3.0	0.15	0.04
Zhou *et al*., 2011	Municipal digested sludge	Packed-bed	Limestone	Nitrate	7.1	3.0	1.19	0.95
Present work	Oil reservoir brine (Coleville enrichment)	Packed-bed	-	Nitrate	10.3	0.4	24.20	17.26
					53.4	3.0	17.50	12.50
Zhou *et al*., 2011	Municipal digested sludge	Packed-bed	Limestone	Nitrite	0.7	3.0	0.23	0.22
					7.1	5	2.3	1.27
Present work	Oil reservoir brine (Coleville enrichment)	Packed-bed	-	Nitrite	10.4	0.6	17.98	13.23
					42.0	3.1	13.49	10.24

5.3 Denitrification and Denitritation with H_2S in a Packed Bed Reactor

The effects of H_2S loading rate (through the variation of gas flow rate) on the removal efficiency and removal rate of H_2S were investigated in a packed bed bioreactor.

5.3.1 Denitrification with H_2S

With CSB medium containing 10 mM nitrate, 4 batch runs were conducted at gas flow rates of 25, 50, 75, and 100 mL min^{-1}. Corresponding gas retention times (GRT) were 9.2, 4.6, 3.1, and 2.3 min, respectively.

The concentration profiles of sulfide, nitrate, nitrite, sulfate, and thiosulfate in the liquid phase and H_2S concentration in the effluent gas are shown in Figure 5.8. In this set of experiments regardless of the applied residence time, the removal of H_2S accompanied by the decrease of nitrate concentration (nitrate reduction) and corresponding increases in nitrite and sulfate concentrations, confirming that the removal of H_2S was biologically accomplished through the autotrophic denitrification process. However, the amount of produced nitrite was less than what was expected from the stoichiometry of nitrate reduction which indicates that nitrate and nitrite were used simultaneously as the electron acceptors during the removal of H_2S (oxidation of H_2S). H_2S removal efficiency remained greater than 98.6% during the entire course of the experiments when gas retention times of 9.2, 4.6, and 3.1 min were applied. When inlet H_2S flow rate increased to 100 mL min^{-1} (the lowest gas retention time of 2.3 min), the concentration of residual H_2S in the outlet gas reached 79.3 ppm in the first 30 min. The activity of bacteria decreased the residual H_2S concentration in the outlet gas to lower than 15 ppm in around 1.6 h and later on to a lower value of 5.3±0.5 ppm which translated to a H_2S removal efficiency

greater than 98.9%. The gaseous H_2S introduced into the bioreactor first dissolves in the liquid phase and then diffuses to the surface of biofilm and gets oxidized by the Coleville enrichment. When a short residence time was applied, the concentration of H_2S in the effluent gas peaked due to the saturation of dissolved sulfide in the liquid phase but eventually the activity of bacteria decreased the concentration of H_2S both in the liquid and in the gas effluent.

During the biooxidation of H_2S, if nitrate is only reduced to nitrite, the molar ratio of the consumed nitrate and the produced sulfur compounds, i.e. sulfate, thiosulfate, or sulfite would be in the range of 3:1 to 4:1 according to Equation 5.10 - 5.12 listed below.

$$H_2S + 4NO_3^- \longrightarrow SO_4^{2-} + 4NO_2^- + 2H^+ \quad (5.10)$$

$$2H_2S + 4NO_3^- \longrightarrow S_2O_3^{2-} + 4NO_2^- + H_2O + 2H^+ \quad (5.11)$$

$$H_2S + 3NO_3^- \longrightarrow SO_3^{2-} + 3NO_2^- + 2H^+ \quad (5.12)$$

If nitrate was reduced to nitrogen gas, the ratio of consumed nitrate and produced sulfate or thiosulfate would be 8:5 (1.6) and the ratio of consumed nitrate and produced sulfite would be 6:5 (1.2) according to Equation 5.13 - 5.15 described below.

$$5H_2S + 8NO_3^- \longrightarrow 5SO_4^{2-} + 4N_2 + 4H_2O + 2H^+ \quad (5.13)$$

$$10H_2S + 8NO_3^- \longrightarrow 5S_2O_3^{2-} + 4N_2 + 9H_2O + 2H^+ \quad (5.14)$$

$$5H_2S + 6NO_3^- \longrightarrow 5SO_3^{2-} + 3N_2 + 3H_2O + 4H^+ \quad (5.15)$$

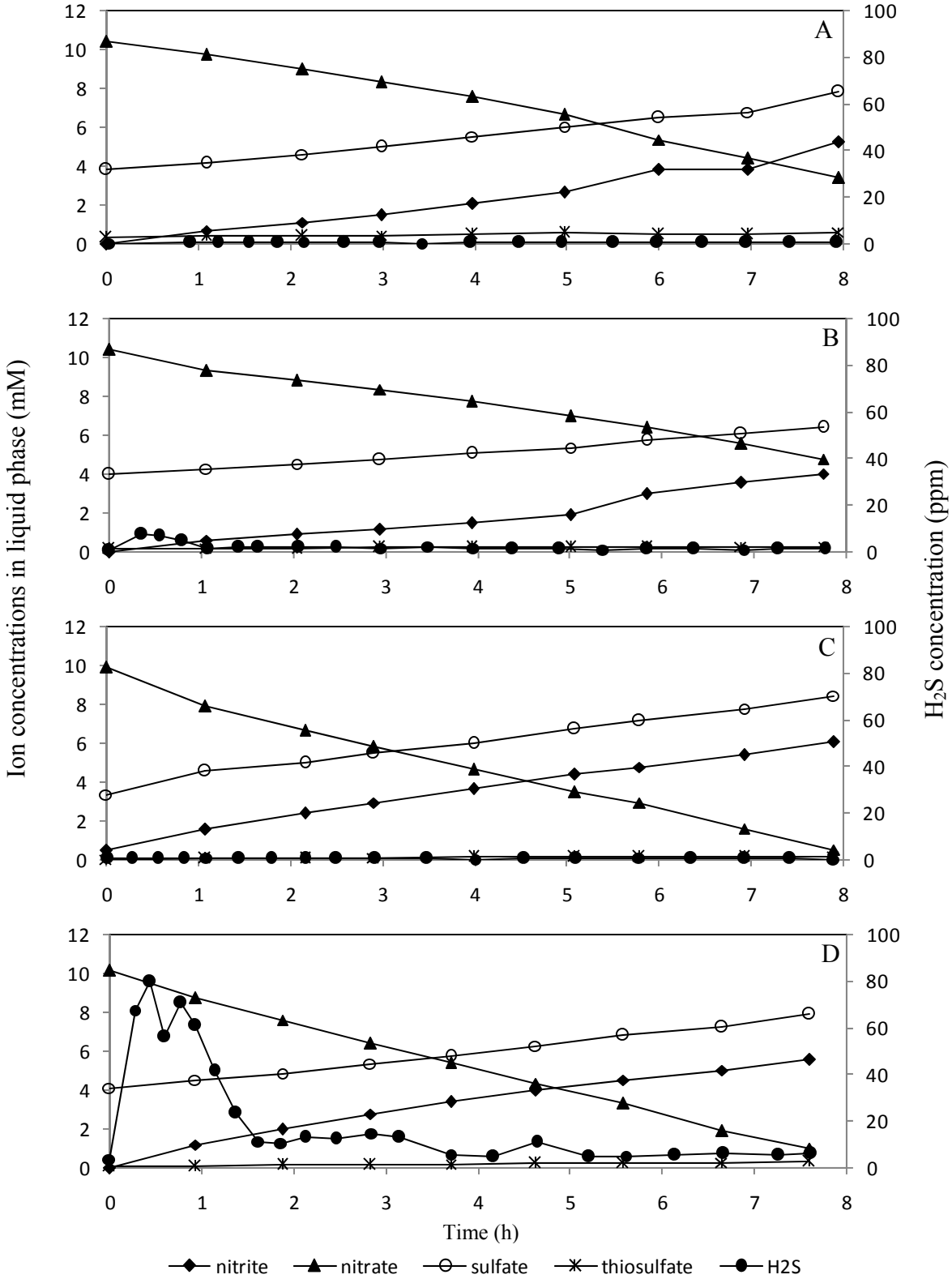

Figure 5.8 The concentration profiles of various ions in the liquid phase and H_2S concentration in the effluent gas as a function of time at gas retention times of (A) 9.2 (B) 4.6 (C) 3.1 (D) 2.3 min with 10 mM nitrate.

The actual ratio of reduced nitrate to produced sulfur compounds ranged from 1.6 to 2.3 and the end product of H_2S oxidation was mainly sulfate (thiosulfate: 0.14-0.27 mM). This confirms again that nitrite, as an intermediate product of nitrate reduction, was partially used as the electron acceptor during the biooxidation of H_2S.

With H_2S retention times ranging from 2.3-9.2 min, nitrate reduction rates were 0.89, 0.69, 1.13, 1.19 mM h^{-1}, respectively and sulfate production rates were 0.48, 0.31, 0.61, 0.50 mM h^{-1}, respectively (Table 5.9). Nitrate reduction rate increased with the decrease of retention time (the increase of H_2S flow rate) due to the supply and oxidation of more H_2S. With the increase of H_2S flow rate from 25 to 50 mL min^{-1}, gas bubbles of H_2S escaped fast from the liquid phase through the gaps amongst the sponge strips, thus a decrease of nitrate reduction rate was observed. After these two runs, more sponge strips were placed into the bioreactor to eliminate the potential bypass of H_2S.

Table 5.9 summarizes the important results obtained in this set of experiments. For all applied gas retention times, nitrite production rate was always lower than nitrate reduction rate implying that part of the produced nitrite was used as the electron acceptor in the desulfurization process. For the same level of nitrate (10 mM), nitrate reduction rates obtained with H_2S were about 2.4-4.1 times higher than those obtained in the batch experiments with elemental sulfur (Table 5.1) and also 1-1.75 times higher than those achieved in the batch experiments in the presence of acetate (Table 5.3). This in some way reconfirmed the order of preference of electron donors for Coleville enrichment as: sulfide > biological produced sulfur > acetate > elemental sulfur.

Table 5.9 Summary of the results obtained in the packed bed reactor for H_2S removal with nitrate

Gas Flow rate (mL min^{-1})	H_2S loading rate (g H_2S m^{-3} day^{-1})	H_2S removal rate (g H_2S m^{-3} day^{-1})	Nitrate / sulfur compounds	Nitrate reduction rate (mM h^{-1})	Nitrite production rate (mM h^{-1})	Sulfate production rate (mM h^{-1})
25	108.3	108.2	1.6	0.89	0.62	0.48
50	216.6	215.7	2.2	0.69	0.51	0.31
75	324.9	324.7	1.8	1.13	0.68	0.61
100	433.3	428.2	2.3	1.19	0.70	0.50

5.3.2 Denitritation with H_2S

Four batch runs with CSB medium containing 10 mM nitrite and feed gas with 508 ppm H_2S were carried out at gas retention times of 2.3, 3.1, 4.6, and 9.2 min. The concentration profiles of sulfide, nitrite, sulfate, and thiosulfate in the liquid phase and the H_2S concentration in the effluent gas are shown in Figure 5.9. Nitrite concentration decreased significantly and sulfate concentration increased propotionally during the course of the experiments. This illustrated the removal of H_2S was accomplished through the autotrophic denitrification process. The increases of thiosulfate concentration (0.09-0.65 mM) were slightly higher than those obtained in the experiments with nitrate. The removal efficiency of H_2S maintained greater than 99.4% for all the tested residence times. Although the performance of the bioreactor in desulfurization with nitrite seems slightly better than that with nitrate, it does not neccesarily mean that nitrite is a more suitable electron acceptor than nitrate. The reason for better performance with nitrite may lie in the fact that the experiments were carried out sequentially (first nitrate then nitrite) and as a result the extent of biomass hold-up (biofilm) in the bioreactor could have increased resulting in the enhancement of the bioreactor performance.

According to the Equation 5.17 and 5.18, the stoichiometric ratio of reduced nitrite to produced sulfur compounds (either sulfate or thiosulfate) is 8:3 (2.7). Thus, the total moles of the produced sulfate and thiosulfate were used in comparing the experimental data with the stoichiometric values. In all four tested retention times, the actual ratio of reduced nitrite to produced sulfur compounds were in the range of 2.2 to 2.5, slightly lower than the stoichiometric value. This discrepancies may be explained by the errors in the measurement of various ion concentrations.

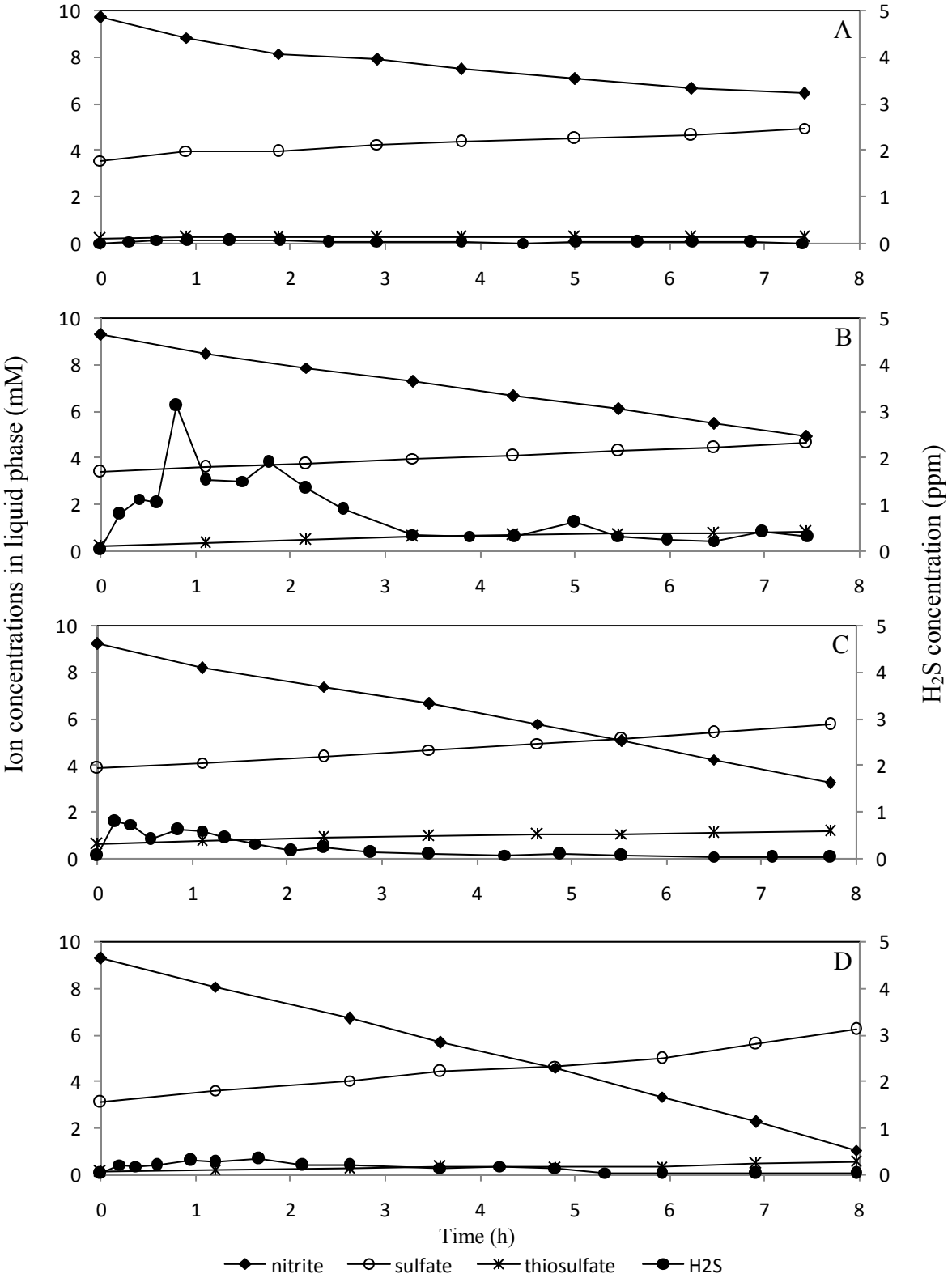

Figure 5.9 The concentration profiles of various ions in the liquid phase and H_2S concentration in the effluent gas as a function of time at gas retention times of (A) 9.2 (B) 4.6 (C) 3.1 (D) 2.3 min with 10 mM nitrite.

$$3H_2S + 8NO_2^- \longrightarrow 3SO_4^{2-} + 4N_2 + 2H_2O + 2OH^- \quad (5.16)$$

$$6H_2S + 8NO_2^- \longrightarrow 3S_2O_3^{2-} + 4N_2 + 5H_2O + 2OH^- \quad (5.17)$$

At H_2S retention times of 9.2, 4.6, 3.1 and 2.3 min, nitrite reduction rates were 0.42, 0.57, 0.75, 1.02 mM h^{-1}, respectively and sulfate production rates were 0.17, 0.16, 0.24, 0.36 mM h^{-1}, respectively (Table 5.10). Nitrite reduction rate and sulfate production rate both increased with the increases of H_2S loading rate as shown in Figure 5.10. Nitrite reduction rates obtained during the oxidation of H_2S were about 2.3-5.7 times higher than those obtained in the autotrophic denitritation batch experiments with sulfur.

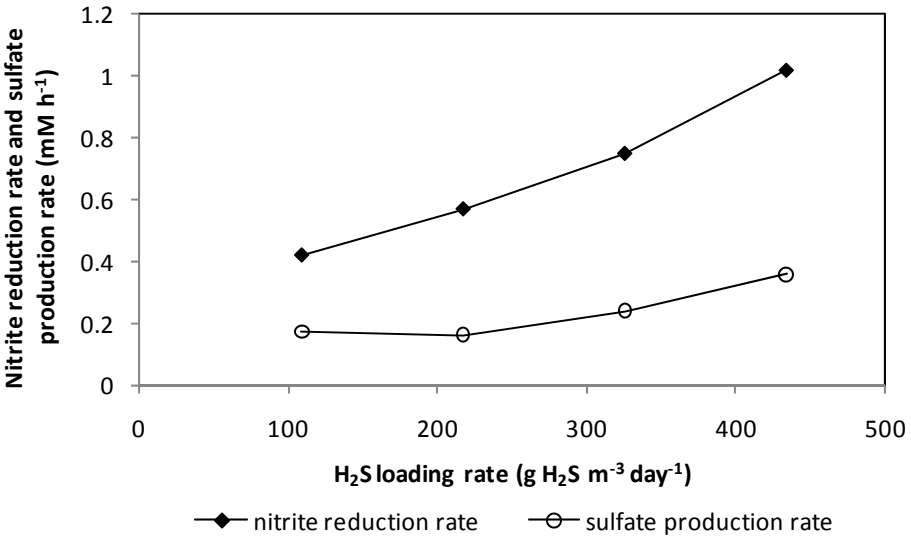

Figure 5.10 Nitrite reduction and sulfate production rates as a function of H_2S loading rate.

Table 5.10 Summary of the results obtained in the packed bed reactor for H_2S removal with nitrite

Flow rate (mL min^{-1})	**H_2S loading rate (g H_2S m^{-3} day^{-1})**	**H_2S removal rate (g H_2S m^{-3} day^{-1})**	**nitrite /sulfur compounds**	**Nitrite reduction rate (mM h^{-1})**	**Sulfate production rate (mM h^{-1})**
25	108.3	108.3	2.2	0.42	0.17
50	216.6	216.3	2.3	0.57	0.16
75	324.9	324.8	2.5	0.75	0.24
100	433.3	433.1	2.3	1.02	0.36

Chapter 6 CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

6.1 Conclusions

Results obtained in the present study combined with those from the previous work by others in our laboratory (An *et al*., 2010) revealed the preference order of electron donors used by Coleville enrichment during the denitrification is: sulfide > biological produced sulfur > acetate > elemental sulfur.

Sulfate productions were closely matched with the theoretical values expected from the stoichiometry on the assumption of nitrite and nitrogen gas as the end products of nitrate reduction. Sodium bicarbonate functioned as an effective buffering agent to neutralize the protons produced during the sulfur-driven autotrophic denitrification as well as the inorganic carbon source for Coleville enrichment. This eliminated the need for addition of lime particles as a neutralizing agent thus increased the volume of the reactor available for the denitrification process. Strong inhibitory effect on bacteria activities imposed by nitrite was observed in both batch and continuous experiments

The highest nitrate and nitrite reduction rates obtained in the batch autotrophic denitrification were 0.32±0.01 mM h^{-1} with 20 mM nitrate and 0.18±0.03 mM h^{-1} with 20 mM nitrite, respectively. In the batch experiments with acetate, the maximum nitrate reduction rate (1.8±0.1 mM h^{-1}) and nitrite reduction rate (0.29±0.01 mM h^{-1}) were much higher.

In the continuous biofilm reactor, nitrate removal rates increased linearly with the increase of nitrate loading rate through the increase of either feed flow rate or feed concentration. Similar trends were observed for the nitrite removal experiment. The highest nitrate removal rate (17.3 mM h^{-1}) was obtained at a nitrate loading rate of 24.2 mM h^{-1} (corresponding residence time: 0.4 h) with a nitrate removal efficiency of 71.3% and a total nitrogen removal efficiency of 9.5%. Complete removal of 10.2±0.3 mM nitrate was achieved for nitrate loading rates up to 3.8 mM h^{-1} without the accumulation of nitrite (corresponding residence time: 2.7 h). The highest nitrite removal rate (13.2 mM h^{-1}) was achieved at a nitrite loading rate of 18.0 mM h^{-1} (corresponding residence time: 0.6 h) with a nitrite removal percentage of 73.6%. Complete reduction of 10.7±0.6 mM nitrite was accomplished at nitrite loading rates up to 5.0 mM h^{-1} (corresponding residence time: 2.1 h).

In the semi-continuous experiments of denitrification and denitritation with H_2S, the removal efficiency of H_2S remained greater than 98.6% and 99.4% with nitrate and nitrite as the electron acceptor, respectively. Nitrate and nitrite reduction rates increased with the increase of H_2S loading rate through the variation of feed gas flow rate. The observed reduction rates were much higher than those obtained in the batch denitrification experiment with elemental sulfur and acetate.

6.2 Recommendations for Future Work

Since the Coleville enrichment has the ability to perform denitrification under both heterotrophic and autotrophic conditions, the effects of small amount of organics addition on the sulfur-based autotrophic denitrification process will be worthy of investigation. The combination of

heterotrophic and autotrophic processes, referred to as mixotrophic denitrification can decrease sulfate production and alkalinity consumption of the sulfur-based autotrophic denitrification and as shown in the present study may eliminate or relieve the inhibition from the produced nitrite.

In the case of simultaneous denitrification and biodesulfurization of H_2S-containing gases, it is vital to study the effect of H_2S concentration on the performance of the process, as in many practical cases the concentrations of H_2S may be higher than that tested in this work. Furthermore, the performance of the system under continuous mode of operation for both liquid and gas feeds needs to be investigated. It is equally important to assess the effects of operating conditions, specially loading rates of H_2S and nitrate or nitrite on the composition of end-products and to determine the optimum conditions which lead to the formation of sulfur as the end product to ensure the permanent removal of sulfur compounds.

Finally, the potential of the Coleville enrichment in the removal of other sulfur compound such as methyl sulfides should be investigated.

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APPENDIX: DATA CALCULATION

A.1 Denitrification and Denitritation with Sulfur in the Batch Experiment

(1) Actual sulfate production (ASP) was calculated by the following equation.

$$ASP = C_{SO4\text{-}e} - C_{SO4\text{-}b} \quad (A.1)$$

Where,

$C_{SO4\text{-}e}$: average sulfate concentration at the end of the experiment (mM).

$C_{SO4\text{-}b}$: average sulfate concentration at the beginning of the experiment (mM).

(2) Theoretical sulfate production (TSP) was calculated by the following equation.

$$TSP = (C_{NO3\text{-}b}\text{-}C_{NO3\text{-}e})/s_1 + (C_{NO2\text{-}h}\text{-}C_{NO2\text{-}e})/s_2 \quad (A.2)$$

Where,

$C_{NO3\text{-}b}$: average nitrate concentration at the beginning of the experiment (mM).

$C_{NO3\text{-}e}$: average nitrate concentration at the end of the experiment (mM).

s_1: stoichiometric ratio of nitrate to sulfur during the reduction of nitrate to nitrite, the value is 3 according to equation 5.1.

$C_{NO2\text{-}h}$: highest nitrite concentration after nitrate conversion to nitrite (mM).

$C_{NO2\text{-}e}$: average nitrite concentration at the end of the experiment (mM).

s_2: stoichiometric ratio of nitrite to sulfur during the reduction of nitrite to nitrogen gas, the value is 2 according to equation 5.2.

(3) Discrepancy between ASP and TSP was calculated as that the difference between ASP and TSP was divided by either ASP or TSP whichever had the larger value.

(4) Reduction rate of nitrate and nitrite was calculated as the slope of linear part in the concentration profiles.

A.2 Denitrification with Acetate in the Batch Experiment

(1) Actual acetate consumption (AAC) was calculated by the following equation.

$$AAC = C_{Ace\text{-}b} - C_{Ace\text{-}e} \quad (A.3)$$

Where,

$C_{Ace\text{-}b}$: acetate concentration at the beginning of the experiment (mM).

$C_{Ace\text{-}e}$: average acetate concentration at the end of the experiment (mM).

(2) Theoretical acetate consumption (TAC) was calculated by the following equation.

$$TAC = (C_{NO3\text{-}b} - C_{NO3\text{-}e})/s_3 + (C_{NO2\text{-}h} - C_{NO2\text{-}e})/s_4 \quad (A.4)$$

Where,

$C_{NO3\text{-}b}$: nitrate concentration at the beginning of the experiment (mM).

$C_{NO3\text{-}e}$: average nitrate concentration at the end of the experiment (mM).

s_3: stoichiometric ratio of nitrate to acetate during the reduction of nitrate to nitrogen gas, the value is 1.6 according to equation 5.4.

$C_{NO2\text{-}h}$: highest nitrite concentration after nitrate conversion to nitrite (mM).

$C_{NO2\text{-}e}$: average nitrite concentration at the end of the experiment (mM).

s_4: stoichiometric ratio of nitrite to sulfur during the reduction of nitrite to nitrogen gas, the value is 2.7 according to equation 5.5.

(3) Discrepancy between AAC and TAC was calculated as that the difference between AAC and TAC was divided by either AAC or TAC whichever had the larger value.

(4) Reduction rate of nitrate and nitrite were calculated the same way as described in A.1.

A.3 Denitrification and Denitritation with Sulfur in the Continuous Biofilm Reactor

(1) The average value of working volume (V_w) of the biofilm reactor was determined to be 94 mL. Prior to beginning the experiments, the void volume was measured as 95.5 mL and at the end of the experiment the void volume was measured as 92.5 mL. To measure the void volume, the reactor was filled with water and then drained completely. The volume of water was measured as the void volume of the biofilm reactor.

(2) Hydraulic retention time (HRT) was calculated by the following equation.

$$HRT = V_w / F \quad (A.5)$$

Where,

V_w: working volume of the reactor (mL).

F: feed flow rate (mL h^{-1}).

(3) Loading rate (LR) was calculated for both nitrate and nitrite. The calculation equation was as following.

$$LR = C_{in}/HRT \quad (A.6)$$

Where,

LR: loading rate (mM h^{-1}).

C_{in}: inlet concentration of nitrate or nitrite (mM).

HRT: Hydraulic retention time (h).

(4) Removal rate (RR) was calculated for both nitrate and nitrite. The calculation equation was as following.

$$RR = (C_{in} - C_{out}) /HRT \quad (A.7)$$

Where,

RR: removal rate (mM h^{-1});

C_{in}: inlet concentration of nitrate or nitrite (mM).

C_{out}: outlet concentration of nitrate or nitrite (mM).

HRT: Hydraulic retention time (h).

(5) Removal efficiency (RE) was calculated for both nitrate and nitrite. The calculation equation was as follows.

$$RE = RR/LR \times 100\% \quad (A.8)$$

Where,

RR: removal rate (mM h^{-1});

LR: loading rate (mM h^{-1}).

(6) Actual sulfate production (ASP') at different loading rate was calculated by the following equation.

$$ASP' = C_{SO4\text{-}t} - C_{SO4\text{-}m} \quad (A.9)$$

Where,

$C_{SO4\text{-}t}$: average sulfate concentration in the top port during the steady state (mM).

$C_{SO4\text{-}m}$: average sulfate concentration in the CSB medium during the steady state (mM).

(7) Theoretical sulfate production (TSP') at different loading rate was calculated by the following equation.

$$TSP' = (C_{NO3\text{-}m}-C_{NO3\text{-}t})/s_1 + [(C_{NO3\text{-}m}-C_{NO3\text{-}t}) + C_{NO2\text{-}m}-C_{NO2\text{-}t}]/s_2 \quad (A.10)$$

Where,

$C_{NO3\text{-}m}$: average nitrate concentration in the CSB medium during the steady state (mM).

$C_{NO3\text{-}t}$: average nitrate concentration in the top port during the steady state (mM).

s_1: stoichiometric ratio of nitrate to sulfur during the reduction of nitrate to nitrite, the value is 3 according to equation 5.1.

$C_{NO2\text{-}m}$: average nitrite concentration in the CSB medium during the steady state (mM).

$C_{NO2\text{-}t}$: average nitrite concentration in the top port during the steady state (mM).

s_2: stoichiometric ratio of nitrite to sulfur during the reduction of nitrite to nitrogen gas, the value is 2 according to equation 5.2.

(8) The ratio of reduced nitrate to produced sulfate was calculated by the following equation.

$$R = (C_{NO3\text{-}m}-C_{NO3\text{-}t})/ (C_{SO4\text{-}t}-C_{SO4\text{-}m}) \quad (A.11)$$

Where,

R: the ratio of reduced nitrate to produced sulfate.

$C_{NO3\text{-}m}$: average nitrate concentration in the CSB medium during the steady state (mM).

$C_{NO3\text{-}t}$: average nitrate concentration in the top port during the steady state (mM).

$C_{SO4\text{-}t}$: average sulfate concentration in the top port during the steady state (mM).

$C_{SO4\text{-}m}$: average sulfate concentration in the CSB medium during the steady state (mM).

A.4 Denitrification and Denitritation with H_2S in the Packed Bed Bioreactor

(1) Gas retention time (GRT) was calculated by the following equation.

$$GRT = V_L/Q_g \quad \text{(A.12)}$$

Where,

GRT: Gas retention time (min).

V_L: Working volume; liquid volume of the bioreactor (mL).

Q_g: Gas flow rate (mL min^{-1}).

(2) Volumetric loading rate (VLR) of H_2S was calculated by the following equation.

$$VLR = Q_g C_{g\text{-}in}/V_L \quad \text{(A.13)}$$

Where,

VLR: Volumetric loading rate (g H_2S m^{-3} day^{-1}).

Q_g: Gas flow rate (mL min^{-1}).

$C_{g\text{-}in}$: Concentration of H_2S in the inlet gas (mg H_2S m^{-3}).

V_L: Working volume; Liquid volume of the bioreactor (mL).

(3) Volumetric removal rate (VRR) of H_2S was calculated by the following equation.

$$VRR = Q_g (C_{g\text{-}in}-C_{g\text{-}out})/V_L \quad \text{(A.14)}$$

Where,

VRR: Volumetric removal rate (g H_2S m^{-3} day^{-1}).

Q_g: Gas flow rate (mL min^{-1}).

$C_{g\text{-}in}$: Concentration of H_2S in the inlet gas (mg H_2S m^{-3}).

$C_{g\text{-}out}$: Concentration of H_2S in the outlet gas (mg H_2S m^{-3}).

V_L: Working volume; Liquid volume of the bioreactor (mL).

(4) Reduction rate of nitrate and nitrite were calculated the same way as described in A.1.

(5) Sulfate production rate (SPR) was calculated as the slope of linear part in sulfate concentration profile.

(6) The ratio of reduced nitrate or nitrite to produced sulfur compounds was calculated by the following equation.

$$R' = (C_b - C_e) / [(C_{SO4\text{-}e} - C_{SO4\text{-}b}) + (C_{S2O3\text{-}e} - C_{S2O3\text{-}b})] \quad (A.15)$$

Where,

C_b: the concentration of nitrate or nitrite at the beginning of the experiment (mM).

C_e: the concentration of nitrate or nitrite at the end of the experiment (mM).

$C_{SO4\text{-}e}$: sulfate concentration at the end of the experiment (mM).

$C_{SO4\text{-}b}$: sulfate concentration at the beginning of the experiment (mM).

$C_{S2O3\text{-}e}$: thiosulfate concentration at the end of the experiment (mM).

$C_{S2O3\text{-}b}$: thiosulfate concentration at the beginning of the experiment (mM).