A COMPARATIVE STUDY OF AUTOTROPHIC AND HETEROTROPHIC DENITRIFICATION USING SULPHIDE AND ACETATE

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ABSTRACT

Sulphide containing streams must be treated before releases to environment due to the toxicity, corrosivity and unpleasant odour of sulphide. Anaerobic chemolithotrophic desulphurization under denitrifying conditions is the preferred process when compared with others like physicochemical processes, photoautotrophic and aerobic chemolithotrophic desulphurizations as the catalysts, high pressure, high temperature, light energy and oxygen are not needed. Another main advantage of this process is that the denitrification can be achieved with desulphurization simultaneously. In this work, the anaerobic chemolithotrophic desulphurization under denitrifying conditions (autotrophic denitrification) and heterotrophic denitrification processes were studied. Desulphurization under denitrifying conditions was studied in continuous stirred tank bioreactors (CSTB), while batch, continuous stirred tank and biofilm reactors were used to investigate the heterotrophic denitrification. The kinetics of desulphurization, autotrophic and heterotrophic denitrifications obtained in different systems and under various conditions were compared.

Using three different feed sulphide concentrations in the range 10-20 mM, a linear relationship between sulphide loading rates and sulphide removal rates was observed in continuous stirred tank reactors, regardless of initial sulphide concentration. The highest sulphide removal rate of 1.79 mM h^{-1} was obtained in CSTB fed with 15 mM sulphide. In these systems cell washout occurred at lower dilution rates as sulphide concentration in the feed was increased from 10 to 20 mM. The ratio of sulphide to nitrate loading rates influenced the composition of the sulphur oxidation end products where higher ratios favored the formation of elemental sulphur and lower ratios promoted the formation of sulphate.

In the batch system initial concentration of nitrate (5 to 50 mM) did not have a notable effect on denitrification process. Nitrate was converted to nitrite first and the produced nitrite was then converted to other gaseous end products such as nitrogen. Increases of temperature

in the range of 15 to 35ºC increased the bacterial growth rate significantly with the value of apparent activation energy for specific growth rate being 60.6 kJ mol^{-1}. Using the experimental data generated in two continuous bioreactors operated with feeds containing 10 and 30 mM nitrate biokinetic coefficients for heterotrophic denitrification were determined. The values of μ_m, K_s, m_s, $Y^M_{X/S}$, k_d for initial nitrate concentrations of 10 and 30 mM were 0.087 and 0.082 h^{-1}, 2.01 and 5.27 mM (NO_3^-), 1.441 and 1.096 mM (NO_3^-) (g biomass) $^{-1}$ h^{-1}, 0.011 and 0.013 g (biomass) (mM $NO_3^-)^{-1}$, and 0.016 and 0.014 h^{-1} respectively. In the biofilm system the linear relationship between nitrate loading rate and nitrate removal rate was observed again for the whole range of tested nitrate loading rate range (up to 183 mM h^{-1}), regardless of the approach used to increase the loading rate (increases in feed flow rate or feed nitrate concentration). The highest nitrate removal rate was 183 mM h^{-1} which was around 194 times higher than that achieved in the continuous stirred tank bioreactor with free cells.

A comparison of the autotrophic and heterotrophic denitrification processes studied in the CSTB system indicated that in case of autotrophic denitrification wash-out occurred suddenly and at a much lower loading rate of 0.75 to 0.96 mM (NO_3^-) h^{-1} for initial sulphide concentrations 10 to 20 mM, while in case of heterotrophic denitrification increase of nitrate loading rate did not have such a drastic effect and removal rate of nitrate decreased slowly with the increases of nitrate loading rate. A comparison of the kinetic data obtained in the biofilm reactor in the present work and those generated for autotrophic denitrification in an earlier work conducted at University of Saskatchewan (Tang, 2008) showed that the dependency of nitrate removal rate on its loading rate were linear in either case and somewhat similar. However, the maximum nitrate removal rate obtained in the heterotrophic system (183 mM h^{-1}) was much higher than that obtained in the autotrophic system with sulphide.

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GLOSSARY AND NOMENCLATURE

BOD	Biological oxygen demand (mg L^{-1})
COD	Chemical oxygen demand (mg L^{-1})
CSB	Coleville synthetic brine
CSTB	Continuous stirred tank bioreactor
CSTR	Continuous stirred tank reactor
IC	Ion chromatography
NCC	Net cathodic compartment
OD	Optical density
μ_m	Maximum specific growth rate (h^{-1})
k_d	Endogenous rate constant (h^{-1})
Ks	Saturation constant (mM)
m_s	Maintenance coefficient (mM g^{-1} h^{-1})
Y	Biomass yield (g mM^{-1})
$Y^M_{X/S}$	Biomass maximum yield coefficient (g mM^{-1})
VRR	Volumetric removal rate (mM h^{-1})
VLR	Volumetric removal rate (mM h^{-1})
D	Dilution rate (h^{-1})
DO	Dissolved oxygen concentration (mg L^{-1})
HRT	Hydraulic retention time (h)
M	Molar concentration: mole per liter
mM	Molar concentration: milimole per liter

CHAPTER 1
INTRODUCTION

Sulphide either in the dissolved form or gaseous H_2S is commonly present in oil, gas and in some wastewaters. Due to toxicity, corrosivity, unpleasant odor and generation of sulphur dioxide upon combustion, sulphide must be removed from oil, gas and contaminated waters prior to use or release. There are a number of options for the treatment sulphide containing streams including physicochemical and biological methods. Three physicochemical methods of Amine, Clause, and LO-CAT processes are well established on the industrial scale (Kohl and Nielsen 1997; Monnery *et al.*, 2000). Biological methods are more promising when compared with the physicochemical processes as high pressures; heat and chemical catalysts are not needed. Another advantage of biological treatment is their suitability for dealing with low concentrations of sulphide and small volumes of sulphide containing streams when compared with physicochemical methods (Tang, *et al.*, 2009).

Several bacterial species with the ability to oxidize sulphide, including photoautotroph, aerobic chemolithotroph and anaerobic chemolithotroph, have been investigated in recent years (Tang, *et al.*, 2009). The prospects for the utilization of anaerobic chemolithotrophic bacteria to treat sulphide containing streams are most promising as light energy (required for phototrophs), oxygen (required for aerobes) are not needed. Another advantage of this system is that desulphurization and denitrification processes can occur simultaneously, with the latter being an important step in treatment of many wastewater.

The problems associated by wastewater containing high nitrate concentration such as eutrophication and toxicity have been recognized for many years. The nitrate removal methods include heterotrophic and autotrophic denitrification. In heterotrophic denitrification, bacteria use organic compounds (like glucose, ethanol or acetate) as electron donors to reduce nitrate to nitrite and then to nitrogen, while in autotrophic denitrification bacteria use sulphide or sulphur as electron donors (anaerobic chemolithotrophic desulphurization). As organic compounds are not required and sulphide and nitrate are removed simultaneously, autotrophic denitrification is the preferred approach in treating sulphide and nitrate containing streams.

The objectives of this work were to evaluate the kinetics of simultaneous desulphurization and denitrification and to compare the processes of autotrophic and heterotrophic denitrifications, using batch, and continuous bioreactors with freely suspended cells and immobilized bacteria (biofilm system). The thesis presented here consists of six chapters

including review of the literature, identifying the knowledge gap, specific objectives of the research, materials and methods, results and conclusions, and recommendations for future work.

CHAPTER 2
LITERATURE REVIEW

2.1 Generation of H_2S and Associated Problems

In addition to natural gas and oil which usually contain sulphur compounds, especially sulphide, various processes and industrial activities contribute to the generation of sulphide containing streams. The enhanced recovery of oil through water injection is a process during which waters contaminated with sulphide are produced in large quantities (Nemati *et al*., 2001a). Sulphate content of injected water can be converted to sulphide by the activity of sulphate reducing bacteria residing in the reservoir (Antonio *et al*., 2000; Nemati *et al*., 2001a; Nemati *et al*., 2001 b). Furthermore, dissolution of metal sulphide or sulphide minerals present in the rock formation could contribute to the sulphide content of oil, gas and produced water (Ollivier and Magot 2005). Another process in which sulphide containing streams are generated is the anaerobic digestion of agricultural wastes and sludge for production of biogas. The produced biogas consists of methane, CO_2 and H_2S (Hansen *et al*., 1998). H_2S contaminated streams could also be generated in wastewater treatment processes, especially during the anaerobic treatment of sulphate containing wastewaters by sulphate reducing bacteria's activity (Van Der Zee 2007, Tang *et al*., 2009). Finally, certain industrial activities such as those in tanneries, pulp and paper production processes result in the production of H_2S contaminated waste streams (Janssen *et al*., 1999).

H_2S is a colorless, poisonous, flammable and corrosive gas which can cause eye irritation, sore throat, cough, and shortness of breath at concentrations around 300 ppm. At elevated levels, H_2S may paralyze the lungs and cause death (ATSDR, 1999). In addition to these health concerns, combustion of fuels containing sulphide results in formation of SO_2 which is a primary air pollutant and the main cause of acidic depositions. The corrosive nature of sulphide also creates severe problems for industry.

2.2 Treatment of Sulphide Containing Streams

The methods used for the treatment of sulphide containing streams can be classified into physicochemical and biological processes. To compare with physicochemical methods which require high pressure, heat and expensive chemical catalysts, biological methods for

sulphide removal are carried out at ambient pressure and temperature without the need for expensive chemicals and catalysts which makes them more promising.

2.2.1 Physicochemical Processes

The classical physicochemical processes for sulphide removal include Amine, Claus and LO-CAT processes which have been investigated well and are used commonly in industrial scale. The following sections describe briefly the principle reactions or processes involved in each physicochemical method.

1) Amine process

In the Amine process, an amine absorbent (i.e. monoethanolamine, diethanolamine, methyldiethanolamine) is used in an absorption column to separate hydrogen sulphide from the contaminated gaseous stream. The rich amine is sent into a stripper column in which sulphide is separated from the amine. The regenerated amine is then reused in absorption column (Speight, 1993; Kohl and Nielsen, 1997). The regeneration of amine in the stripper column is an energy consuming process that makes this method cost sensitive.

2) Claus process

In the Claus process, oxygen is used to oxidize sulphide to elemental sulphur. Two main reactions occur in the Claus process (reactions 1 and 2) are described below (Kohl and Nielsen 1997; Monnery, 2000; Larraz 2002).

$$H_2S + \frac{3}{2}O_2 \rightarrow SO_2 + H_2O \qquad (2\text{-}1)$$

$$2H_2S + SO_2 \rightarrow 3S + 2H_2O \qquad (2\text{-}2)$$

In reaction (1), part of the hydrogen sulphide (usually one third) is oxidized to sulphur dioxide by oxygen. In the second reaction, the remaining sulphide is oxidized by the produced sulphur dioxide in the first reaction and elemental sulphur is formed. The produced elemental sulphur is then removed as a byproduct by precipitation methods. The amount of supplied oxygen is one of the most important factors in proper operation of the Claus process. The supplied oxygen should be adjusted that only 1/3 part of hydrogen sulphide is oxidized to sulphur dioxide. The produced sulphur dioxide then can oxidize the remaining hydrogen sulphide (2/3 part) to elemental sulphur. In a Clause process, high sulphide removal rate

around 95% can be achieved (Kohland Nielsen 1997). However, one should note that the use of a catalyst such as titanium catalyst is essential in this method (Larraz 2002).

3) LO-CAT process

In the LO-CAT process, ferric iron is used as an oxidizing agent for the conversion of sulphide to elemental sulphur. The fundamental reactions in the LO-CAT process are as follows (Kohl and Nielsen 1997).

$$2Fe^{3+} + HS^- \rightarrow 2Fe^{2+} + S + H^+ \quad (2\text{-}3)$$

$$2Fe^{2+} + \frac{1}{2}O_2 + H_2O \rightarrow 2Fe^{3+} + 2OH^- \quad (2\text{-}4)$$

Ferric iron and hydrogen sulphide are mixed in the first reactor. Hydrogen sulphide is oxidized to elemental sulphur and ferric iron is reduced to ferrous iron. The elemental sulphur is then removed from the mixture as a byproduct. In the second reactor, oxygen is supplied into the solution containing ferrous iron. Ferrous iron is oxidized back to ferric iron and is reused back in the first reactor for oxidation of sulphide to elemental sulphur (Kohl and Nielsen 1997).

2.2.2 Biological Processes

Biological processes in general rely on the oxidation of sulphide either directly by a microorganism or indirectly through the use of ferric sulphate as an oxidizing agent and regeneration of the oxidizing agent through a biological process. Biological processes are carried out at ambient temperature and pressure and do not need expensive chemical catalysts when compared with physicochemical processes. In addition, biological approach can be used to treat small volumes of waste streams and those containing low level of sulphide. This is in contrast with physicochemical methods which are feasible mainly for the treatment of large volumes of contaminated streams, containing high concentrations of sulphide (Tang, *et al.*, 2009).

2.2.2.1 Indirect biooxidation of sulphide

The indirect biological method relies on ferric iron as an oxidizing agent for conversion of sulphide to elemental sulphur. During this process ferric iron is reduced to ferrous iron. The bacterial species which have the ability to oxidize ferrous iron to ferric iron are then used to

regenerate the oxidizing agent. The reactions involved are as follows (Pagella and De Faveri, 2000).

$$2Fe^{3+} + HS^- \rightarrow 2Fe^{2+} + S + H^+ \quad (2\text{-}3)$$

$$Fe^{2+} \xrightarrow{\text{bacteria}} Fe^{3+} + e^- \quad (2\text{-}5)$$

Acidithiobacillous ferrooxidans, Leptospirillum ferrooxidans and *Sulpholobus acidocaldarium* have the ability to biooxidize ferrous ion to ferric ion (Pagella and Faveri, 2000; Madigan *et al*., 2003).

2.2.2.2 Direct biooxidation of sulphide

Some species of bacteria have the ability to oxidize sulphide to elemental sulphur or sulphate directly under proper conditions. These bacteria include photoautotrophs which use light as energy, aerobic chemolithotrophs which use oxygen to oxidize sulphide, and anaerobic chemolithotrophs which use nitrate or nitrite to oxidize sulphide.

1) Photoautotrophic oxidation of sulphide

Some photoautotrophic bacteria have been shown to oxidize hydrogen sulphide to elemental sulphur using light as energy and carbon dioxide as electron acceptors and carbon sources. The reaction which is referred to as van Niel's reaction is shown below (Madigan *et al*., 2003; Janssen *et al*., 1999).

$$2H_2S + CO_2 \xrightarrow{\text{light}} 2S + H_2O + \text{Carbohydra te} \quad (2\text{-}6)$$

The photoautotrophic bacteria with sulphide-oxidizing ability include purple sulphur bacteria and green sulphur bacteria. Most purple sulphur bacteria store the produced elemental sulphur inside their cells. Further oxidation of elemental sulphur results in the release of sulphate from the cells (Madigan *et al*., 2003). *Chromatium, Thioalkalicoccus, Thiorhodococcus, Thiocapsa, Thiocystis, Thuicoccus, Thiospirillum, Thiodictyon* and *Thiopedia* belong to purple sulphur bacteria. Unlike most purple sulphur bacteria, with green sulphur bacteria the produced sulphur resides outside cells (Madigan *et al*., 2003). This character is very valuable to sulphide removal system as sulphur can be easily removed from the treated stream. *Chlorobium, Prosthecochloris, Pelodictyon, Ancalochloris* and *Chloroherpeton* are typical examples of green sulphur bacteria.

2) Aerobic chemolithotrophic oxidation of sulphide

Some chemolithotrophic bacteria can oxidize sulphide to elemental sulphur or sulphate under aerobic conditions. Oxygen is used as an electron acceptor and itself is reduced to H_2O. Sulphide is used as an electron donor and oxidized to elemental sulphur or sulphate. The biooxidation of sulphide under aerobic conditions is carried out according to the reactions 7, 8 and 9 (Madigan *et al*., 2003). *Acidithiobacillus, Bacillus, Beggiatoa, Pseudomonas, Thiobacillus* and *Xanthobacter* are typical examples of aerobic chemolithotrophic sulphide oxidizing bacteria (Friedrich *et al*., 2001).

$$H_2S + \frac{1}{2}O_2 \rightarrow S + H_2O \quad (2\text{-}7)$$

$$S + \frac{3}{2}O_2 + H_2O \rightarrow SO_4^{2-} + 2H^+ \quad (2\text{-}8)$$

$$H_2S + 2O_2 \rightarrow SO_4^{2-} + 2H^+ \quad (2\text{-}9)$$

3) Anaerobic chemolithotrophic oxidation of sulphide

Certain chemolithotrophs can use sulphide as electron donor and energy source and nitrate or nitrite as electron acceptor under anaerobic condition. Desulphurizaton and denitrification can proceed simultaneously in the same system and the main reactions involved are summarized below (Davidova *et al*., 2001; Cardoso *et al*., 2006; Gadekar *et al*., 2006; Tang *et al*., 2009).

$$5S^{2-} + 2NO_3^- + 12H^+ \rightarrow 5S + N_2 + 6H_2O \quad (2\text{-}10)$$

$$3S^{2-} + 2NO_2^- + 8H^+ \rightarrow 3S + N_2 + 4H_2O \quad (2\text{-}11)$$

$$S^{2-} + NO_3^- + 2H^+ \rightarrow S + NO_2^- + H_2O \quad (2\text{-}12)$$

$$S^{2-} + 4NO_3^- \rightarrow SO_4^{2-} + 4NO_2^- \quad (2\text{-}13)$$

$$S^{2-} + 1.6NO_3^- + 1.6H^+ \rightarrow SO_4^{2-} + 0.8N_2 + 0.8H_2O \quad (2\text{-}14)$$

The reactions for oxidation of elemental sulphur to sulphide are as follows:

$$S + 3NO_3^- + H_2O \rightarrow SO_4^{2-} + 3NO_2^- + 2H^+ \quad (2\text{-}15)$$

$$5S + 6NO_3^- + 8H_2O \rightarrow 5H_2SO_4 + 6OH^- + 3N_2 \quad (2\text{-}16)$$

$$S + 2NO_2^- \rightarrow SO_4^{2-} + N_2 \quad (2\text{-}17)$$

Thiobacillus denitrificans, Thiomicrospira denitrificans, Thiomicrospira sp. CVO and a *Beggiatoa* enrichment culture have shown the ability to oxidize sulphide to elemental sulphur

or sulphate under anaerobic (denitrifying) conditions (Nemati *et al*., 2001; Viaopoulou *et al*., 2005; Kamp, 2006; Tang *et al*., 2009).

The anaerobic chemolithotrophic sulphide oxidation is most promising method in all biodesulphurization processes as sulphide and nitrate removals can proceed in the same system simultaneously. Chemical reagents, light energy and oxygen are not required when compared with indirect biological, photoautotrophic and aerobic chemolithotrophic sulphide removal methods, respectively. Finally sulphide oxidation under denitrifying conditions is the main process for the in situ removal of H_2S from oil reservoirs experiencing souring (Nemati *et al*., 2001) that makes this bio-process more promising than the other bio-desulphurization processes.

2.3 Generation of Nitrate, Associated Problems and Denitrification Methods

In order to evaluate the simultaneous desulphurization and denitrification process properly, a study comparing this process with the heterotrophic denitrification is valuable and is one of the objectives of the present work. Heterotrophic denitrification is an important process in the typical treatment of many nitrogen containing wastewaters. The following sections provide an overview of heterotrophic denitrification and recent research work in this area.

2.3.1 Generation of Nitrate and Associated Problems

Nitrate containing streams are generated mainly during oxidation of ammonia during the nitrification process in municipal wastewater treatment plants. As we know, municipal wastewater contains organic compounds including those containing nitrogen. Removal of organics during the activated sludge process (oxidation of organics by aerobic bacteria) results in formation of ammonia and ammonia was also generated during the wastewater transportation to wastewater plants. In most wastewater plants, ammonia is removed from the wastewater by bacterial oxidation of ammonia to nitrate under aerobic conditions (nitrification process). The produced nitrate is then converted to nitrogen under anaerobic conditions by another group of microorganisms (denitrification process). The reactions involved in the nitrification process are shown below (reactions 18 and 19).

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+ \qquad (2\text{-}18)$$

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + 2H^+ \qquad (2\text{-}19)$$

The second sources of nitrate containing wastewater are certain industrial processes. Glass and Silverstein (1999) reported certain industries, like manufacturing of fertilizers, metal finishing and nuclear plants, can generate high nitrate concentration wastewaters. Finally nitrate containing streams are also generated as part of agricultural activities when nitrogen containing fertilizers are used. Different with municipal and industrial wastewater, the nitrate containing wastewater (leachate) generated in the agriculture sector are difficult to be collected and are usually make their way to the underground or other natural bodies of water. Although chemical and biological processes for the removal of organics (COD and BOD) have been studied in details for a long time and very well understood, treatment of nitrate containing wastewaters has been studied only for three decades.

One of the main problems associated with nitrate containing streams is the health problem since high nitrate (or nitrite) concentrations can deteriorate drinking water quality and have potential hazards to aquatic animals in those waters contaminated by nitrate or nitrite. Strict environmental regulations for nitrate disposal are in place and permissible level for nitrate in drinking water in USA 10 ppm (NO_3^- - N) (US EPA, 1987). According to Canadian Guideline Limit Sample (Safe Drinking Water Foundation, 1998), nitrate concentration in drinking water should be lower than 10.2 mg (NO_3^- - N) L^{-1}. The second problem caused by the release of high nitrate concentration into a natural body of water is the eutrophication (overgrowth of green blue alga which damages the water ecology system). Nitrate and phosphorus are reported as the two most important contributors to the eutrophication of water bodies and most researchers believe that nitrate plays a more important role than phosphorus in eutrophication process. Removing nitrate from wastewater (denitrification process) before the wastewater release is the main method to control eutrophication problem. Lastly, the release of high nitrate concentration wastewater into nature water bodies does often cause the incompletely denitrification and NO and N_2O are the most common products in incompletely denitrification process. These compounds are reported as green house gases for global warming and should be removed. As these associated problems of nitrate, how to remove nitrate economically is worthy to be studied further.

2.3.2 Denitrification Methods

The treatment methods for nitrate containing streams include physical-chemical denitrification and biological denitrification approaches. Reverse osmosis, ion exchange, chemical catalysis are typical physicochemical methods for the removal of nitrate (Leakovic

et al., 1996; Shrimali and Singh, 2001; Fernandez-Nava, 2008). Compared with biological denitrification, most physicochemical methods, like reverse osmosis and ion exchange, only remove nitrate from the contaminated stream and the treated concentrated nitrate effluent still has to be dealt with, while in biological approaches nitrate is converted to gaseous nitrogen a completely harmless end product to environment. Another obvious disadvantage of physicochemical denitrification methods is the higher costs when compared with biological approaches as energy, chemical agents and catalysts are always required in physicochemical denitrification methods. As a result, biological methods are broadly used in industrial scale and physicochemical methods are only used in a few special cases.

The biological denitrification has been used broadly in the recent thirty years as a cost effective approach for complete removal of nitrate and its conversion to gaseous nitrogen. The biological nitrate removal can be achieved through heterotrophic denitrification or autotrophic denitrification using organic carbon compounds or sulphide as electron donors, respectively. The autotrophic denitrification which is also referred to as anaerobic chemolithotrophic desulphurization (or simultaneous desulphurization and denitrification) has been described in details in earlier section (Section 2.2.2.2) of this chapter. The following sections provide information on heterotrophic denitrification.

Under anaerobic conditions or low dissolved oxygen concentration (dissolved oxygen concentration lower than 1 mg L^{-1}), certain heterotrophic bacteria have the ability to reduce nitrate or nitrite to gaseous nitrogen. These bacteria include *Brevundimonas*, *Pseudomonas*, *Agrobacterium*, *Achromobacter* and *Phyllobacterium*. The heterotrophic denitrification with organic carbon (like acetate, glucose) as carbon and energy source proceeds through the following reactions (Honda and Osawa, 2002).

$$6NO_3^- + C_6H_{10}O_2 \rightarrow 3N_2 + 6CO_2 + 2H_2O + 6OH^- \quad (2\text{-}20)$$

$$10NO_2^- + C_6H_{10}O_2 \rightarrow 5N_2 + 6CO_2 + 10OH^- \quad (2\text{-}21)$$

Under anaerobic conditions, the nitrate or nitrite reducing bacteria use the electrons produced during the oxidation of organic compounds to reduce nitrate or nitrite to gaseous nitrogen. The organic carbon compounds are used as electron donors as well as energy source and nitrate and nitrite are used as electron acceptors in these reactions. Many kinds of organic carbon compounds, like acetate, methanol, ethanol, cellulosic compounds and high COD containing effluent, have been reported to be used in anaerobic heterotrophic denitrification by nitrate and nitrite reducing bacteria. Due to availability and suitability municipal wastewater containing high COD concentration is broadly used as organic carbon

source for heterotrophic denitrification which also accomplishes the removal of COD from such wastewaters and reduces the operation costs.

A large number of research works have been done on heterotrophic denitrification and its industrial application specially in wastewater treatment plants and high nitrate removal efficiencies (higher than 95%) have been achieved under optimal conditions. The most important factors influencing heterotrophic denitrification include temperature, pH, dissolved oxygen concentration, type and concentration of organic compounds, nitrate concentration, biomass retention time, as well as inhibitory effect of nitrite at high concentrations or other toxic material (Henze *et al*., 1997; Yoo *et al*., 1999; Zhao *et al*., 1999; Dhamole *et al*., 2007; Blackburne *et al*., 2008).

As mentioned earlier, oxidation of ammonia as part of wastewater treatment is one of the main processes contributing in formation of nitrate. According to the final products, nitrification can be divided into nitritation (oxidation of ammonia to nitrite) and nitrification (oxidation of ammonia to nitrate). A large number of aerobic bacteria have the nitrification ability including *Nitrosomonas europea, Nitrobacter agilis, Nitrosococcus mobilis, Nitrospira sp.*. The main factors affecting the nitrification process include biomass retention time, pH, alkalinity, ammonium nitrogen concentration, dissolved oxygen concentration, temperature and inhibition effects of high nitrite concentration or other toxic compounds (Hansen *et al*., 1998; Yoo *et al*., 1999; Zhao *et al*., 1999; Dhamole *et al*., 2007; Blackburne *et al*., 2008). One point should be noted that removal of nitrogen (in form of ammonia) through conversion to nitrite ($NH_3 \rightarrow NO_2^- \rightarrow N_2$) is more economical than that occurring by conversion to nitrate ($NH_3 \rightarrow NO_2^- \rightarrow NO_3^- \rightarrow NO_2^- \rightarrow N_2$). Blackburne (2008) pointed out that the required aeration and COD could be reduced by 25 and 40%, respectively if nitrogen removal (nitrification) through nitrite is used instead of that through nitrate. However, the problem associated with nitrogen removal via nitrite is the generation and accumulation of nitrite which could impose severe inhibitory effect on both nitrifying bacteria and the bacteria which are used in the heterotrophic denitrification step. Identifying bacteria tolerating high level of nitrite and development of the processes for simultaneous nitritation and denitrification are some of the challenges which should be addressed in future research in this field.

2.4 Kinetics of Desulphurization and Denitrification Processes

2.4.1 Desulphurization Kinetics

1) Photoautotrophic desulphurization kinetics

Many sulphide removal systems based on photoautotrophic sulphide oxidation have been investigated by researchers. Kobayashi *et al*., (1983) achieved 95% sulphide removal in a packed column inoculated with photosynthetic bacteria with a sulphide loading rate 107 mg day^{-1} and at a retention time 24 h. In a submerged system, 98% sulphide removal rate was achieved with sulphide loading rate of 36.2 mg L^{-1} h^{-1} and a retention time of 0.66 h. Using *Chlorbium thiosulphatophilum* performances, an immobilized-cell bioreactor and a free-cell bioreactor were compared by Kim and Chang (1991). Sulphide removal rates 0.26 and 0.11 μmol min^{-1} $(mg\ protein)^{-1}$ L^{-1} were achieved in immobilized-cell and free-cell bioreactors, respectively. Henshaw *et al*., (1998) reported a sulphide removal rate of 3.2 mg L^{-1} h^{-1} in a suspended growth CSTR inoculated by *Chlorobium limicola.* An enrichment of green sulphur bacteria was used in a biofilm reactor by Hurse and Keller (2004). The maximum sulphide removal rate 2.08 g $(m^2)^{-1}$ d^{-1} was achieved at flow rate in the range 1.11 to 1.18 mL min^{-1} with a maximum sulphide concentration of 11.5 mg L^{-1}.

2) Aerobic chemolithotrophic desulphurization kinetics

Sulphide removal with *Thiobacillus denitrificans* in a small scale reactor was studied by Sublette and Sylvester (1987). The authors reported complete sulphide removals at a loading rate 4-5 mM (S^{2-}) $(g\ biomass)^{-1}$ h^{-1}. Annachhatre *et al*., (2001) studied the effects of dissolved oxygen concentration on sulphide removal rate in a fluidized bed reactor. The results showed sulphate was the main product at DO concentration higher than 0.1 mg L^{-1}, while sulphur was the main product at DO concentration lower than 0.1 mg L^{-1}. Huang *et al*., (1996) used two biofiltration systems with *Thiobacillus* sp. CH11 and *Pseudomonas Putida* CH11, respectively to investigate the aerobic chemolithotrophic removal of sulphide. Sulphide removal rates around 95% were achieved in both systems at flow rate ranging from 18 to 93 L h^{-1} with 60 ppm H_2S. Sulphide removal rates in a bio-trickling filtration were studied by Datta *et al*., (2007) over a range of temperatures (40 – 70°C). The maximum sulphide removal rate of 40 g (H_2S) $(m^3)^{-1}$ h^{-1} was obtained at 70°C.

3) Anaerobic chemolithotrophic desulphurization kinetics

Considering several advantages already described, anaerobic biooxidation of sulphide has been the subject of a large number of studies (Tang *et al*., 2009). A mixed microbial culture originated from the produced water of Coleville oil field in Saskatchewan, Canada was used by McComas *et al*., (2001) for oxidation of sulphide. The maximum sulphide removal rate of 5.8 mM (H_2S) (g biomass)$^{-1}$ h^{-1} was achieved with this enrichment culture. In addition, the enrichment culture was more tolerant of extremes in pH, salinity and elevated temperature when compared with *Thiobacillus denitrificans.* Using *Thiomicrospira* sp. CVO, Gadekar *et al*., (2006) studied biological removal of sulphide in batch and continuous systems. In the continuous system, complete sulphide removals were achieved at sulphide loading rates up to 1.6 mM h^{-1} and the maximum volumetric removal rate 2.4 mM h^{-1} was achieved at loading rate 3.2 mM h^{-1} with sulphide conversion 76%. Simultaneous desulphurization and denitrification by *Thiobacillus denitrificans* have been investigated by Wang *et al*., (2005). Sulphide removal around 99% was achieved using sulphide to nitrate ratios of 3 to 5 and an initial sulphide concentration of 100 mg L^{-1}. The 90% removal of nitrate and 65% removal of sulphide were achieved in a continuous stirred tank reactor with nitrate loading of 0.2 kg (N) (m^3)$^{-1}$ d^{-1} and sulphide loading of 0.042-0.294 kg (S^{2-}) (m^3)$^{-1}$ d^{-1} by Avila *et al*., (2004).

2.4.2 Denitrification Kinetics

1) Heterotrophic denitrification

Heterotrophic denitrification under anaerobic conditions proceeds through a set of sequential reactions as described below (equation 22) with the oxidation state of nitrogen in each compound is presented in the bracket (Feleke and Sakakibara, 2002; Killingstad *et al*., 2002). The electron required for reduction of nitrate and other intermediates are supplied through oxidation of some suitable organic compounds such as acetate, starch, glucose, cellulosic materials such as bark, wood chip, and organic containing wastewater.

$$\overset{(-5)}{NO_3^-} \rightarrow \overset{(+3)}{NO_2^-} \rightarrow \overset{(+2)}{NO}(\text{gaseous}) \rightarrow \overset{(+1)}{N_2O}(\text{gaseous}) \rightarrow \overset{(0)}{N_2}(\text{gaseous}) \qquad (2\text{-}22)$$

Malgorzata *et al*., (2006) reported the maximum denitrification rate of 3.841 mg (N-NO_3^-) (mg biomass)$^{-1}$ h^{-1} in batch operation using 1.67 mg (CH_3COOH) (mg N)$^{-1}$ with municipal waste water sludge as microbial culture. Alexandre *et al*., (2006) achieved nitrate removal rates of 0.8 and 0.4 kg (N) day^{-1} (m^3)$^{-1}$ in a sequencing batch reactor and a continuous reactor (chemostat), respectively. Ficara and Canziani (2007) established a lab-scale sequencing batch reactor (SBR) to study heterotrophic denitrification. The maximum denitrification rate

obtained in the SBR was 16.3 ± 1.2 mg (N) (g VSS)$^{-1}$ h^{-1} with acetate as a carbon source. Soto *et al*., (2007) reported a maximum specific rate $k_{(NO_3^-)}$=12.7 mg (NO_3^--N) (g VSS)$^{-1}$ h^{-1} and a saturation constant $K_{S(NO_3^-)}$=0.47 mg (NO_3^--N) L^{-1} for denitratation (nitrate reduction to nitrite) and $k_{(NO_2^-)}$=13.8 mg (NO_2^--N) (g VSS)$^{-1}$ h^{-1} and saturation constant of $K_{S(NO_2^-)}$=0.36 mg (NO_2^--N) L^{-1} for denitritation (nitrite reduction to gaseous N_2). Canto *et al.,* (2008) established a sequencing batch biofilm reactor for simultaneous nitrification and denitrification. The reactor was fed with ammonium and operated at low dissolved oxygen concentration (as 2 mg L^{-1}). Total nitrogen removal of 80% and 97% of ammonium removal were achieved with the high reaction rate 86.5 mg (N-NH_4^+) L^{-1} d^{-1} obtained at optimal conditions.

Heterotrophic denitrification of nitrate containing stream (9032 ppm NO_3^--N) was investigated in a sequencing batch reactor by Dhamole *et al.,* (2007) and complete nitrate removal was achieved in 6 h. Dold *et al.,* (2008) compared the growth kinetics of denitrifying bacteria with growing on different organic carbon sources. The acetate utilizing organisms has the highest maximum specific growth rate of 4.0 d^{-1}, while methanol utilizing organism showed a maximum specific growth rate of 1.3 d^{-1}. The highest nitrate reaction rate was around 9.0 mg (NO_3^--N) (g VSS)$^{-1}$ h^{-1} in batch experiments using acetate. Fernandez-Nava *et al.,* (2009) reported the maximum specific denitrification rate 48 mg (NO_3^--N) (g VSS)$^{-1}$ h^{-1} in a sequencing batch reactor fed with a high sugar concentration wastewater. Complete nitrate and COD removals were achieved over a period of 4 to 6 h.

Recently microbial fuel cell type bioreactors have been used for denitrification. The maximum volumetric nitrate removal rate of 0.084 mg (NO_3^--N) (cm^2 electrode surface area)$^{-1}$ day^{-1} was achieved in a microbial fuel cell using glucose as organic food (Jia *et al*., 2008). Virdis (2008) established a microbial fuel cell type reactor by feeding carbon containing effluent to the anode and nitrate containing effluent into the cathode. The maximum removal rates for COD and nitrate were 2 kg (COD) (m^3 NCC)$^{-1}$ d^{-1} and 0.41 kg (NO_3^--N) (m^3 NCC)$^{-1}$ d^{-1}, respectively. Clauwaert *et al.,* (2007) reported the highest nitrate removal rate of 0.146 kg (NO_3^--N) (m^3)$^{-1}$ d^{-1} at a current of 58 A (m^3)$^{-1}$ (net cathodic compartment) and a cell voltage of 0.075 V for a microbial fuel cell fed with acetate as carbon source.

2) Autotrophic denitrification

Simultaneous removal of nitrate and sulphide (autotrophic denitrification) has been investigated in a number of studies. Avila *et al*., (2004) reported 90% removal of nitrate accompanied by 65% removal of sulphide in a continuous stirred tank reactor with nitrate

loading of 0.2 kg (N) $(m^3)^{-1}$ d^{-1} and sulphide loading of 0.042-0.294 kg (S^{2-}) $(m^3)^{-1}$ d^{-1}. Beristain-Cardoso, *et al*., (2008) used a mixed culture consisted of autotrophic and heterotrophic nitrate reducing bacteria in an inverse fluidized bed reactor. Complete removal (100%) of nitrate, sulphide and acetate were achieved with sulphide /nitrate /acetate molar ratio of 13 / 100 / 85. The gaseous nitrogen yield was calculated as 0.81 g (N_2) (g NO_3^--N)$^{-1}$ and nitrate removal rate was 229 ± 15 mg L^{-1} d^{-1}. Chen *et al.,* (2008) established a high-rate expanded granular bed reactor with autotrophic and heterotrophic denitrifying mixed culture. With sulphide/ nitrate/ acetate molar ratio of 5/ 6/ 7.56, the highest removal rates for sulphide, nitrate and acetate were 6.09 kg (S^{2-}) $(m^3)^{-1}$ day^{-1}, 2.92 kg (NO_3^--N) $(m^3)^{-1}$ day^{-1} and 7.48 kg (acetate) $(m^3)^{-1}$ day^{-1}, respectively. Sierra-Alvarez *et al.,* (2007) established a packed-bed bioreactor supplied with sulphur and limestone granules (ratio 1:1, v/v) to investigate the process of autotrophic denitrification. Complete nitrate removal (100%) and a nitrate removal rate of 21.6 mM (L reactor)$^{-1}$ d^{-1} were achieved with an influent nitrate concentration of 7.3 mM. Two reactors, up-flow anoxic hybrid growth reactor and up-flow anoxic suspended growth reactor, were used by Byun *et al*., (2008) for the autotrophic denitrification of nitrate at loading rates of 0.15 to 0.40 kg (NO_3^-) $(m^3)^{-1}$ d^{-1}. A similar nitrate removal of 90% was achieved in both reactors. The ratio of sulphate production to nitrate removal ranged from 1.5 to 2.1 mg (SO_4^{2-}) (mg NO_3^-)$^{-1}$, a value close to what is expected from the stoichiometry of nitrate reduction and sulphide oxidation. The kinetic coefficients for autotrophic denitrification were calculated as μ_{max} =0.097 h^{-1}, k_d =0.0021 h^{-1}, k_s =200 mg (NO_3^-) L^{-1} and Y =0.31 mg (MLVSS) (mg NO_3^-)$^{-1}$.

Furumai *et al.,* (1996) and Reyes-Avila *et al.,* (2004) reported that the denitrification rates for conversion of nitrite to gaseous nitrogen via heterotrophic pathway are faster than that via autotrophic pathway, but for conversion of nitrate to nitrite the opposite is through (i.e. denitrification rate via heterotrophic pathway is lower than that via autotrophic pathway).

As indicated earlier a large number of researches have been conducted in the field of denitrification with both autotrophic and heterotrophic cultures. Table 2-1 provides a summary of the recent literature data on autotrophic and heterotrophic denitrification processes. Included in this table are the source of microbial cultures, bioreactor configuration, operating conditions such as pH, temperature and nitrate concentration and finally the performance of the reactor in terms of volumetric removal rate of nitrate or nitrogen. The variations in the microbial cultures and experimental conditions applied in each work complicate the accurate assessment and as such careful consideration is required when comparing the kinetic data reported in different work.

Table 2-1 Operating conditions and biokinetics of nitrate removal in various bioreactors with autotrophic or heterotrophic denitrification bacteria

Reference	Bacteria or culture source	Bioreactor configuration	Matrix for biofilm establishment	Electron donor	Carbon source	T (℃)	pH	Treated influent	Removal rate	Rem-oval (%)	End products
Komorowska-Kaufman, *et al.*, 2006	Heterotrophs	Bardenpho reactor	-	Acetate	Acetate	15	7	Concen-tration: 7.0 mg ($N\text{-}NO_3^-$) L^{-1}	3.324 mg ($N\text{-}NO_3^-$) (mg Biomass)$^{-1}$ h^{-1}	-	-
Ruiz, *et al.*, 2006	Heterotrophs	Upflow sludge blanket reactor	-	Acetate	Acetate	23	7.85	Loading: 5 kg ($N\text{-}NO_2^-$) $(m^3)^{-1}$ d^{-1}	4.67 kg ($N\text{-}NO_2^-$) $(m^3)^{-1}$ d^{-1}	93.5	N_2
Sumino, *et al.*, 2006	Heterotrophs	Up-flow reactor	Porous fabric carriers	Acetate	Acetate	30	-	Loading: 0.4-0.6 kg ($N\text{-}NO_3^-$) $(m^3)^{-1}$ d^{-1}	0.48 kg ($N\text{-}NO_3^-$) $(m^3)^{-1}$ d^{-1}	80-94	N_2
Clauwere, *et al.*, 2007	Heterotrophic denitrifiers	Microbial fuel cell	-	Acetate	Acetate	22	7.0	Loading: 0.16 kg ($N\text{-}NO_3^-$) (m^3 NCC)$^{-1}$ d^{-1}	0.037 g ($N\text{-}NO_3^-$) (g VSS)$^{-1}$ d^{-1}	92	N_2

(Continued)

Reference	Bacteria or culture source	Bioreactor configuration	Matrix for biofilm establishment	Electron donor	Carbon source	T (℃)	pH	Treated influent	Removal rate	Rem-oval (%)	End products
Dhamole, *et al.*, 2007	Fresh sludge in fertilizer industry	Sequencing batch reactor	-	Acetate	Acetate	23	7.2	Concen-tration: 9032 mg ($N\text{-}NO_3^-$) L^{-1}	2.909 g ($N\text{-}NO_3^-$) L^{-1} h^{-1}	-	N_2, NO_2^-
Sierra-Alvarez, *et al.*, 2007	Chemo-lithotrophic denitrifers	-	Granular element sulphur and limestone grit	Sulphur	CO_2	30	7.0	Loading: 18.1 mM ($N\text{-}NO_3^-$) L^{-1} d^{-1}	26.4 mM ($N\text{-}NO_3^-$) (m^2 sulphur)$^{-1}$ d^{-1}	95.9	N_2
Tugtas, *et al.*, 2007	Autotrophic denitrifers	Batch	-	Sulphide	CO_2	35	7.0	Concen-tration: 75 mg ($N\text{-}NO_3^-$) L^{-1}	-	100	NO, N_2O, N_2
Beristain-Cardoso, *et al.*, 2008	Denitrifying sludge	Inverse fluidized bed	Low-density polyethylene	Acetate and sulphide	Acetate and CO_2	32	8.0	Loading: 16 mM L^{-1} d^{-1}	0.228 g ($N\text{-}NO_3^-$) L^{-1} h^{-1}	100	N_2

(Continued)

Reference	Bacteria or culture source	Bioreactor configuration	Matrix for biofilm establishment	Electron donor	Carbon source	T (℃)	pH	Treated influent	Removal rate	Rem-oval (%)	End products
Canto, *et al.,* 2008	Anaerobic heterotrophic biomass	Sequencing batch biofilm reactor	Acrylic	Sucrose, starch, cellulose, meat extrat, soybean oil	Sucrose, starch, cellulose, meat extrat, soybean oil	30	7.0	Concen-tration: 50 mg (NH_4^--N)L^{-1}	0.079 g ($N\text{-}NH_4^+$) L^{-1} h^{-1}	39.4	N_2
Chen, *et al.,* 2008	Culture collected in EGSB reactor	High-rate expanded granular bed reactor	-	Sulphide and acetate	CO_2 and acetate	28	7.6	Loading: 0.17-4.08 kg ($N\text{-}NO_3^-$) $(m^3)^{-1}$ d^{-1}	0.17-1.99 kg ($N\text{-}NO_3^-$) $(m^3)^{-1}$ d^{-1}	48.8-100	N_2
Dold, *et al.,* 2008	Methanol-utilizing heterotrophs	Batch reactor	-	Methanol	Methanol	13-20	7.0-7.3	Concen-tration: 100 mg ($N\text{-}NO_3^-$) L^{-1}	9.0 mg ($N\text{-}NO_3^-$) $(g\ VSS)^{-1}$ h^{-1}	-	-

(Continued)

Reference	Bacteria or culture source	Bioreactor configuration	Matrix for biofilm establishment	Electron donor	Carbon source	T (℃)	pH	Treated influent	Removal rate	Rem-oval (%)	End products
Fernanda-Nava, *et al.*, 2008	Sludge from landfill and sewage plant	Sequencing batch reactor	-	COD	COD	20	7.1	Concen-tration: 455 mg ($N\text{-}NO_3^-$) L^{-1} and 69 mg ($N\text{-}NO_2^-$) L^{-1}	7.3 mg ($N\text{-}NO_3^-$) $(g\ VSS)^{-1}$ h^{-1}	100	N_2
Fernanda-Nava, *et al.*, 2008	Sludge from a landfill plant	Sequencing batch reactor	-	COD	COD	20	8.5	Concen-tration: 2500 mg (NO_3^-) L^{-1}	42-48 mg ($N\text{-}NO_3^-$) $(g\ VSS)^{-1}$ h^{-1}	-	-
Li, *et al.*, 2008	Sludge from municipal wastewater plant	Membrane bioreactor	Hollow-fiber membrane	Glucose, starch	Glucose, starch	23	-	Concen-tration: around 90 mg (TN) L^{-1}	-	63.1	-
Rezaee, *et al.*, 2008	*Pseudomonas stutzeri* (heterotroph)	Continuous suspended granule reactor	Microbial cellulose	COD	COD	25	7.0	Loading: 1.61 kg ($N\text{-}NO_3^-$) $(m^3)^{-1}$ d^{-1}	1.61 kg ($N\text{-}NO_3^-$) $(m^3)^{-1}$ d^{-1}	100	N_2O, N_2

(Continued)

Reference	Bacteria or culture source	Bioreactor configuration	Matrix for biofilm establishment	Electron donor	Carbon source	T (℃)	pH	Treated influent	Removal rate	Rem-oval (%)	End products
Chen, *et al.,* 2009	Autotrophic and heterotrophic denitrifiers	Expanded granular sludge bed reactor	-	Sulphide and acetate	CO_2 and acetate	28	7.6	Loading: 2.6 kg (N-NO_3^-) $(m^3)^{-1}$ d^{-1}	2.35 kg (N-NO_3^-) $(m^3)^{-1}$ d^{-1}	92	N_2, NO_2^-
Ghafari, *et al.,* 2009	Auto-hydrogeno-trophs	Sequencing batch reactor	-	H_2	CO_2	25	7.5, 8.0	Concen-tration: 40 mg (N-NO_2^-) L^{-1}	25 mg (N-NO_2^-) (g MLVSS)$^{-1}$ h^{-1}	-	N_2
Kalyuzhnyi, *et al.,* 2009	Heterotrhophs, autotrophs	DEAMOX reactors	Granular sludge	COD , sulphide	COD, CO_2	35	4.0-5.7	Loading: 1000 mg (total nitrogen) L^{-1} d^{-1}	900 mg (total nitrogen) L^{-1} d^{-1}	90	N_2
Li, *et al.,* 2009	Autotrophs and heterotrophs	Anaerobic attached-growth bioreactor	Sponge cubes	Sulphide, glucose	CO_2, glucose	30	7.0	Loading: 475.2 mg (N-NO_3^-) L^{-1} d^{-1} and 180 mg (N-NO_3^-) L^{-1} d^{-1}	475.2 mg (N-NO_3^-) L^{-1} d^{-1} and 180 mg (N-NO_3^-) L^{-1} d^{-1}	100	N_2

(Continued)

Reference	Bacteria or culture source	Bioreactor configuration	Matrix for biofilm establishment	Electron donor	Carbon source	T (℃)	pH	Treated influent	Removal rate	Rem-oval (%)	End products
Ma, *et al.,* 2009	Sludge from municipal wastewater plant	Pilot-scale continuous pre-denitrification plant	-	COD from sewer line	COD	21	7.5	Concen-tration: 78.2 mg (TN) L^{-1}	-	73.9	-
Shen, *et al.,* 2009	Sludge from pharmaceutical industry	Anoxic/oxic membrane bioreactor	Hollow-fiber membrane module	Acetate	Acetate	30	7.5-8.5	Loading: 0.12 g (N-NO_3^-) L^{-1} h^{-1}	324 mg (N-NO_3^-) (g VSS)$^{-1}$ h^{-1}	100	N_2
Walters, *et al.,* 2009	Heterotrophic denitrifers	Stirred biofilm batch reactor	Polyhydro-xybutyrate and poly-caprolactone	Glucose	Glucose	21	7.0	Concen-tration: 60 mg (N-NO_3^-) L^{-1}	0.01 g (N-NO_3^-) (g VLSS)$^{-1}$ d^{-1}	-	-
Wang, *et al.,* 2009	Denitrification bacteria	Continuous biofilm reactor	Fiber threads (80% cotton and 20% terylene)	Methanol	Methanol	20-30	7.0-7.5	Concen-tration: 106 mg (N-NO_3) L^{-1}	13 mg (N-NO_3) L^{-1} h^{-1}	99	N_2

CHAPTER 3
RESEARCH OBJECTIVES

As mentioned in the literature review chapter, chemolithotrophic biological sulphide removal technologies are prominent when compared with other approaches including physicochemical, indirect biological, and photoautotrophic bacterial methods as high temperature, high pressure, chemical catalysts (required for physicochemical methods) and light (required for photoautotrophic methods) are not required. Chemolithotrophic biooxidation of sulphide under aerobic conditions has been investigated extensively using various species of sulphide oxidizing bacteria, especially those belonging to *Thiobacilli* genus. However, risks from operation of the reactor system for the treatment of gaseous streams such as biogas under oxygen rich environment is a concern which should not be overlooked. Chemolithotrophic biooxidation of sulphide under denitrifying conditions reduces this risk and eliminates the cost associated with the aeration (An *et al*., 2009). Furthermore, understanding the anaerobic biooxidation of sulphide with nitrate is also important since it has been identified as one of the main mechanisms in the control of souring in oil reservoirs subjected to nitrate amendment (An *et al*., 2009). One of the major challenges in application of biooxidation of sulphide identified in the literature is the low level of sulphide which can be tolerated by the bacterial species used in previous works (1-2 mM). The microbial culture which is used in this work is a sulphide oxidizing and nitrate reducing culture enriched from the produced water of a Canadian oil reservoir and is shown to be able to oxidize sulphide at concentrations as high as 16 mM. In this research, therefore, biooxidation of sulphide under denitrifying conditions was studied using this microbial consortium.

In order to provide a better understanding of the denitrification process by this microbial culture, the heterotrophic denitrification with acetate as energy source and electron donor was also investigated in the batch, CSTB (continuous stirred tank bioreactor), biofilm reactors. The reaction rate, removal ratio and biological kinetics obtained during the heterotrophic and autotrophic denitrification processes were compared. The essential information regarding the process of denitrification is an important step in treatment of conventional wastewaters containing nitrogen.

The specific objectives and various phases of this research are as following:

1) To study the biological removal of the dissolved sulphide from a liquid phase under denitrifying conditions and to verify the effects of initial sulphide concentration (10, 15 and 20 mM), and sulphide to nitrate loading ratio (from 0.1 to 3.5) on the kinetics of the reaction and composition of the end products using continuous stirred tank bioreactors.

2) To study the process of heterotrophic denitrification in the absence of sulphide using acetate as the energy and carbon source. The effects of initial nitrate concentration (5, 10, 15, 30 and 50 mM), ratio of nitrate to acetate (from 1.7 to 10) and temperature (15, 20, 25, 30 and 35ºC) on the kinetics of heterotrophic denitrification have been investigated in batch system. The effects of low and high feed concentrations (10 and 30 mM) have been investigated in CSTB systems with freely suspended cells and kinetics parameters have been evaluated. Utilization of immobilized cells as a means to improve the rate of denitrification was also investigated in a packed-bed bioreactor and effects of increases of loading rate by increasing feed flow rate (0.5 to 200 mL h^{-1}) or feed concentration (30, 50, 75, 100 mM of each acetate and nitrate) were studied too. This part of the research allowed us to compare the process of denitrification in the presence and absence of sulphide (autotrophic and heterotrophic denitrifications).

CHAPTER 4
MATERIAL AND METHODS

The following sections describe the experimental set-up, procedures and the measurement methods.

4.1 Microbial Culture and Medium

A mixed microbial culture of sulphide oxidizing and nitrate reducing bacteria enriched from the produced water of the Coleville oil field in Saskatchewan was used in this work for simultaneous removal of sulphide and nitrate (autotrophic denitrification). The same culture was used as an inoculum to develop the suitable culture for heterotrophic denitrification.

4.1.1 CSB Medium

Coleville Synthetic Brine (CSB) was used as the medium for the maintenance and growth of the microbial culture. The composition of CSB medium is as follows: sodium chloride 7.0 g L^{-1}; magnesium sulphate 0.68 g L^{-1}; calcium chloride 0.24 g L^{-1}; ammonium chloride 0.02 g L^{-1}; potassium phosphate 0.027 g L^{-1}; sodium acetate 0.68 g L^{-1}; potassium nitrate 1.0 g L^{-1}; sodium bicarbonate 1.9 g L^{-1}; 0.5 mL L^{-1} of 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 distilled water); resazurin 0.5 mL L^{-1}; Tris Buffer 6.057 g L^{-1}. The pH of the medium was adjusted to 7.0-7.5 using 2.0 or 4.0 M HCl solution.

4.1.2 Culture Conditions

The enrichment culture was maintained in 125 mL serum bottles. For culture maintenance, 100 mL CSB medium was added to each serum bottle. The medium in each bottle was purged with sterilized nitrogen gas (filtered by a 0.2 µm Supor® membrane syringe filter) for 30 minutes. The bottles were then sealed with rubber septum and aluminum caps and autoclaved for 30 minutes at 121°C to ensure sterilization. When the medium was cooled to room temperature (23°C), 0.5 mL of 1 M sterilized Na_2S (filtered by a 0.2 µm Supor® membrane syringe filter) was added to the bottle to achieve a sulphide concentration around 5 mM. The pH was readjusted to 7.0-7.5 using 2.0 M sterilized HCl (filtered by a 0.2 µm

Supor® membrane syringe filter). Finally, 10 mL of a two weeks old enrichment culture was added to the bottle as an inoculum. The bottle was kept at room temperature (23°C). The established microbial culture was then used as inoculum in subsequent subculturing as described above. Subculturing was carried out on a bi-weekly basis to maintain an active enrichment culture.

For maintenance of the heterotrophic denitrifying culture, CSB medium with no sulphide was used as medium and the Coleville enrichment described above was used as an inoculum. All the procedures and conditions used for maintenance of the cultures and subculturing was similar to those described above (desulphurization culture).

4.1.3 Medium Used in CSTB and Biofilm Reactor

CSB medium with the composition described earlier (Section 4.1.1) and containing desired sulphide and nitrate concentrations (for desulphurization experiments) or nitrate and acetate concentrations (for heterotrophic denitrification experiments) was used in experiments conducted in CSTB and biofilm reactor. Medium was prepared in a 4 L glass flask containing 3 L of medium which was autoclaved 30 min at 121°C. After cooling to room temperature (23°C), the medium was purged by sterilized nitrogen gas to remove dissolved oxygen. For desulphurization experiments, the required amount of 1 M sterilized Na_2S solution was added to the medium using a syringe to achieve the desired sulphide concentration and pH was readjusted to 7.0-7.5 by adding 4 M sterilized HCl solution. The medium was then transferred into either a 5 L (for the low flow rates of the feed) or 10 L (for high flow rates of the feed) sterilized collapsible medium bag by introducing pressurized sterilized nitrogen gas into the flask. Using collapsible bag, experiments can be kept under anaerobic condition more easily. The bag was connected to the bioreactor using tygon tubings. For heterotrophic denitrification experiments, CSB medium contained the desired acetate and nitrate concentration was used. Except adding Na_2S solution and readjusting pH, the other procedures were the same with that described above.

4.2 Experimental Set-Ups and General Procedures

Three experimental systems including batch, continuous bioreactor with free cells (CSTB) and continuous bioreactor with immobilized cells (biofilm reactor) were used in this work. Heterotrophic denitrification was studied in all three systems but oxidation of sulphide under

denitrifying conditions was only investigated in the continuous bioreactor with free cells, as batch and biofilm system had been investigated in an earlier work (Tang, 2008).

4.2.1 Batch Experiments

Batch experiments were conducted in 125 mL serum bottles containing 100 mL CSB mediums with desired acetate and nitrate concentrations (heterotrophic denitrification experiments). Medium was purged with nitrogen gas and autoclaved following the procedures described earlier. After reaching to room temperature, 10 mL (10% v/v) of a three days old enrichment culture grown in a medium containing 10 mM nitrate and 10 mM acetate was added to the serum bottles containing the designated nitrate and acetate concentrations. The bottles were maintained at the designated temperature and sampled regularly (every two hours for low concentration experiments to daily for high concentration experiments). Prior to sampling, each serum bottle was shaken gently, and then 1.5 mL of sample was taken using a syringe. The optical density, acetate, nitrite and nitrate concentrations were determined in the sample. For each investigated conditions, experiments were conducted in duplicates. The average data calculated based on the results of duplicated experiments was used. Standard deviation for these measurements was also calculated and used as error bar in presenting the results. A typical serum bottle used in batch experiment is shown in Figure 4-1.

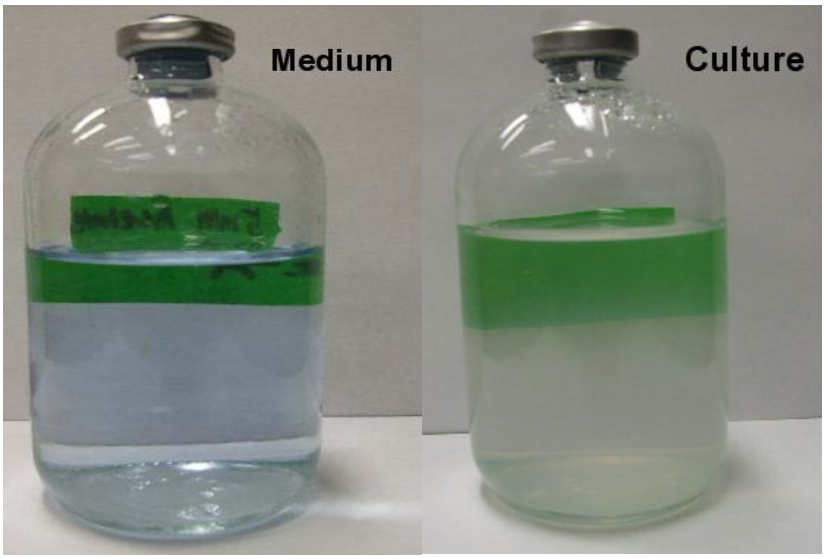

Figure 4-1 Batch experimental set-up used to maintenance cultures and study heterotrophic denitrification (left: medium prior to inoculation; right: growing culture)

4.2.2 Experiments in Continuous Stirred Tank Bioreactor (CSTB)

Three CSTB systems as shown in Figure 4-2 were set up to study simultaneous removal of sulphide and nitrate (autotrophic denitrification) and heterotrophic denitrification. Each system consists of a stirred tank bioreactor with a working volume of approximately 230 mL. Medium containing sulphide and nitrate (for desulphurization experiments) or acetate and nitrate (for heterotrophic denitrification experiments) at the desired concentrations was pumped into the bioreactor continuously using a variable speed peristaltic pump and effluent was transferred into the effluent bottle through an overflow tube. The volumetric loading rate of sulphide or nitrate was controlled by adjusting the flow rate of the feed. A magnetic stirrer was used to achieve mixing and maintain all the particles (biomass or sulphur) in suspension.

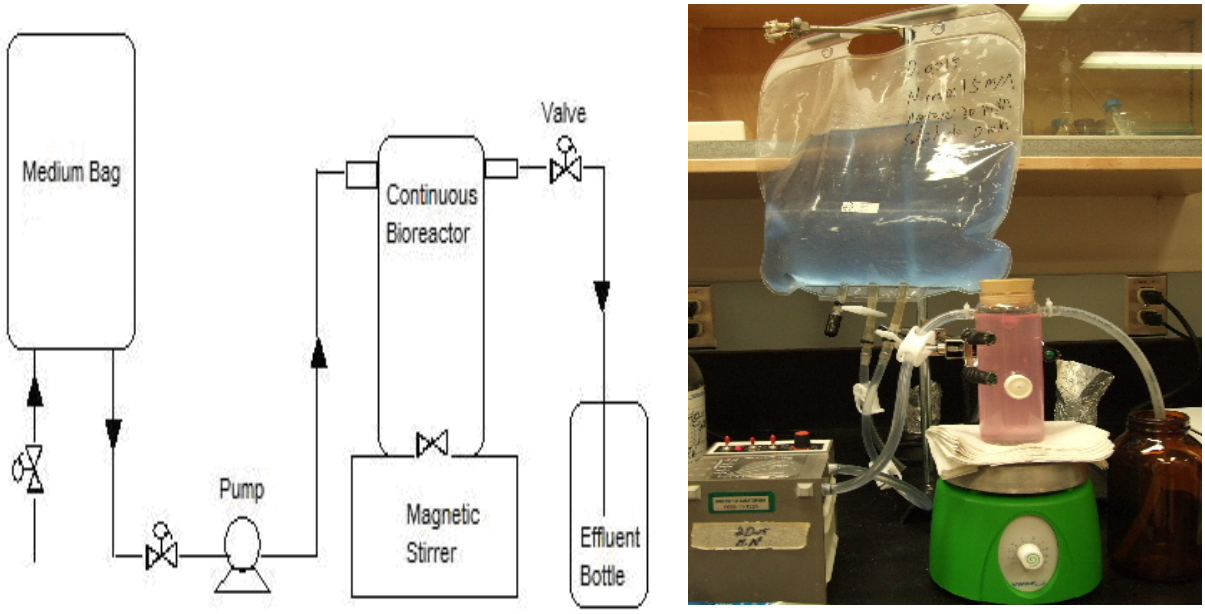

Figure 4-2 Flow diagram and picture of the CSTB experimental set-up used to study autotrophic and heterotrophic denitrifications

The bioreactors were operated batchwise initially to allow the growth of bacteria and establishment of a sizable biomass. At this stage each bioreactor was filled with CSB medium containing 5 mM sulphide and 10 mM nitrate for desulphurization with nitrate experiments (autotrophic denitrification) or 10 mM nitrate and 10 mM acetate but no sulphide for heterotrophic denitrification using the peristaltic pump (the level of other ingredients of the CSB medium were the same as normal CSB medium as described in Section 4.1.1). The pump was then stopped and the bioreactor was inoculated with 30 mL of three days old enrichment culture, growing either on sulphide and nitrate (for autotrophic

denitrification) or acetate and nitrate (for heterotrophic denitrification). During the batch operation the sulphide concentration (for desulphurization experiments) or nitrate concentration (for heterotrophic denitrification experiments) was monitored daily. When complete removal of either sulphide (for desulphurization experiments) or nitrate (for heterotrophic denitrification experiments) was achieved, the bioreactor was switched to continuous mode. During the continuous operation flow rate of the feed was increased stepwise until cell wash-out happened. At each flow rate sufficient time was given so the system reached the steady state conditions. Steady state was assumed to be established when complete removal of sulphide (desulphurization with nitrate) or nitrate (heterotrophic denitrification) was observed in the bioreactor or when the residual sulphide or nitrate concentration in the bioreactor was constant (changed less than 5%) over a period of at least two days. The experiments were carried out at room temperature (23°C). At each sampling event, a 1.0 mL syringe was used to remove 0.5 mL sample through the rubber septum located in the middle of each bioreactor. The concentrations of sulphide, acetate, nitrite, nitrate, sulphate and thiosulphate, or acetate, nitrite and nitrate were measured for desulphurization or heterotrophic denitrification, respectively. Another sample (5 mL) was taken for sulphur and protein determination in desulphurization experiments or for optical density measurement in heterotrophic experiments. The concentration of sulphide was determined on a daily basis and in some cases twice a day. The concentrations of acetate, nitrite, nitrate, sulphate and thiosulphate, sulphur and protein, optical density as well as pH were determined at each flow rate in three consecutive days following the establishment of steady-state conditions. The average value calculated based on these repeated sampling at steady state condition were used in presenting the results. Standard deviation for these measurements was also calculated and used as error bar.

4.2.3 Experiment in Biofilm Reactor

A packed-bed bioreactor (biofilms reactor) as shown in Figure 4-3 was set up to study the heterotrophic denitrification process and to assess the potential for improving the removal rate of nitrate.

The bioreactor was consisted of a glass column with a diameter of 4 cm, and a height of 36 cm. Three sampling ports were devised at 12.5 cm intervals along the length of the column. The three sampling ports were sealed using rubber septum. A polymeric mesh pad was used at the bottom of the reactor to support the carrier matrix used for the establishment of a

biofilm. The carrier matrix utilized in this experiment was quartz sand with a mesh size of -50 to +70 and an average diameter of 225 μm. The bioreactor had a working volume (void volume) of approximately 41.8 mL. Bioreactor was operated batchwise initially. During the batch operation, the bioreactor was filled with CSB medium containing 10 mM nitrate and 10 mM acetate using a peristaltic pump. The bioreactor was then inoculated by injecting 10 mL of a three day old enrichment culture (grown on CSB medium containing 10 mM nitrate and 10 mM acetate) to each port. The bioreactor was switched to continuous mode when nitrate concentration in the samples taken from each port was negligible. During the continuous operation, CSB medium containing desired acetate and nitrate concentrations was introduced into the bioreactor using the peristaltic pump. Effluent was removed from the top of the bioreactor via an overflow tube. Samples (0.3 mL) were taken from each port using 1.0 mL syringe and analyzed for nitrate, nitrite and acetate concentrations on a daily basis. The flow rate of the feed was increased stepwise (increments around 0.5 mL h^{-1}) until a decrease in performance of the system in terms of nitrate removal rate was observed. Steady state was assumed to be established when complete conversion of nitrate in the reactor was observed or when the residual nitrate concentration in the reactor changed by less than 10% over a period of at least two-three days. The acetate, nitrite, nitrate concentrations from all three ports, as well as pH and optical density of the effluent were determined at each flow rate for three consecutive days following the establishment of steady-state conditions. The average value calculated based on the measurements after establishment of steady state conditions were used in presenting the results. Standard deviation was also calculated and used as error bar together with these results.

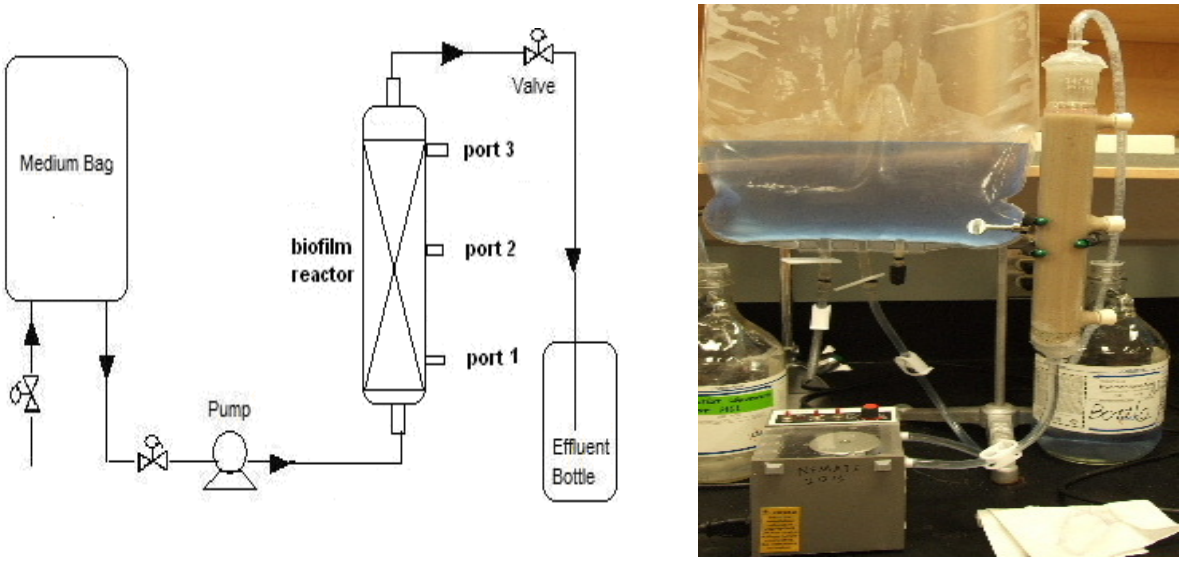

Figure 4-3 Flow diagram and picture of the experimental set-up used to study biological removal of nitrate in a biofilm reactor

4.3 Parameters Investigated

The general procedures described in Sections 4.2 were applied in all the experiments. The following section describes the exact parameters and conditions which were investigated in this research work.

4.3.1 Autotrophic Denitrification with Sulphide in CSTB

1) Effects of initial sulphide concentration

To study the effects of initial sulphide concentration, three initial sulphide concentrations of 10 mM, 15 mM and 20 mM were chosen. The highest level was chosen based on the previous research results which indicated that sulphide at concentrations around or higher than 20 mM had a strong inhibition effect (Tang, 2008). Three identical CSTB systems as described above were set-up and each operated with a designated sulphide concentration according to the procedure described earlier. In these experiments the ratio of sulphide to nitrate concentration were kept constant at 2 (i.e. the concentrations of nitrate in the reactors run with 10, 15 and 20 mM sulphide were 5, 7.5 and 10 mM, respectively). These experiments were run at room temperature (23°C).

2) Effects of sulphide to nitrate ratio

The effects of sulphide to nitrate ratios (0.25, 0.5, 1, 2, 4 and 6) were studied using two CSTB systems operated at low and high dilution rates of 0.15 and 0.75 h^{-1} (corresponding to flow rates of 3.5 and 17.2 mL h^{-1}, respectively) at room temperature (23°C). This allowed us to assess the effect of residence time (loading rate) as well. The initial operation of these two bioreactors (batchwise and continuous) was exactly the same as that described for the effects of sulphide concentration (Section 4.2.2). When the flow rates reached to 3.5 and 17.2 mL h^{-1} in these two reactors respectively, the flow rates were kept constant. The CSB medium containing desired sulphide to nitrate ratio was pumped into the bioreactor. In all the experiments sulphide concentration was kept at 15 mM and nitrate concentration was changed to achieve a desired sulphide to nitrate ratio. With each sulphide to nitrate ratio enough time was given to reach the steady state, and then the next ratio was tested. Sampling and monitored parameters were the same as those described earlier. All the sulphide to nitrate ratios as listed above were tested in both bioreactors.

4.3.2 Heterotrophic Denitrification in Batch System

1) Effects of nitrate and acetate concentrations

Effects of nitrate and acetate concentrations on heterotrophic denitrification were investigated by conducting two sets of experiments at room temperature (23°C). In the first set, nitrate was the limiting substrate where 5, 10, 15, 20, 30, 50 mM nitrate with 30 mM acetate were used. In the second set, acetate served as the limiting substrate where 50 mM nitrate and 5, 10, 15, 20, 30 mM acetate concentrations were used.

2) Effect of temperature

Effect of temperature (15, 20, 25, 30 and 35°C) on the heterotrophic denitrification was evaluated using CSB medium containing 30 mM acetate and 20 mM nitrate. Another set of experiments was conducted at higher concentration of 30 mM acetate and 50 mM nitrate, but this time only selected temperatures (15, 20 and 30°C) were tested, due to time constraint.

All the batch experiments were run in duplicates. Control experiments were also conducted under exact conditions in the absence of bacteria (no inoculation).

4.3.3 Heterotrophic Denitrification in CSTB System

Effects of volumetric loading rate of nitrate on heterotrophic denitrification were investigated in two identical CSTB systems operated at room temperature (23°C). One bioreactor was fed with CSB medium containing 10 mM acetate and 10 mM nitrate, and the other CSTB was operated with CSB medium containing 30 mM acetate and 30 mM nitrate. This allowed us to evaluate the possibility of treatment of high nitrate concentration in the CSTB system.

4.3.4 Heterotrophic Denitrification in Continuous Biofilm Reactor

1) Effect of volumetric loading rate (increase of feed flow rate)

To investigate the effects of volumetric loading rate on heterotrophic denitrification, CSB medium containing 30 mM acetate and 15 mM nitrate was introduced into the reactor. The flow rate was increased stepwise until a decrease in performance of the system in terms of nitrate removal rate was observed.

2) Effect of volumetric loading rate (increase of feed concentration)

The effect of volumetric loading rate was also investigated in the same biofilm reactor at a constant flow rate of 50 mL h^{-1} and increasing the concentration of nitrate and acetate in the feed. This experiment was followed with the above biofilm experiments and the flow rate was decreased to 50 mL h^{-1} and kept constant after then. The CSB medium containing 30, 50, 75, 100 and 150 mM (both acetate and nitrate) was pumped into the reactor. With each medium, enough time was given for the system to reach to the steady state conditions and then next medium was tested.

4.4 Analytical Methods

4.4.1 Sulphide Concentration

The sulphide concentration was determined using a spectrophotometric method as described by Cord-Ruwisch (Cord-Ruwisch 1985). A 5.0 mM acidic copper sulphate solution containing 0.8 g L^{-1} of copper sulphate and 4.1 mL L^{-1} of HCl (36.5-38%) was prepared first. Then 0.1 mL of the sample containing sulphide was added to 0.9 mL of 5.0 mM acidic copper sulphate solution. The absorbance of the mixture was measured at 480 nm using a spectrophotometer (SHIMADZU UVmini-1240 spectrophotometer). A calibration curve already generated using the same procedure and standard sodium sulphide solutions (0-10 mM) was used to determine the concentration of sulphide in the unknown samples.

4.4.2 Acetate, Nitrite, Nitrate, Sulphate and Thiosulphate Concentrations

The concentrations of acetate, nitrite, nitrate, sulphate and thiosulphate were determined using ion chromatography. A Dionex Ion Chromatograph (ICS-2500) with a conductivity detector (CD25A) equipped with an IonPac CG5A guard column and an IonPac CS5A analytical column was used. The eluent was 1.0 mM KOH and the flow rate of the eluent was set at 1.5 mL h^{-1}. The software used to operate the ion chromatograph was Chromeleon version 6.60 (Dionex Corporation, Sunnyvale, CA, U.S.A.). The system was calibrated using standard solutions of acetate, nitrite, nitrate, thiosulphate and sulphate with concentrations of 5, 10, 15 and 20 ppm. The standard deviation associated with measurements of nitrate, nitrite, sulphate, thiosulphate and acetate, determined by repeated analysis of 5 samples of known concentrations for each ion were 0.017, 0.009, 0.053, 0.100, and 0.039 ppm, respectively.

4.4.3 Biomass Measurement in Desulphurization Experiments

In the desulphurization experiments, measurement of biomass concentration through determination of optical density was not possible due to the presence of sulphur particles. Therefore, measurement of protein concentration in the samples was used to estimate the biomass concentration. The protein concentration was determined using a Coomasie Plus Bradford Assay Kit (Pierce, Rockford Illinois, U.S.A.). A calibration curve was generated first by treating the standard solutions of bovine serum albumin with the reagent and measuring the absorbance of the resulting mixture at 595 nm using a SHIMADZU UVmini-1240 spectrophotometer. To measure the protein concentration in sample, 1 mL sample solution was sonicated for 2 minutes at 10 Watts using a BRANSON Model 450 Sonifier (Branson Ultrasonics, Dansbury, CT, U.S.A.). The sample was then mixed with the assay reagent, and the absorbance was measured at 595 nm. Using the calibration curve generated by standard solutions, the protein concentration in the sample was calculated. Assuming that protein forms approximately 50% of the biomass weight (Bratbak and Dundas, 1984), these protein measurements were converted to biomass concentration.

4.4.4 Sulphur Estimation

To assess the extent of produced sulphur in the desulphurization experiments, the concentration of suspended particles were determined. Sulphur concentration was then calculated by subtracting the biomass concentration from the suspended solid concentration.

To determine the concentration of suspended particles, 5 mL sample was put into a weighted micro tube and centrifuged for 5 min at 9180 ×g (Microfuge 18 Centrifuge, Beckman Coulter, USA). The supernatant liquid was removed and the micro tube was dried in an oven (35ºC, one week). The difference between the weight of the empty tube and that containing dried particles was determined and used to calculate the concentration of suspended particles and sulphur.

4.4.5 Biomass Concentration in Heterotrophic Denitrification Experiments

Due to absence of sulphur particles during the heterotrophic nitrification, measurement of optical density was used as a means to determine the biomass concentration. First a calibration curve relating the optical density to biomass concentration was generated by measuring the absorbance (at 620 nm) of cell culture samples containing known cell dry weight concentrations. The culture used for calibration was a three-day old batch culture

with the CSB medium containing 30 mM acetate and 50 mM nitrate which was diluted with reverse osmosis water at ratios of 1:0; 4:1; 1:1; 1:3 and 1:4. The dry weight of the cells (methods as described in Section 4.4.4) and optical density (620 nm, SHIMADZU UVmini-1240 spectrophotometer) were determined in these samples and used to generate the calibration curve.

4.4.6 pH Measurement

For pH measurement, 2.0 mL sample was taken and pH was determineded by a pH meter (PerpHecT Meter, Models 330, Thermo Orion, USA).

4.5 Statistical Analysis of Results

As described earlier, in this study every set of batch experiments were conducted in duplicates. The average values of the data obtained in the repeated experiments were presented as final results. The standard deviation associated with data was calculated and presented as error bar.

For experiments conducted in the continuous bioreactors (CSTB and biofilm reactor), following the establishment of steady state at each applied conditions three additional samplings were conducted in three consecutive days and the average value of these data was used as the final result. The standard deviation associated with the data was also calculated and presented as error bar. Where applicable the value of regression coefficient was calculated and included as part of results.

CHAPTER 5
RESULTS AND DISCUSSION

The results and discussion of autotrophic denitrification with sulphide and heterotrophic denitrification with acetate in batch, CSTB and biofilm systems were presented in the following sections.

5.1 Autotrophic Denitrification with Sulphide in CSTB

5.1.1 Effects of Feed Sulphide Concentration

1) Concentration profiles

The concentration profiles of various ions in CSTB operated with different sulphide concentrations (10, 15 and 20 mM) are shown in Figure 5-1 (sulphide, nitrate and sulphate concentration profiles) and Figure 5-2 (acetate, nitrite and thiosulphate concentration profiles). For feed with 10 mM sulphide system, sulphide and nitrate residual concentrations observed in the reactor and effluent were zero for dilution rates up to 0.15 h^{-1}, indicating the complete removal percentages of nitrate and sulphide. Further increase of the dilution rate (a slight increase of 0.02 h^{-1}) led to cell washout and a sudden increase of concentrations of sulphide and nitrate which over a period of 48 h reached to values close to those in the feed. At the lowest tested dilution rate a sulphate concentration around 8 mM was observed. The increase of dilution rates caused a decrease in concentration of sulphate from 8 mM to a value around 3.5 mM. Considering the concentration of sulphate in the medium (2.8 mM) it can be concluded that at lower dilution rates most of the sulphide was converted to sulphate, while at higher dilution rates which corresponded to shorter residence time sulphur was the main product.

Nitrite concentration was around zero for the entire range of applied dilution rates, indicating that during the oxidation of sulphide, nitrate was likely reduced to nitrogen at the sulphide to nitrate ratio 2. Thiosulphate concentration was very low (0.54 mM) over the entire range of applied dilution rates. One should be noted that the medium fed to the bioreactor contained this level of thiosulphate possibly due to spontaneous oxidation of sulphide in the medium bag containing the feed. Acetate concentration did not change significantly (the average decrease in acetate concentration was 1.5 mM) over the entire

range of applied dilution rates, indicating that the microbial culture relied on CO_2 (supplied as carbonate) as a carbon source and sulphide as an energy source.

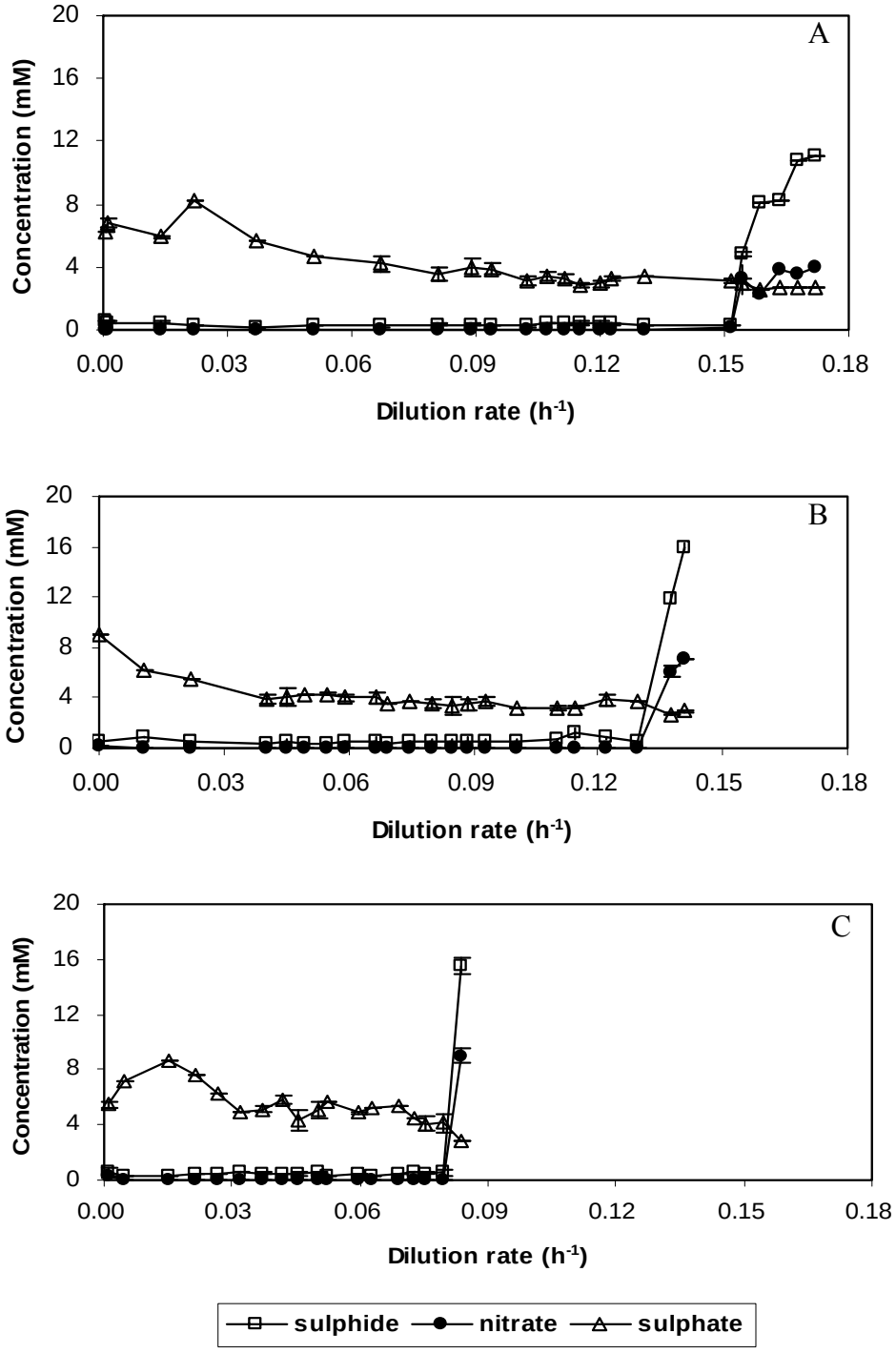

Figure 5-1 Concentration profiles of sulphide, nitrate and sulphate in CSTB operated with 10 (A), 15 (B) and 20 (C) mM sulphide in feeds

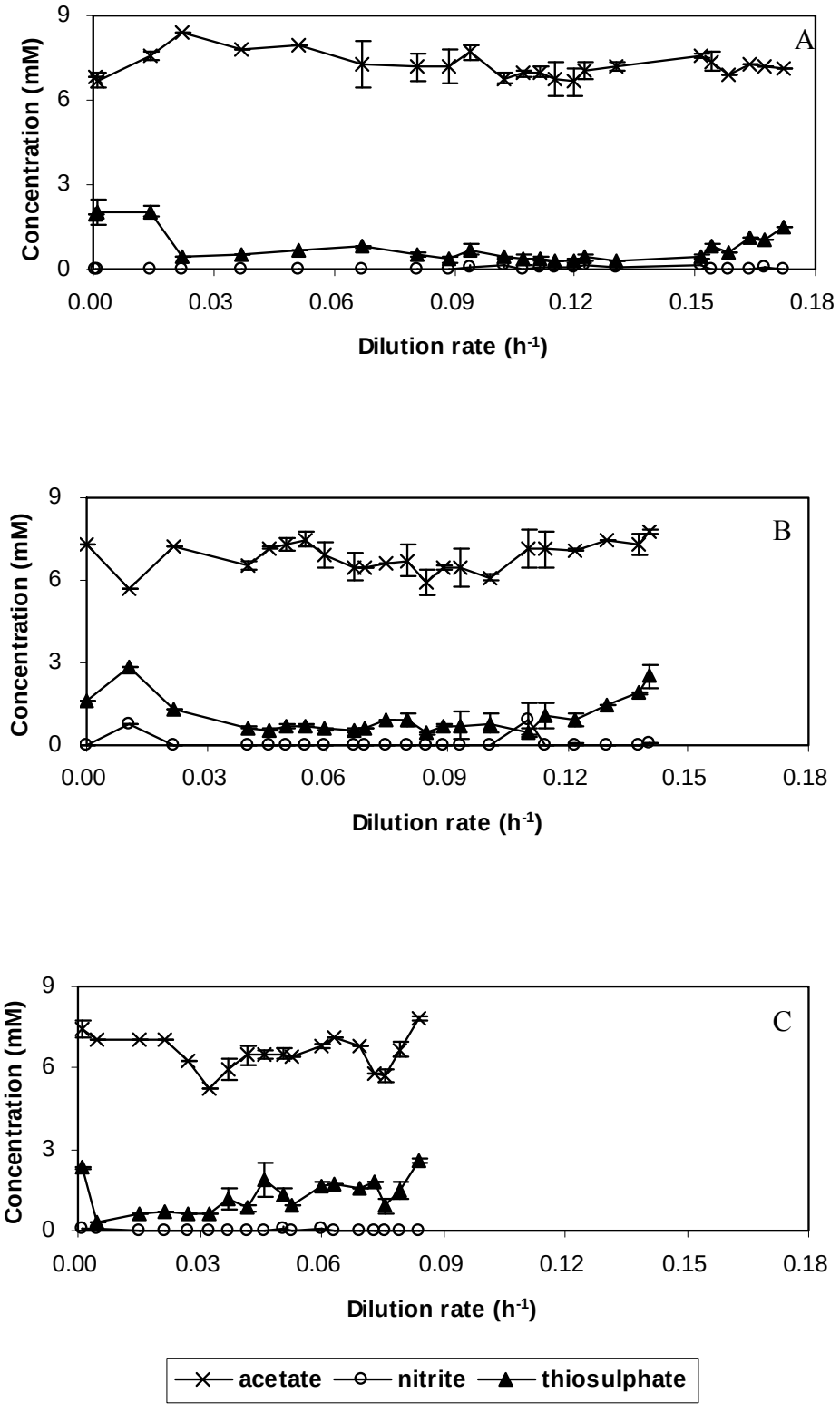

Figure 5-2 Concentration profiles of acetate, nitrite and thiosulphate in CSTB operated with 10 (A), 15 (B) and 20 (C) mM sulphide in feeds

The maximum protein concentration in this run was around 50 mg L^{-1} observed at a dilution rate of 0.12 h^{-1}. The protein concentration dropped to zero when the dilution rate was increased to 0.17 h^{-1}, an indication of cell washout. Effluent pH varied in the range 7.5 to 8.1 for dilution rates below or around 0.15 h^{-1} and decreased to 7.0 (same pH value as the in feed medium) when washout occurred.

The concentration profiles in the CSTB operated with 15 and 20 mM sulphide showed similar patterns to those observed in the reactor with 10 mM sulphide. For reactor with 15 mM sulphide, the critical value of dilution rate at which wash out occurred was 0.13 h^{-1}. The complete sulphide and nitrate removal percentages were achieved up to dilution rate 0.13 h^{-1} and a slight increase of dilution rate (0.01 h^{-1}) led to cell washout. The maximum protein concentration in this run was around 180 mg L^{-1} observed at a dilution rate of 0.08 h^{-1} and protein concentration decreased to zero with cell washout. In the reactor operated with 20 mM sulphide, the washout of cell occurred at a much lower dilution rate of 0.08 h^{-1} and the complete sulphide,nitrate removals were achieved when dilution rate was lower than 0.08 h^{-1}. In this run a slight increase of dilution rate of 0.004 h^{-1} lead to complete washout of the cells, indicating CSTB with initial sulphide concentration 20 mM was more sensitive to variation of dilution rate when compared with CSTBs operated with 10 and 15 mM. Sulphate concentration was around 5.0 mM, except for dilution rates below 0.02 h^{-1} where sulphate concentrations of 7-8 mM were observed. Sulphate concentration in this run was lower than those observed in the previous runs (10 and 15 mM sulphide in the feed) at the same dilution rate condition. This observation indicated that when higher feed sulphide concentration was used, a smaller portion of sulphide was converted to sulphate and sulphur was the main end product. The maximum protein concentration in this run was around 100 mg L^{-1} observed at a dilution rate of 0.07 h^{-1}. It should be indicated that in order to assess the reproducibility of the data we have repeated the experimental runs and the results are shown in Figure 5-3 (only the sulphide, nitrate and sulphate concentrations profile in CSTB with 20 mM sulphide feed was chosen according to the space limitation requirement of thesis) which indicates the reasonable reproducibility of the results.

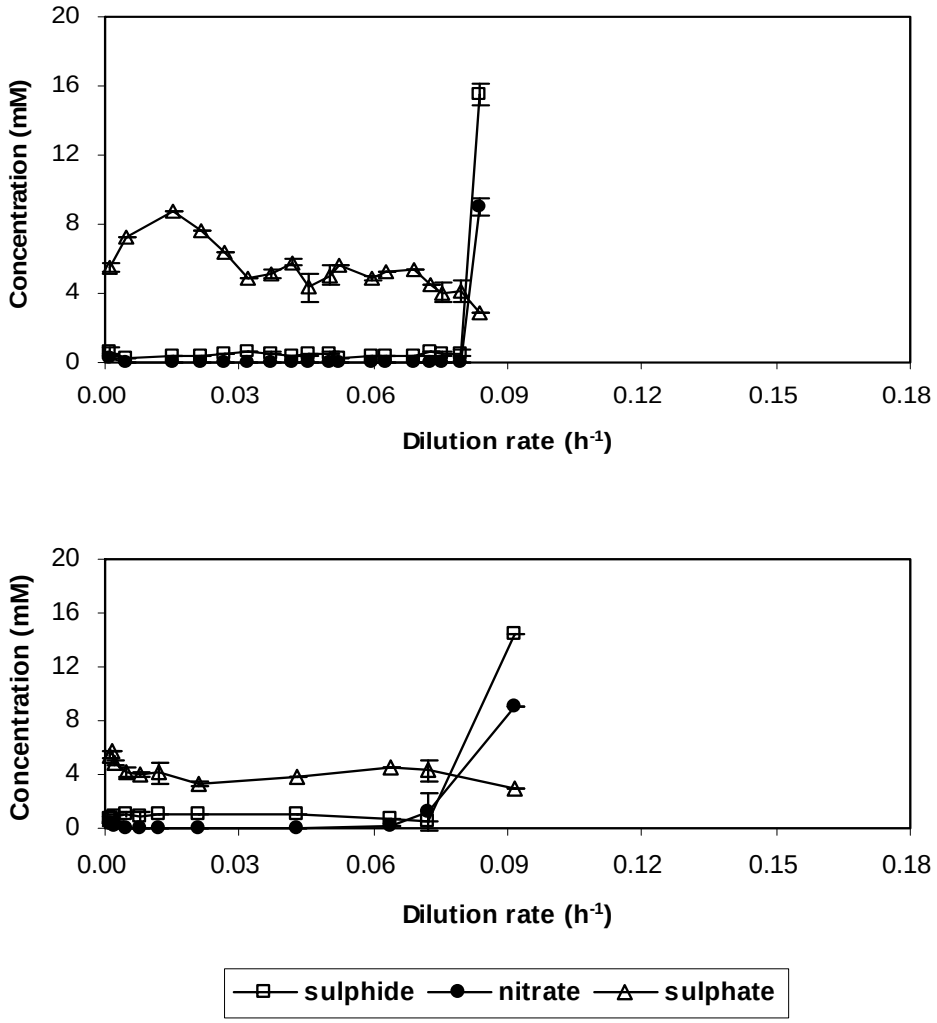

Figure 5-3 Concentration profiles of sulphide, nitrate and sulphate in CSTB operated with 20 mM sulphide in feeds (repeated runs)

2) Removal percentage and removal rate

The dependency of sulphide and nitrate removal rates on their respective loading rates in the bioreactors operated with 10, 15, 20 mM initial sulphide concentrations are compared in Figure 5-4. The observed sulphide removal rates in these reactors had the similar dependencies on sulphide loading rate, regardless of initial concentration of sulphide. However, it should be noted that the maximum sulphide removal rate (1.80 mM h^{-1}) occurred in the reactor operated with 15 mM sulphide. Furtheremore, washout of the cells and sudden decrease in removal rate occured at a higher sulphide loading rate of 1.87 mM h^{-1} when compared with those observed in the reactors operated with initial sulphide concentrations of 10 and 20 mM (1.46 and 1.51 mM h^{-1}, respectively). The nitrate removal rates showed similar dependencies on nitrate loading rate, regardless of initial concentration of sulphide or

nitrate. However, the highest nitrate removal rate of 0.92 mM h^{-1} obtained in the CSTB operated with 15 mM sulphide. In addition, washout of the cells and sudden decrease in removal rate also occured at a higher nitrate loading rate of 0.93 mM h^{-1} when compared with those observed in the reactors operated with initial sulphide concentrations of 10 and 20 mM (0.74 and 0.75 mM h^{-1}, respectively).

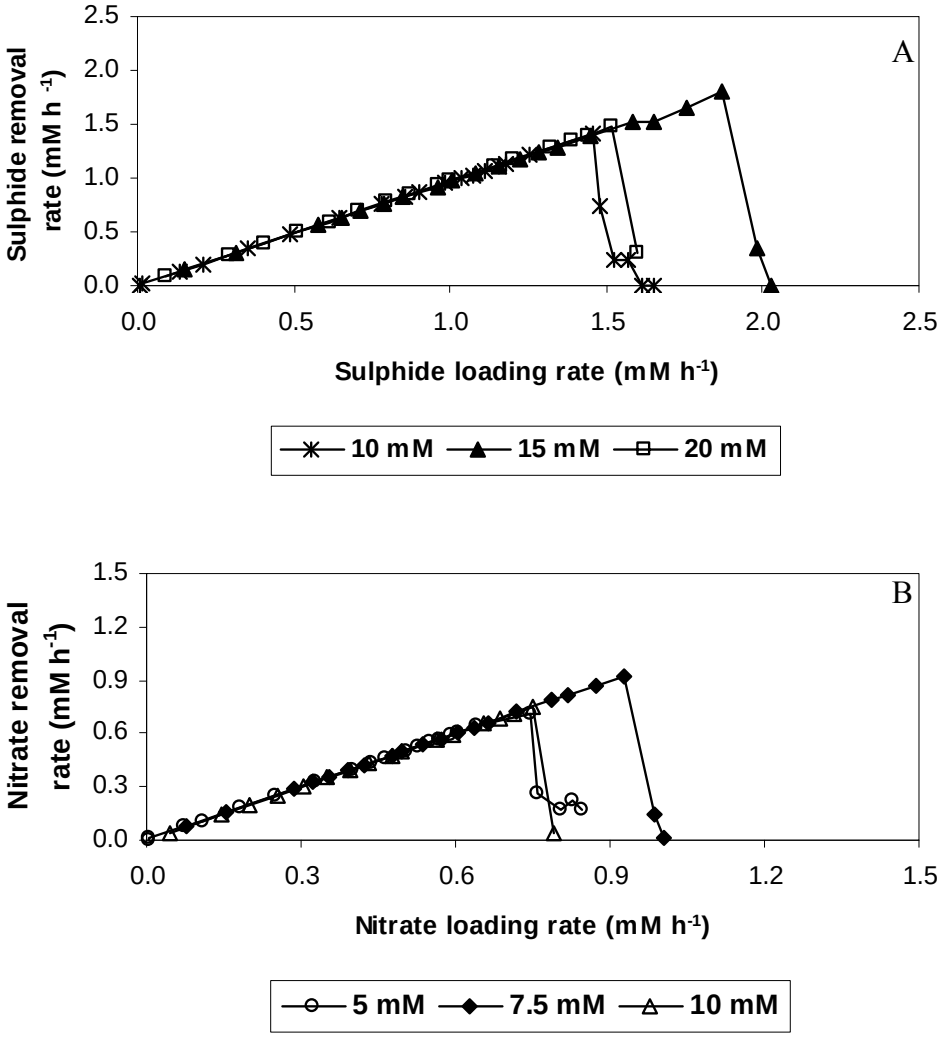

Figure 5-4 Effects of loading rate on sulphide (A) and nitrate (B) removal rates in the CSTB operated with different sulphide concentrations

The dependency of sulphide and nitrate removal percentages on sulphide and nitrate loading rates are shown in Figure 5-5. Close to 100% sulphide and nitrate removals percentages were achieved in all three reactors when loading rate was below or close to the critical loading rates at which cell washout occurred. The removal percentages for both

sulphide and nitrate decreased to zero suddenly when the loading rates increased slightly above the critical value.

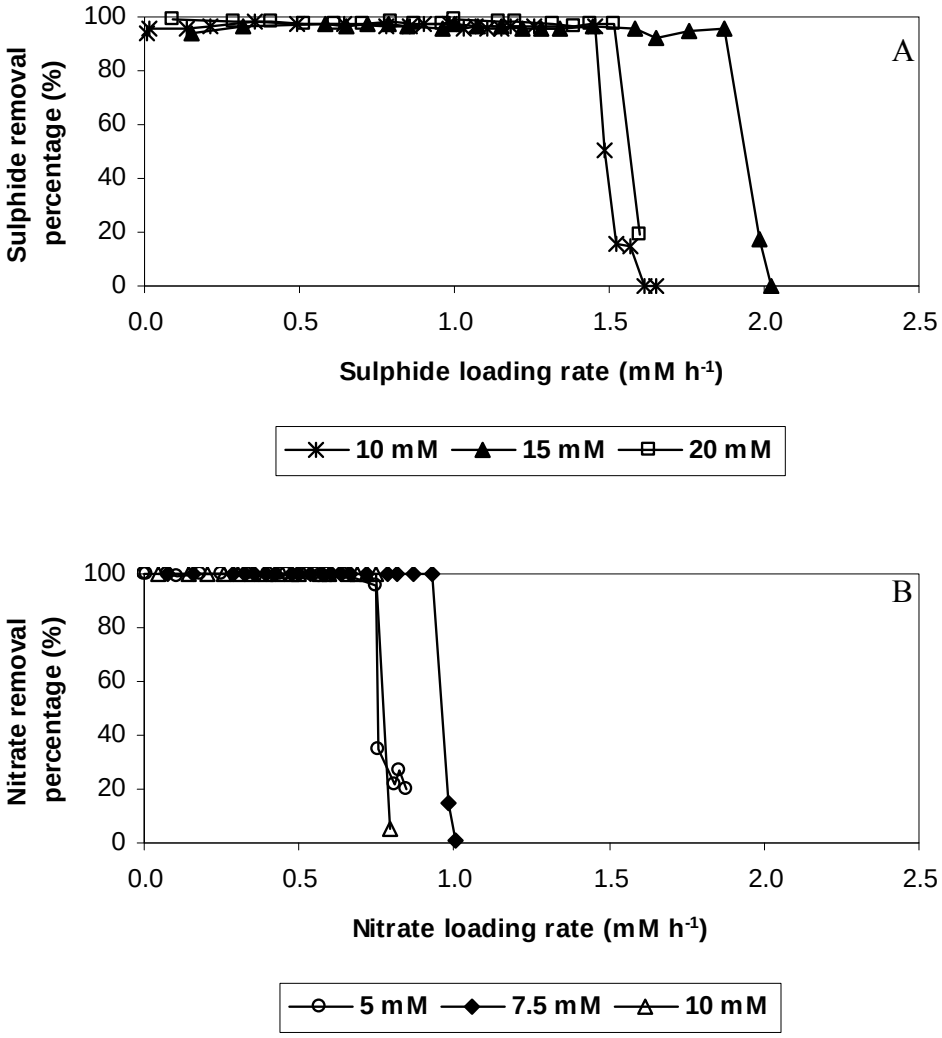

Figure 5-5 Effects of loading rate on sulphide (A) and nitrate (B) removal percentages in the CSTB operated with different sulphide concentrations

3) Conversion of sulphide to sulphate (composition of end products)

The dependency of percentage of sulphide converted to sulphate on sulphide loading rate is shown in Figure 5-6. Regardless of the initial sulphide concentration in the feed, a similar pattern was observed. With the increases of sulphide loading rates (decrease of residence time), the percentage of sulphide converted to sulphate decreased and sulphur became the dominant end product. In the reactor operated with 10 mM sulphide, the highest percentage of sulphide converted to sulphate was 43% observed at 0.01 mM h^{-1} (the lowest tested value)

sulphide loading rate and the percentage decreased to 0.9% when the highest loading rate of 1.48 mM h^{-1} was applied. Similar results were achieved in the other two systems with 15 and 20 mM sulphide. The results obtained indicated that through controlling sulphide loading rate, the composition of the end product can be controlled toward the production of desired product.

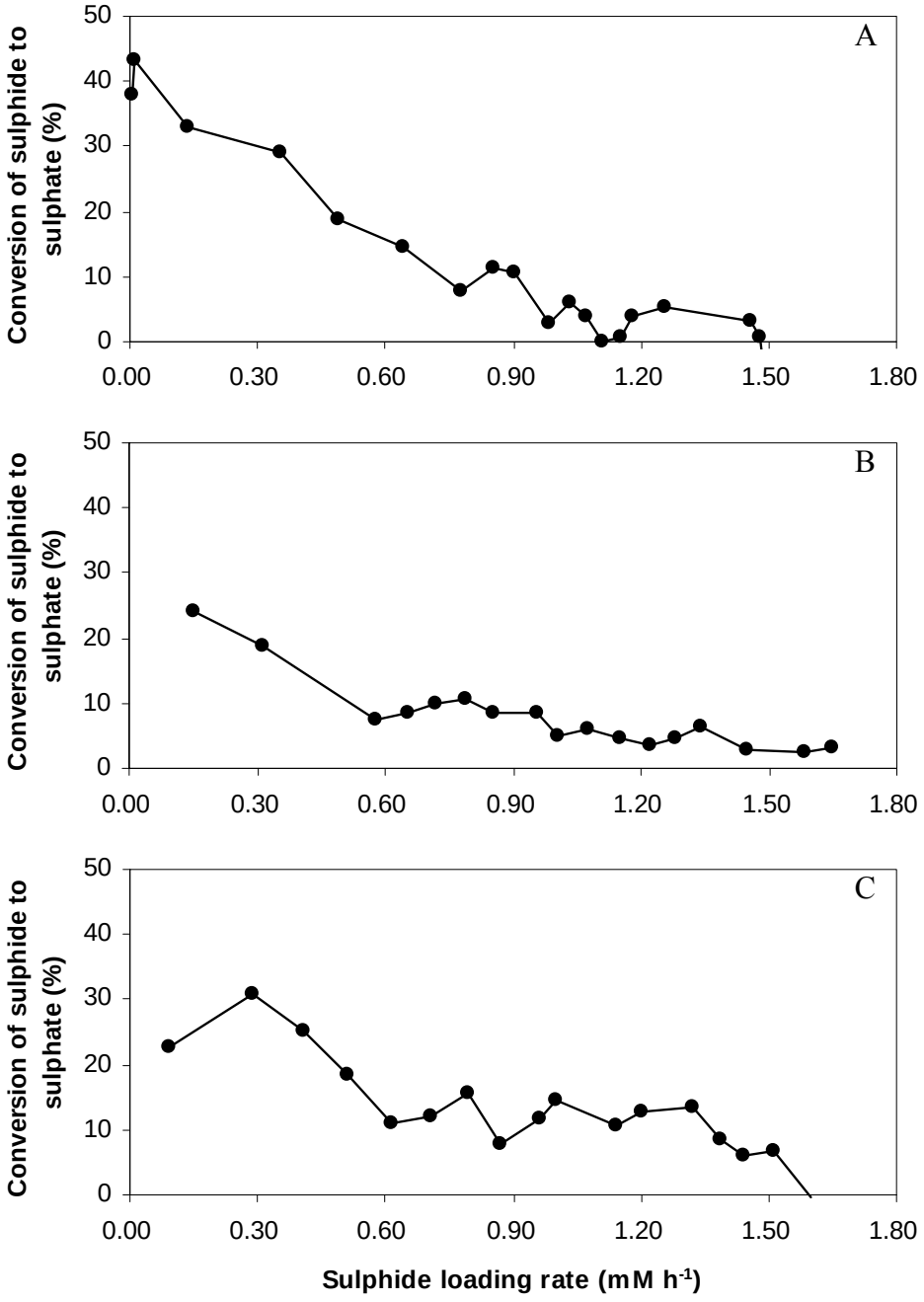

Figure 5-6 Effects of sulphide loading rate on the percentage of sulphide converted to sulphate in three CSTBs operated with A: 10 mM sulphide; B: 15 mM sulphide; C: 20 mM sulphide in the feed

5.1.2 Effects of Sulphide to Nitrate Ratios

1) Concentration profiles

The concentrations profiles of various ions obtained in the CSTBs with constant dilution rates 0.015 h^{-1} at different sulphide to nitrate ratio are shown in Figure 5-7. In this system, the residual sulphide concentration was very low (lower than 0.6 mM) at sulphide to nitrate ratios from 0.10 to 3.39 (corresponding the sulphide removal higher than 98%), while almost 1 mM sulphide was left when sulphide to nitrate ratio was 5.34, due to limitation of nitrate (nitrate is the sole electron acceptors for sulphide oxidation in this system). The sulphate concentration increased with the decreases of sulphide to nitrate ratio that confirmed more sulphide was oxidized to sulphate when there was sufficient nitrate in the system. Thiosulphate concentration did not show obvious changes for the entire range of applied ratio and its value remained constant and around the concentration in the feed. Acetate concentration kept decreasing and eventually dropped to zero with the decrease of sulphide to nitrate ratio which indicated a shift in metabolism of microbial culture. Under low sulphide to nitrate ratio conditions, due to lack of sufficient sulphide (electron donor) acetate was used to reduce the remaining nitrate (heterotrophic denitrification). In other words the bacteria switched from the autotrophic metabolism to the heterotrophic one. Another important conclusion was that when both organic and inorganic electron donors (acetate and sulphide, respectively) were provided, the bacteria used inorganic electron donor (sulphide) to reduce nitrate first and following the consumption of sulphide organic electron donor (acetate) was used. Residual nitrate (around 89 mM) was only present in the bioreactor at the lowest ratio of 0.1 which corresponded to the highest level of nitrate in the feed (152 mM). Reduction of nitrate results in formation of nitrite only at 0.25 and 0.1 ratios and at concentrations of 28 and 30 mM, respectively.

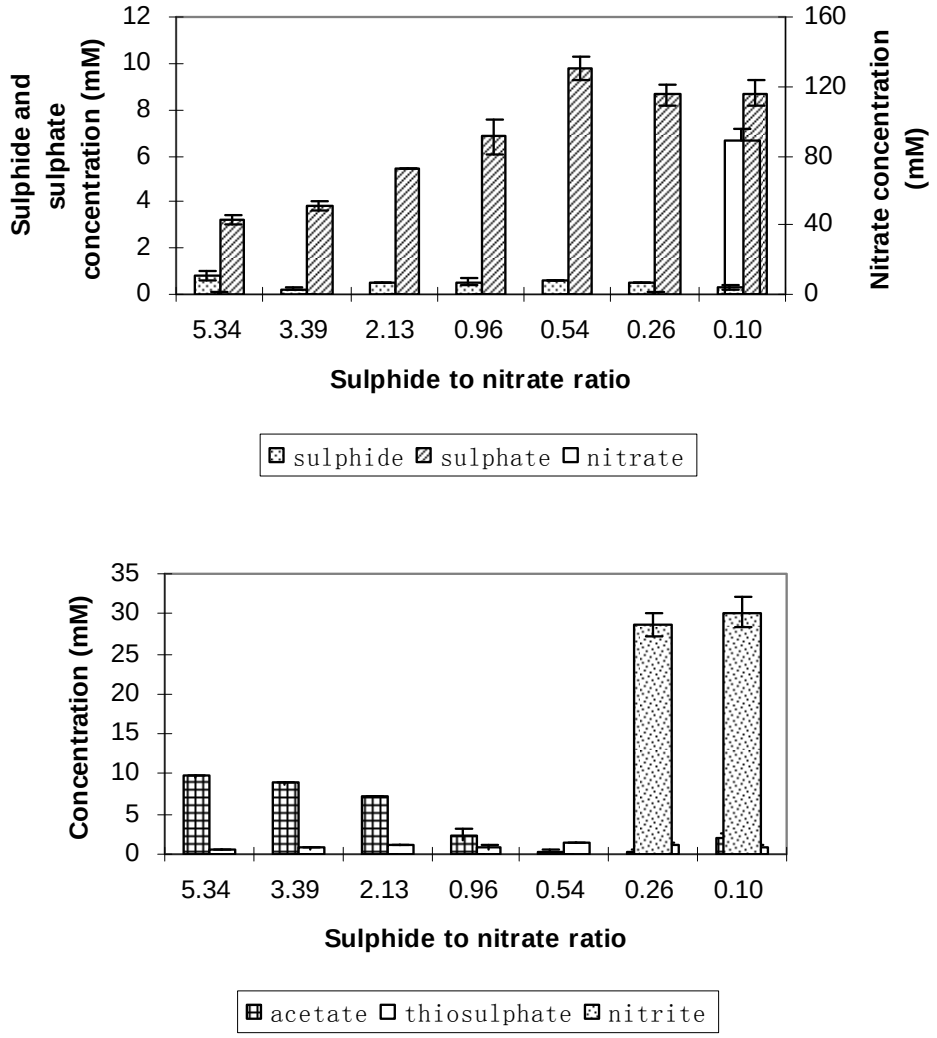

Figure 5-7 Concentration profiles of various ions in CSTBs operated with different sulphide to nitrate ratios in the feed at dilution rate 0.015 h^{-1}

The concentration profiles in the CSTB operated at dilution rate of 0.075 h^{-1} were shown in Figure 5-8 and were different from that at 0.015 h^{-1}. Cell washout happened at sulphide to nitrate ratio of 3.5, potentially due to combination effects of short residence time and limited nitrate supply. Sulphide removal percentage with higher than 96% was achieved at other applied ratios and sulphate concentration increased with decreases in sulphide to nitrate ratio. But at similar ratio, the conversion of sulphide to sulphate was lower than that achieved in the reactor with dilution rate 0.015 h^{-1}, confirming that a shorter retention time favored the formation of sulphur. In contrast with previous run, acetate concentration showed a very small fluctuation and was close to that in the feed, proving that culture did not use acetate for nitrate reduction even when residue nitrate concentration was not zero after the complete

sulphide oxidation. The reason for this different behavior could be likely due to short retention time as that was the only difference between these two reactors. Residual nitrate concentrations of 7.3, 9.9, 30.8 and 126.1 were measured at the ratios of 1, 0.5, 0.25 and 0.1, respectively.

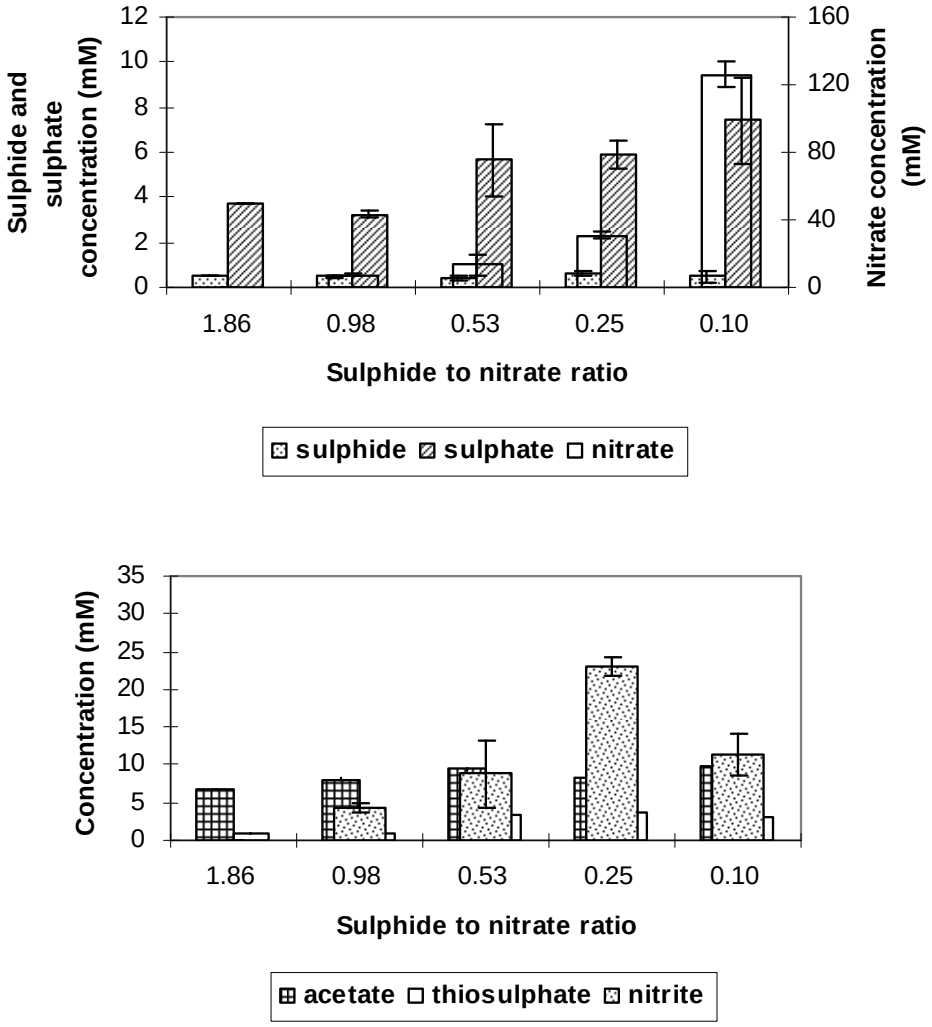

Figure 5-8 Concentration profiles of various ions in CSTBs operated with different sulphide to nitrate ratios in the feed at dilution rate 0.075 h^{-1}

2) Reaction rate and removal percentage

In both reactors operated at dilution rates of 0.015 and 0.075 h^{-1} (corresponding to the sulphide loading rate 0.23 and 1.12 mM h^{-1} respectively), almost complete desulphurization was achieved at all tested sulphide to nitrate ratios and the sulphide removal rate showed same dependency on its loading rate established in the previous runs with different feed

sulphide concentrations. Sulphide removal rates were 0.22 and 1.08 mM h^{-1} in the reactors operated at 0.015 and 0.075 h^{-1}, respectively.

The nitrate removal rate and nitrate removal percentage as a function of nitrate loading rate are shown in Figure 5-9. In the reactor operated at 0.015 h^{-1}, a linear relationship between nitrate removal rate and nitrate loading rate was observed for nitrate loading rates up to 0.86 mM h^{-1}. Further increase of nitrate loading did not increase its removal rate. Complete nitrate removal percentage (100%) was achieved with nitrate loading rates up to 0.86 mM h^{-1} and nitrate removal percentage decreased with further increases of nitrate loading rate. A similar linear relationship between nitrate removal and loading rates (for loading rates up to 4.34 mM h^{-1}) was observed in reactor operated at 0.075 h^{-1}. Nitrate removal percentage was decreased with the increases of nitrate loading rate in this reactor. The highest nitrate removal rate of 2.03 mM h^{-1} was achieved at the nitrate loading rate 4.34 mM h^{-1}, with corresponding removal percentage being 47%.

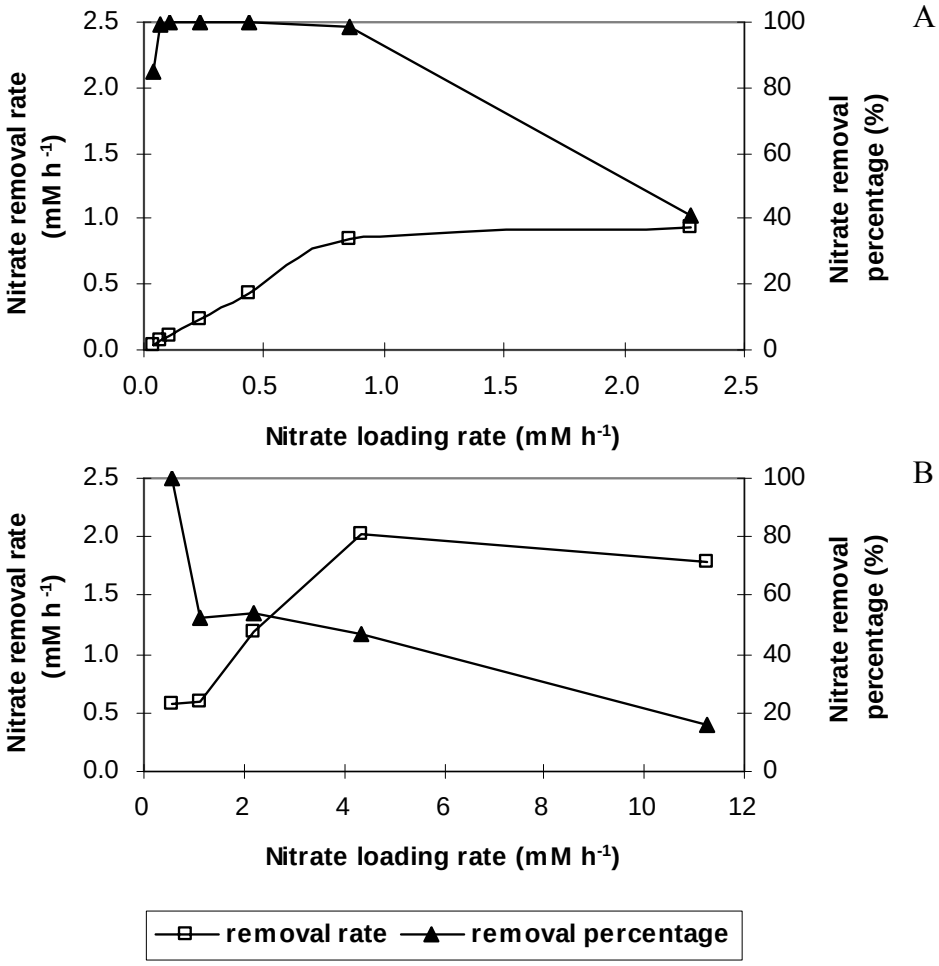

Figure 5-9 Nitrate removal rate and removal percentage in CSTB operated with feeds containing different sulphide to nitrate ratios (A: dilution rate 0.015 h^{-1}; B: dilution rate 0.075 h^{-1})

3) Conversion of sulphide to sulphate

The percentage of sulphide converted to sulphate as a function of sulphide to nitrate ratio in the reactors operated at 0.015 and 0.075 h^{-1} are shown in Figure 5-10. A similar trend was achieved in both reactors and the conversion of sulphide to sulphate decreased (from 40% to zero) with the increases of sulphide to nitrate ratios from 0.1 to 5.3 due to unavailability of sufficient nitrate at higher ratios. At the same sulphide to nitrate ratio, the conversion of sulphide to sulphate in the reactor operated at 0.015 h^{-1} was higher than that operated at 0.075 h^{-1}, clearly to longer residence time of the former. The results obtained in these experiments in general indicated that both sulphide to nitrate ratio and residence time (volumetric loading rate) can be used to control the composition of the end products toward the desired product whether sulphur or sulphate.

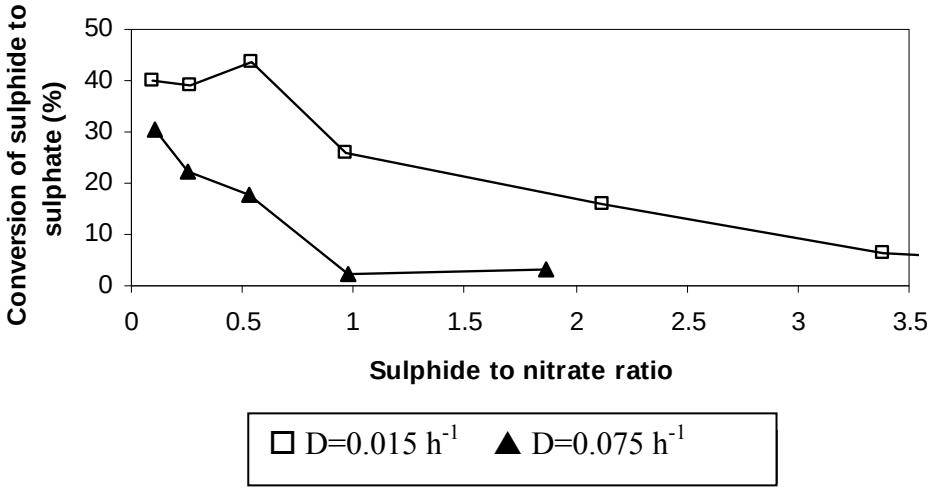

Figure 5-10 Effects of sulphide to nitrate ratio on the conversion of sulphide to sulphate

5.2 Heterotrophic Denitrification with Acetate

5.2.1 Batch System

5.2.1.1 Effects of initial concentrations

1) Nitrate initial concentration

Concentration profiles for denitrification in CSB medium with 30 mM acetate and 5, 10, 15, 20, 30, 50 mM nitrate are shown in Figure 5-11 and Figure 5-12. Lag phase ranging from 4-22 h was observed during the heterotrophic denitrification with the longer lag phases occurred at higher initial nitrate concentrations. The removal of nitrate resulted in formation and accumulation of nitrite at a rate almost similar to that of nitrate removal. When nitrate

concentration decreased to zero, nitrite concentration began to decrease due to reduction of nitrite by bacteria. These results showed that during the batch heterotrophic denitrification nitrate was reduced to nitrite firstly, and then the produced nitrite was reduced further to other gaseous nitrogen compounds, like NO, N_2O and N_2 (Tugtas, *et al*., 2007 and Rezaee, *et al*., 2008).

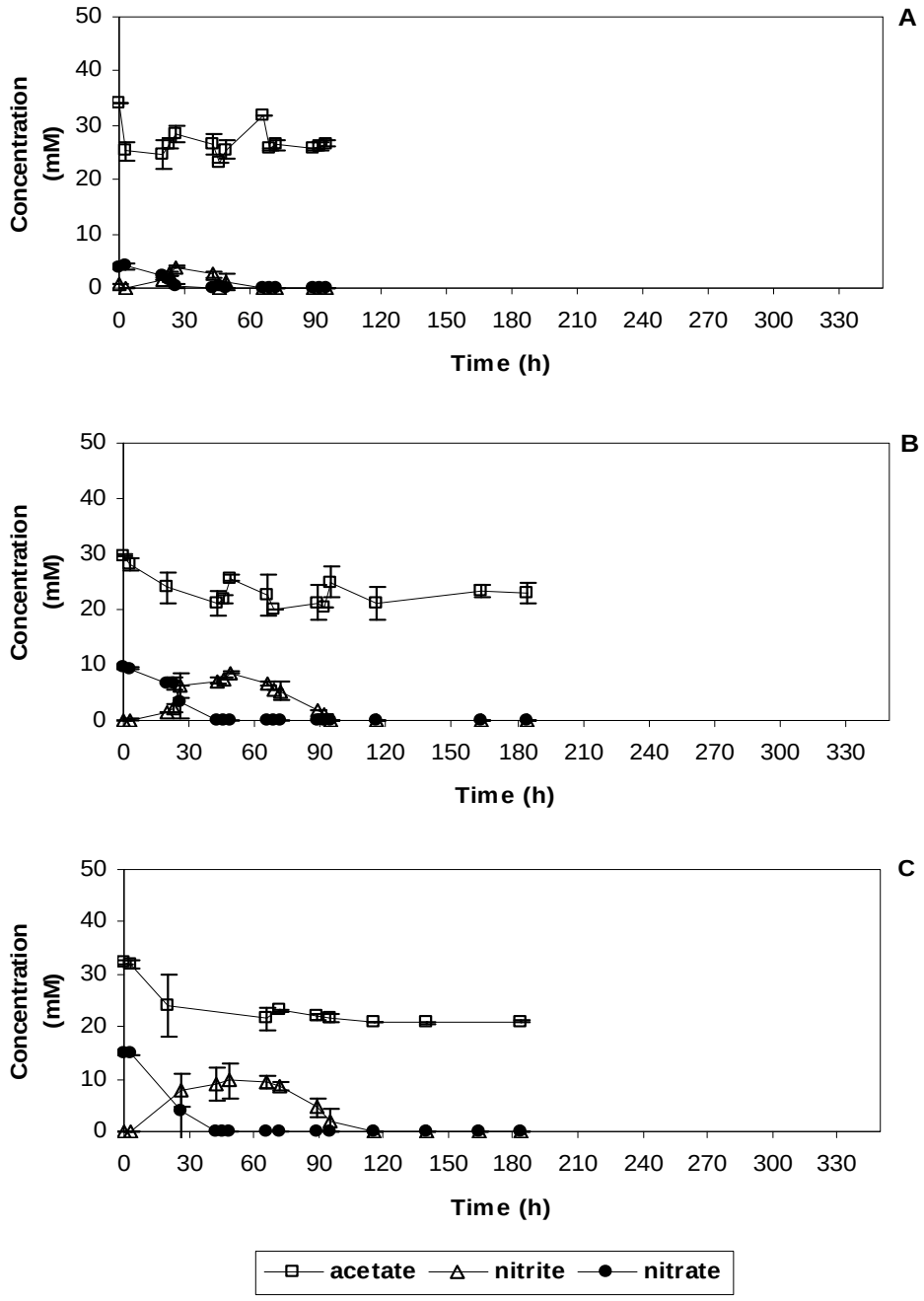

Figure 5-11 Concentration profiles during nitrate removal in batch system; 30mM acetate and (A) 5, (B) 10 and (C) 15 mM nitrate

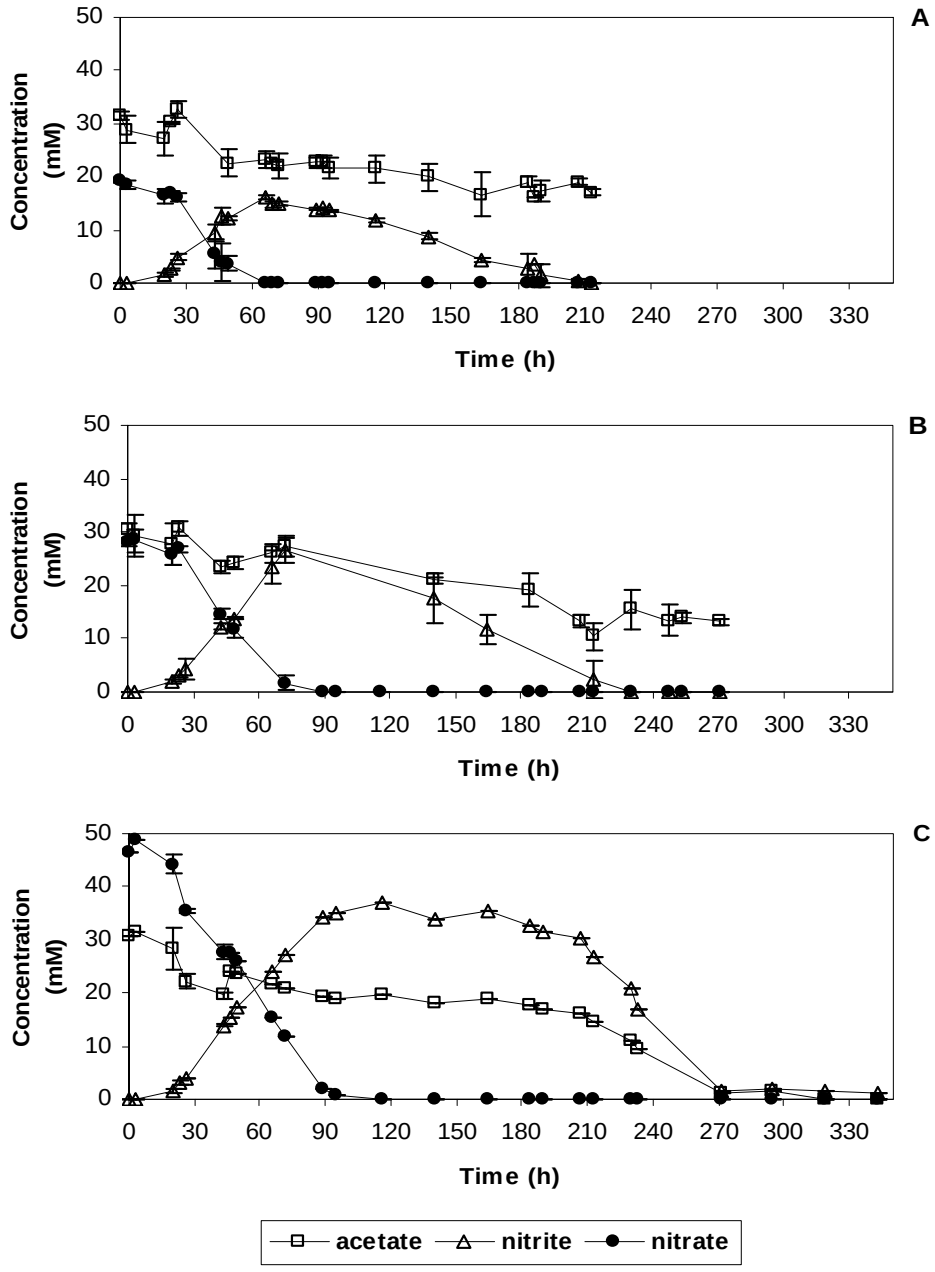

Figure 5-12 Concentration profiles during nitrate removal in batch system; 30mM acetate and (A) 20, (B) 30 and (C) 50 mM nitrate

With exception of the system containing 50 mM nitrate (Panel C in Figure 5-12), complete denitrifications were achieved in all the cases (Figure 5-11 and Panel A, B in Figure 5-12). The system with a nitrate concentration of 50 mM (C in Figure 5-12), contained a small amount of residual nitrite following the complete consumption of the provided acetate. It seemed that if sufficient acetate provided at the start of the experiment, complete

denitrification would have been achieved in this case too. Our experimental results when investigating the effect of acetate concentration confirmed this.

The specific growth rate and biomass yield were calculated using the data obtained in these experiments (the details in Appendix; Figure A-1 to Figure A-6). The average specific growth rate was determined to be 0.030±0.002 h^{-1} for the experiments with 5-20 mM nitrate and 0.016±0.002 h^{-1} for the experiments with 30-50 mM nitrate. The calculated biomass yield was in the range 0.016- 0.085 g (biomass) (g nitrate)$^{-1}$. For heterotrophic denitrificaiton with 5, 10, 15, 20 and 30 mM nitrate (Figure 5-11 and Panel A, B in Figure 5-12), the ratio of consumed amount of acetate to nitrate was in the range of 0.5-0.6.

According to the following reactions which describe simultaneous acetate oxidation and nitrate reduction, in addition to nitrite other gaseous compounds including NO, N_2O and N_2 are the possible intermediates or end products of denitrification process (Tugtas, *et al*., 2007 and Rezaee, *et al*., 2008).

$$C_2H_3O_2^- \rightarrow 2CO_3^{2-} + 8e \quad (5\text{-}1)$$

$$NO_3^- + 2e \rightarrow NO_2^- \quad (5\text{-}2)$$

$$NO_2^- + e \rightarrow NO \quad (5\text{-}3)$$

$$NO + e \rightarrow 0.5\ N_2O \quad (5\text{-}4)$$

$$0.5\ N_2O + e \rightarrow 0.5\ N_2 \quad (5\text{-}5)$$

2) Different acetate concentrations

Concentration profiles of CSB medium with 50 mM nitrate and 5, 10, 15 mM acetate are shown in Figure 5-13. Contrary to previous set of experiments, in this case acetate was the limiting nutrient for bacterial growth and nitrate was provided in excess. As can be seen with 5 and 10 mM acetate (panels A and B, respectively), following complete utilization of acetate denitrification stopped and nitrate and nitrite concentrations did not change further which was due to insufficient amount of acetate in the medium. With increases of acetate concentration in the medium (15 mM for both C and D), all 50 mM nitrate was reduced to nitrite. But no further nitrite reduction occurred in this system as there was no acetate left to provide the electrons required for nitrite reduction. For acetate concentration in the range 5-15 mM, average specific growth rate was 0.015±0.001 h^{-1} (the details in Appendix; Figure A-1 to Figure A-6). The biomass yield decreased from 0.065 to 0.028 g (biomass) (g nitrate)$^{-1}$ as acetate concentration increased from 5 to 15 mM.

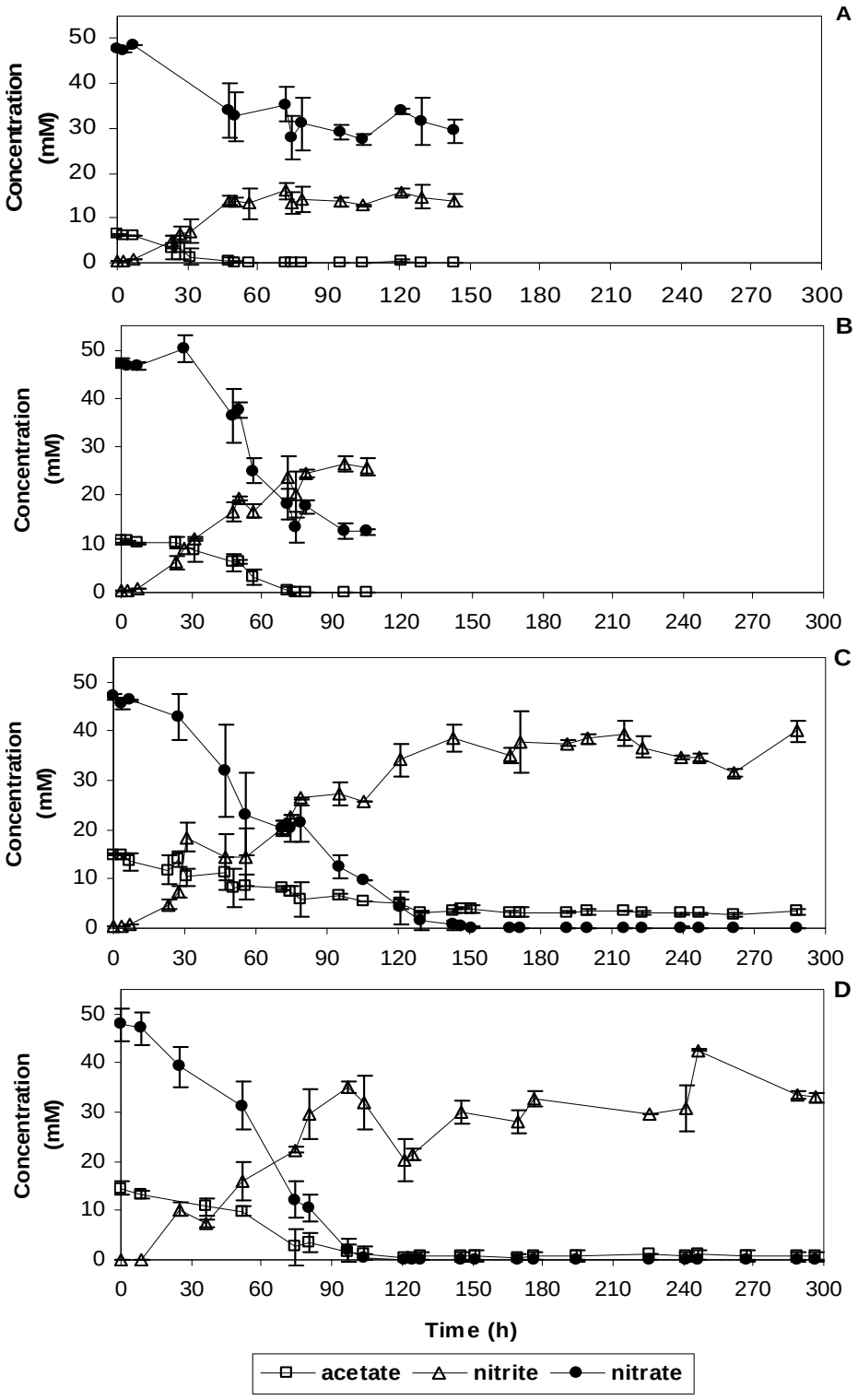

Figure 5-13 Concentration profiles during nitrate removal in batch system; 50mM nitrate and (A) 5, (B) 10, (C) 15 and (D) 15 mM acetate (Panels C and D show the results of duplicate runs)

The concentration profiles in the experiments with 50 mM nitrate and 20, 30 mM acetate were shown in Figure 5-14. In these experiments, all the nitrate (50 mM) was reduced to nitrite (maximum concentration around 35 mM), but nitrite reduction did not occur even though there was still 10 mM residual acetate in the system. The inhibitory effects of nitrite at high concentrations which is a known phenomenon may explain this incomplete denitrification. The average specific growth rates was calculated as 0.013±0.002 h^{-1} and yield of biomass increased from 0.012 to 0.029 g (biomass) (g nitrate)$^{-1}$ as acetate concentration increased from 20 to 30 mM (the details in Appendix; Figure A-1 to Figure A-6).

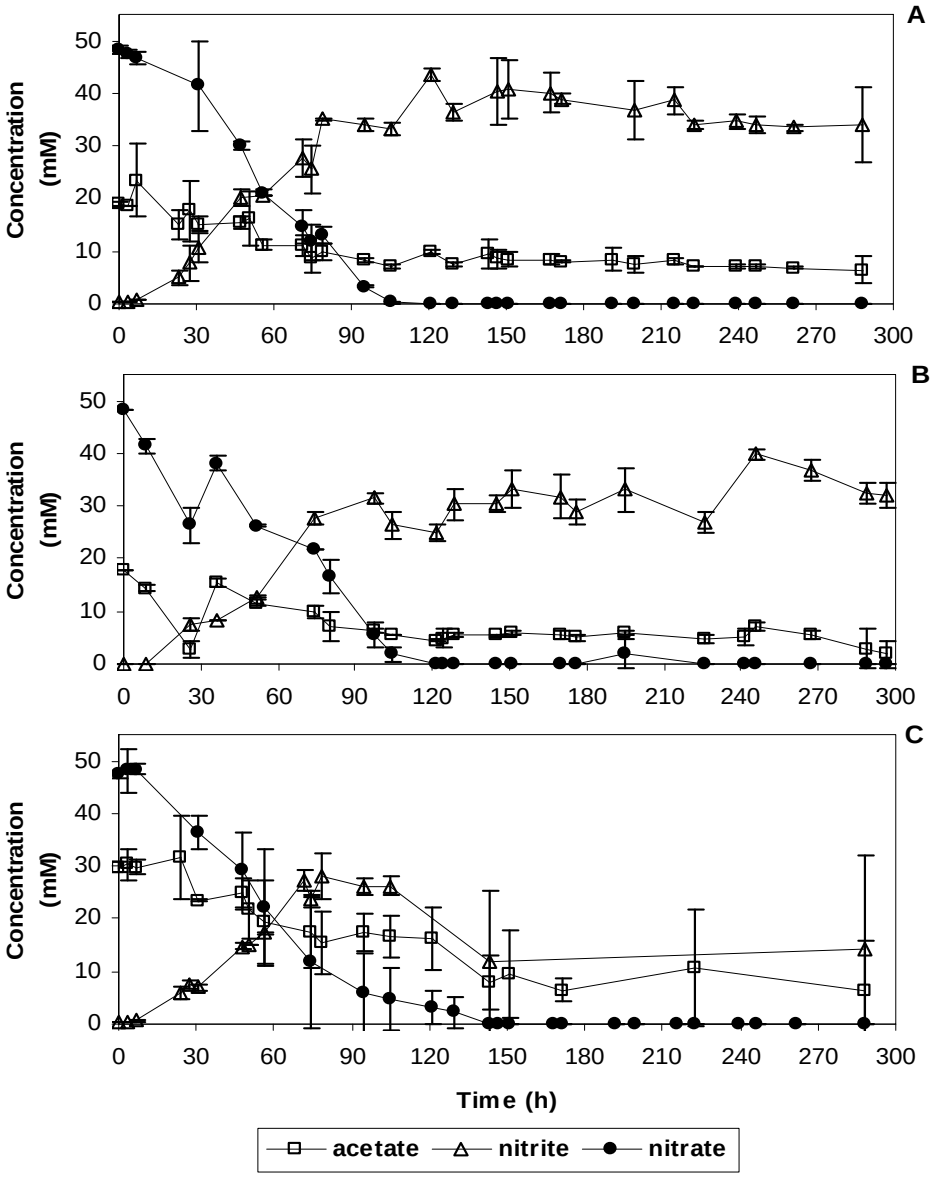

Figure 5-14 Concentration profiles during nitrate removal in batch system; 50mM nitrate and (A) 20, (B) 20 and (C) 30 mM acetate (Panels A and B show the results of duplicate runs)

5.2.1.2 Effects of temperature

Concentration profiles of heterotrophic denitrification in CSB medium containing 50 mM nitrate and 30 mM acetate at different temperatures are shown in Figure 5-15.

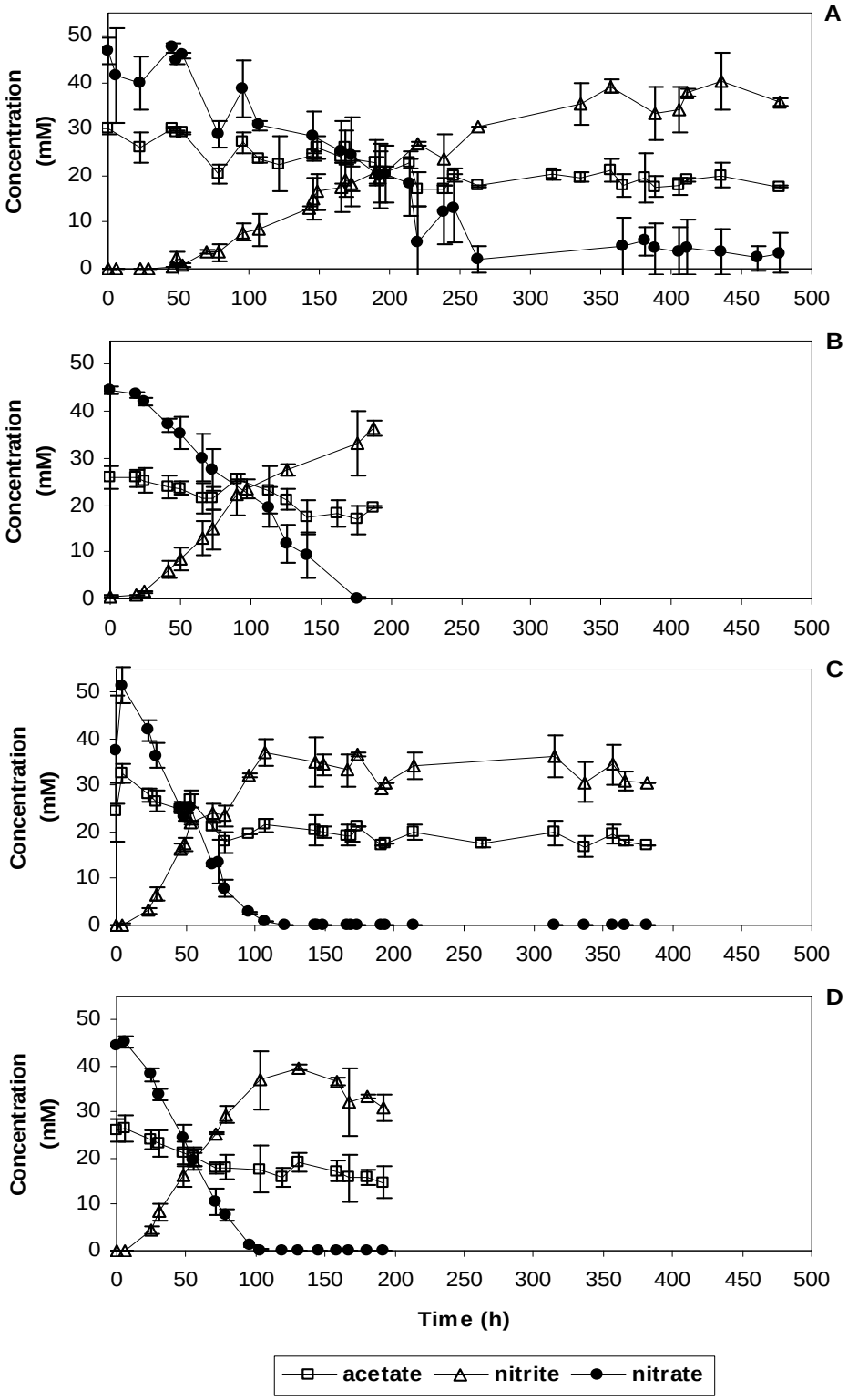

Figure 5-15 Concentration profiles during nitrate removal in batch system with 50 mM nitrate and 30 mM acetate; (A) 15, (B) 20, (C) 30 and (D) 30°C (Panels C and D show the results of duplicate runs)

In all tested temperature nitrate was completely removed, while nitrite concentrations increased and then leveled out at a value close to 40 mM. Decrease in acetate concentration occurred throughout the experiments. This indicates once again that the denitrifying bacteria used acetate (electron donors) to reduce nitrate (electron acceptors) to nitrite. However, accumulation of nitrite at concentrations around 35 to 40 mM inhibited the denitrification and acetate, nitrate and nitrite concentrations remained constant. These results were in agreement with those observed in the previous experiments in which inhibitory effect of nitrite at high concentration prevented the progress of denitrification.

Data presented in Figure 5-15 also indicated that the rate of heterotrophic denitrification increased with increases in temperature. Under the operating temperature of 15°C (Figure 5-15, Panel A), the reduction of 50 mM nitrate to nitrite took almost 400 h. At 20°C (Figure 5-15, Panel B), same process took 180 h and with 30°C (Figure 5-15, Panel C and D), only 100 h were required. It can be concluded that increasing the temperature in the range 15°C to 30°C accelerated the heterotrophic denitrification significantly.

The concentration profiles for denitrification in CSB medium containing 20 mM nitrate and 30 mM acetate (lower concentrations) at different temperatures are shown in Figure 5-16 and Figure 5-17. Since nitrate concentration in the medium was only 20 mM, the produced nitrite concentration did not exceed 20 mM. Thus, no nitrite inhibition occurred and complete denitrification was achieved in these experiments. The ratio of consumed acetate to nitrate was calculated and ranged from 0.5 to 0.6. Generally, nitrate reduction rate increased with increasing the temperatures in the range of 15°C to 35°C. The required time for reduction of 20 mM nitrate were 360 h, 210 h, 80 h and 60 h at 15°C, 25°C, 30°C and 35°C, respectively. As can be seen in the data presented in Figure 5-16 (15, 20 and 25°C, respectively), concentration of produced nitrite reached to a maximum value around 20 mM (close to initial concentration of nitrate). This confirmed again that under these conditions nitrate was reduced to nitrite first, and then the produced nitrite was reduced to other intermediates or end products. On the other hand, the maximum nitrite concentration at 30 and 35°C (Figure 5-17) was only round 12 mM. This implies that when temperature was in the range of 30°C to 35°C, the reduction of nitrate and nitrite were occurring simultaneously.

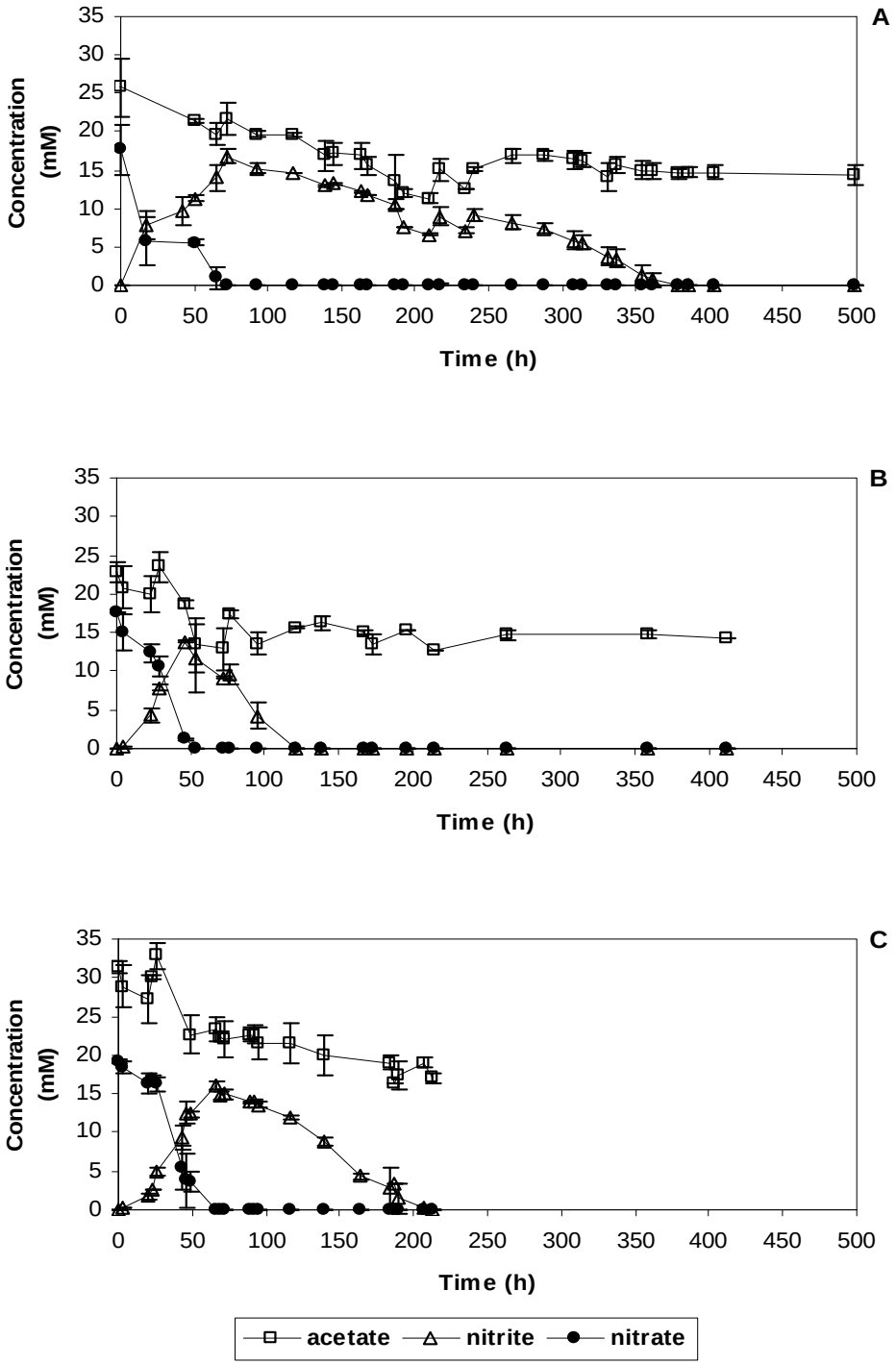

Figure 5-16 Concentration profiles during nitrate removal in batch system with 20 mM nitrate and 30 mM acetate; (A) 15, (B) 20 and (C) 25°C

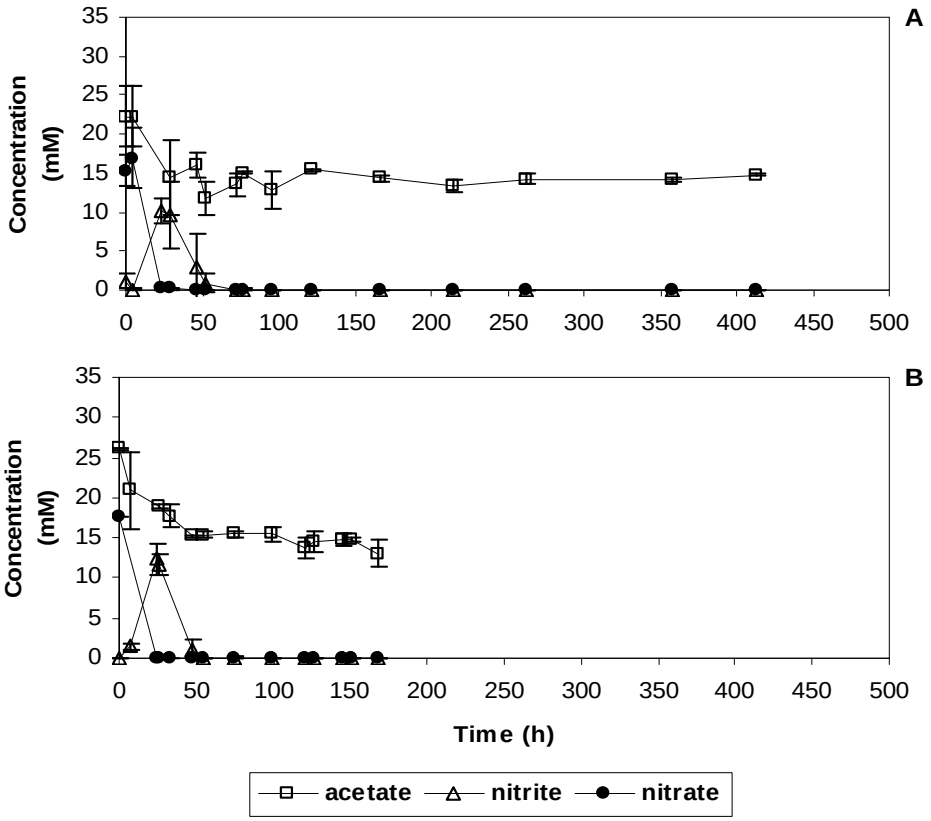

Figure 5-17 Concentration profiles during nitrate removal in batch system with 20 mM nitrate and 30 mM acetate; (A) 30 and (B) 35°C

The specific growth rates and biomass yield calculated based on the results of these experiments (equation 5-6 and 5-7) are shown in Table 5-1 (the calculation processes in Appendix; Figure A-1 to Figure A-6).

$$\ln \frac{X}{X_0} = \mu_{net} t \qquad (5\text{-}6)$$

$$Y = -\frac{\Delta X}{\Delta S} \qquad (5\text{-}7)$$

Table 5-2 Kinetics coefficients of denitrification calculated at different temperatures

Temperature °C	Specific Growth Rate, μ_{net} h^{-1}	Yield, Y g (biomass) (g nitrate)$^{-1}$
15	0.011	0.054
20	0.012	0.030
25	0.031	0.030
30	0.029	0.030
35	0.039	0.085

In all cases medium contained 30 mM acetate and 20 mM nitrate.

The specific growth rates increased from 0.011 to 0.039 h^{-1} when temperature increased in the range of 15 to 35°C. Using the the Arrhenius plot (Figure 5-18), the activation energy for microbial growth was calculated to be 60.6 kJ mol^{-1}. It is clear that increasing temperature accelerated the growth rates of the denitrifying bacteria (in the 15-35ºC range). As can be seen the biomass yield was in the range of 0.030 to 0.085 g (biomass) (g nitrate) $^{-1}$, with no clear dependency on temperature. The Arrhenius equation is given below:

$$\mu = Ae^{-\frac{Ea}{RT}} \qquad (5\text{-}8)$$

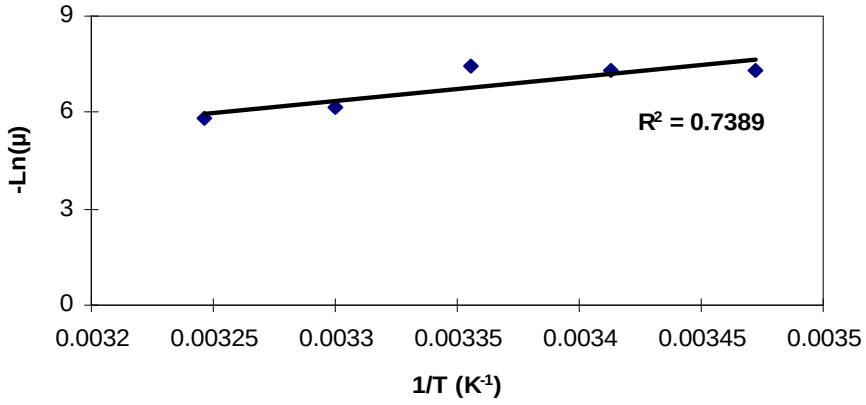

Figure 5-18 Arrhenius plot showing the effect of temperature on specific growth rate of heterotrophic denitrifying bacteria

5.2.2 CSTB System

Concentration profiles in CSTB reactors with media containing 10 mM of each nitrate and acetate, and 30 mM of each nitrate and acetate are shown in Figure 5-19. Both reactors showed similar biomass, acetate, nitrate and nitrite concentration profiles. With increases of the dilution rates, biomass concentration decreased to zero and cell washout happened at a dilution rate of 0.11 h^{-1} in both reactors. One should note that dilution rate was a more important variable than initial concentration and a dilution rate of 0.11 h^{-1} was determined as the critical value for heterotrophic denitrification with acetate, regardless of the initial concentrations of acetate and nitrate in the feed.

The maximum biomass concentrations were 0.1 and 0.25 g L^{-1} for CSTB reactors with feed concentrations of 10 and 30 mM, respectively. In both cases, there was no residual nitrate or nitrite at dilution rates lower than 0.02 h^{-1}. Residual nitrate and nitrite accumulated in the reactor effluent at a dilution rate of 0.03 h^{-1} and higher. With further increases in the

dilution rate, nitrite concentration kept decreasing and nitrate concentration kept increasing until cell washout occurred.

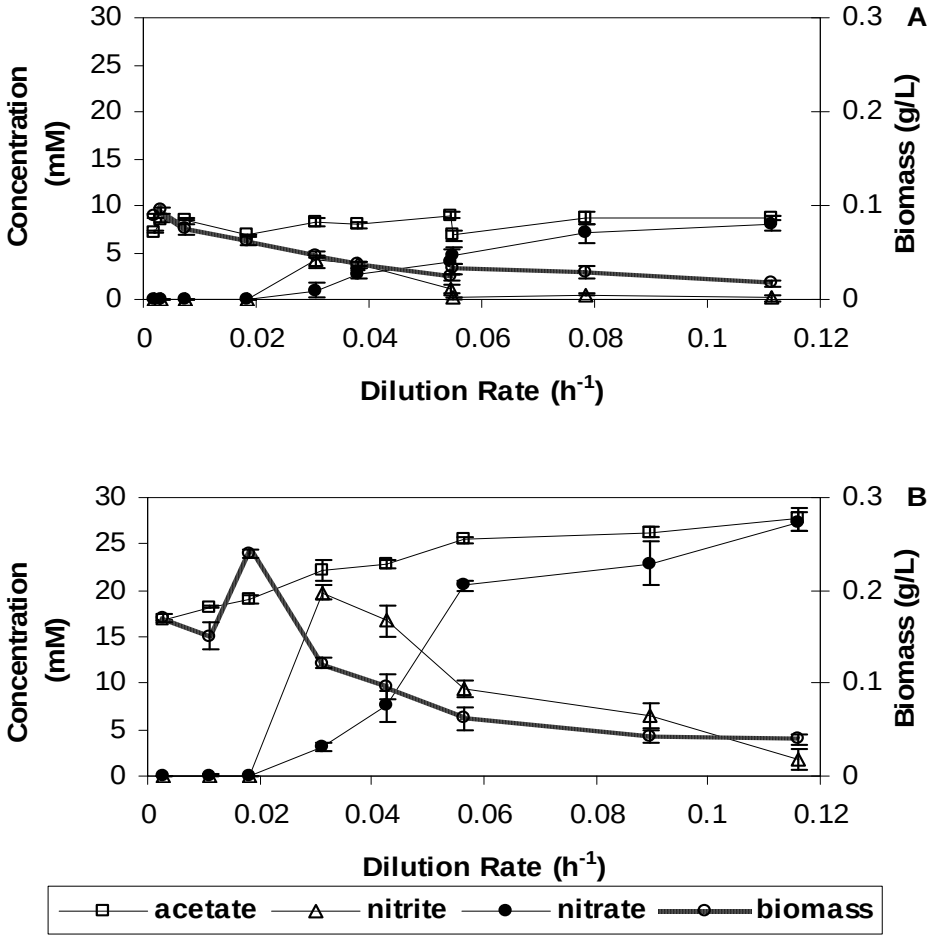

Figure 5-19 Concentration profiles in CSTB reactors (A: CSB medium with10 mM nitrate and 10 mM acetate; B: CSB medium with 30 mM nitrate and 30 mM acetate)

Figure 5-20 includes the removal percentages and removal rates of nitrate obtained in CSTB systems with 10 and 30 mM each nitrate or acetate. In these experiments, complete denitrification was achieved when nitrate loading rates were lower than 0.21 and 0.59 mM h^{-1}, and the maximum nitrate removal rates were 0.32 and 0.94 mM h^{-1} with corresponding nitrate loading rate 0.55 and 1.26 mM h^{-1} for systems with 10 mM and 30 mM each ions, respectively.

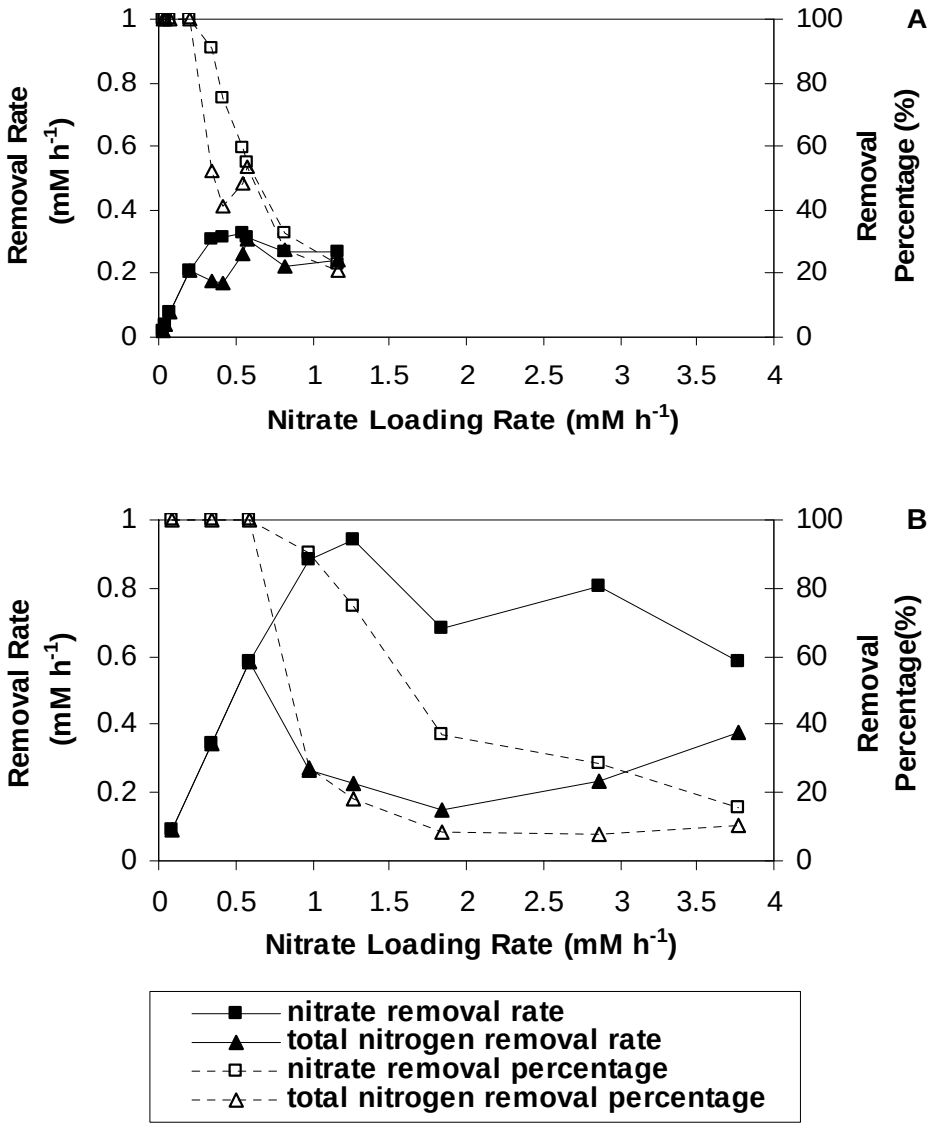

Figure 5-20 Nitrate removal rate and nitrate removal percentage in CSTB reactors (A: CSB medium with10 mM nitrate and 10 mM acetate; B: CSB medium with 30 mM nitrate and 30 mM acetate)

Monod expression is one of the most common empirical equations describing the microbial growth (Equations 5-9 to 5-12). Using the data generated in continuous reactors kinetic Monod coefficients were calculated for denitrification and presented in Table 5-2 (calculation details listed in the Appendix Parts Figure A-7 and Figure A-8). μ_m and K_s were calculated using Lineweaver-Burk Plot. As can be seen it appeared that nitrate concentration in the feed only affected the value of K_s with a higher of saturation constant obtained when higher nitrate concentration was used.

$$\mu_g = \frac{\mu_m S}{K_s + S} \quad (5\text{-}9)$$

$$\mu_g = \mu_{net} + k_d = D + k_d \quad (5\text{-}10)$$

$$\frac{1}{Y^{AP}_{X/S}} = \frac{1}{Y^{M}_{X/S}} + \frac{m_s}{D} \quad (5\text{-}11)$$

$$m_s = \frac{k_d}{Y^{M}_{X/S}} \quad (5\text{-}12)$$

Table 5-2 Kinetics coefficients for microbial growth calculated experimental data obtained in the CSTB operated with CSB media with different acetate and nitrate concentrations

Medium	μ_m h^{-1}	K_s mM (NO_3^-)	m_s mM (NO_3^-) (g biomass)$^{-1}$ h^{-1}	$Y^M_{X/S}$ g (biomass) (mM NO_3^-)$^{-1}$	k_d h^{-1}
10 mM (nitrate and acetate)	0.087	2.01	1.441	0.011	0.016
30 mM (nitrate and acetate)	0.082	5.27	1.096	0.013	0.014
Methanol (Gaudy, 1980)	0.008-0.083	4-10	-	-	-

5.2.3 Continuous Biofilm Reactor

5.2.3.1 Effects of loading rate (increasing feed flow rate)

The biofilm reactor performance was far better than the CSTB reactor from the point of view of handling higher flow rates and achieving far higher removal rates at much higher loading rates (shorter residence times), as shown in Figure 5-21 and Figure 5-22. With flow rates as high as 200 mL h^{-1} (corresponding to retention time 0.21 h and nitrate loading rate of 153 mM h^{-1}), there was no residual nitrate and nitrite at port 3 (Panel B, Figure 5-21) and the maximum nitrate removal rate 153 mM h^{-1} with total nitrogen removal was 100% (Panel B, Figure 5-22) (corresponding to the highest nitrate removal rate 0.94 mM h^{-1} achieved in CSTB system). In samples taken from port 1 (Panel A, Figure 5-21), there was no residual nitrate or nitrite when feed flow rate was lower than 50 mL h^{-1}. With further increases of

flow rate, the residual nitrate and nitrite appeared and kept increasing with the increases in flow rate in this region of bioreactor.

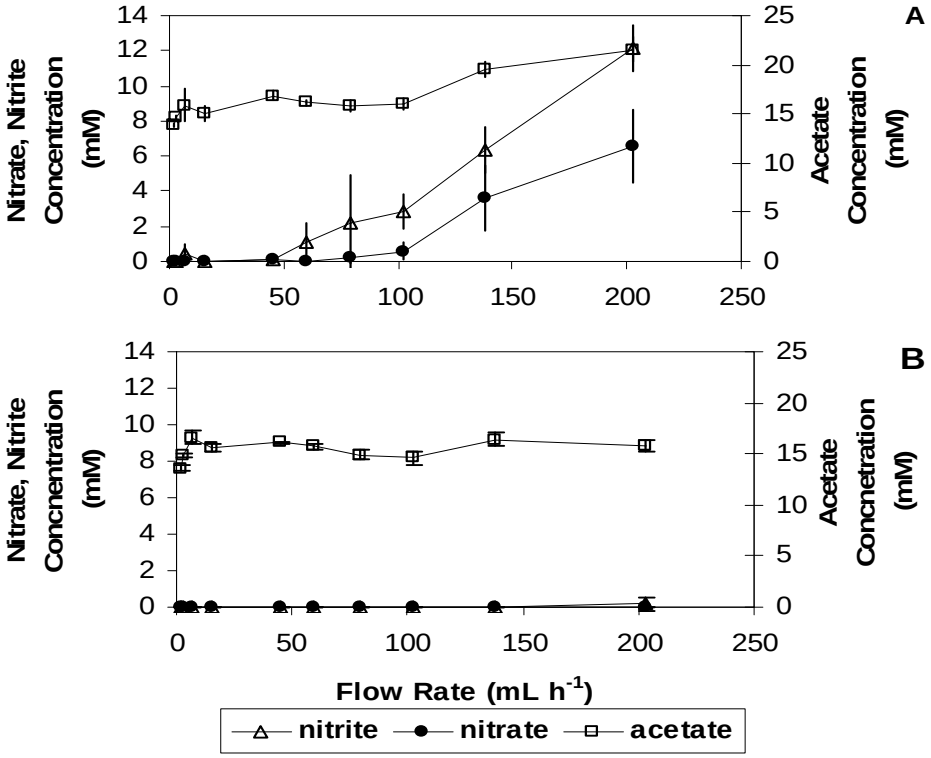

Figure 5-21 Concentration profiles in the biofilm reactor operated with CSB medium containing 30 mM nitrate and 30 mM acetate with increasing flow rate (A: port1; B: port3)

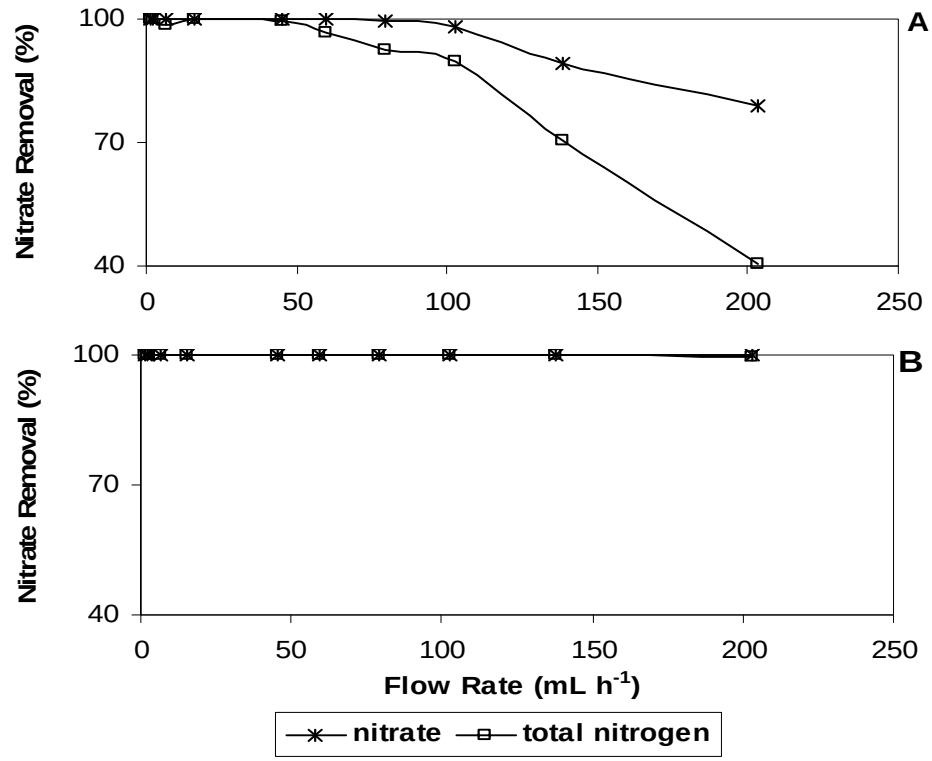

Figure 5-22 Nitrate removal percentages in the biofilm reactor operated with CSB medium containing 30 mM nitrate and 30 mM acetate with increasing flow rate (A: port1; B: port3)

5.2.3.2 Effects of loading rate (increasing feed concentration)

The concentration profiles and nitrate removal percentages for biofilm reactor operated with CSB medium containing higher concentrations of acetate and nitrate are shown in Figure 5-23 and Figure 5-24. The residual acetate, nitrate and nitrite concentrations of port 1 increased with the increase of feed concentration (Panel A, Figure 5-23). Nitrate removal percentage and total nitrogen removal percentage were in the range of 79%-100% and 41%-100% respectively in port 1 (Panel A, Figure 5-24). In this experiment the flow rate and consequently residence time were kept constant at 50 mL h^{-1} and 1.17 h^{-1}, respectively and the only variable was the volumetric loading rate of nitrate and acetate. Residual nitrate concentration was zero in port 3 for the entire range of applied loading rates and 100% nitrate removal was achieved. However, residual nitrite was observed in the bioreactor effluent when nitrate concentrations above 75 mM were used (Panels B, Figure 5-23 and Figure 5-24).

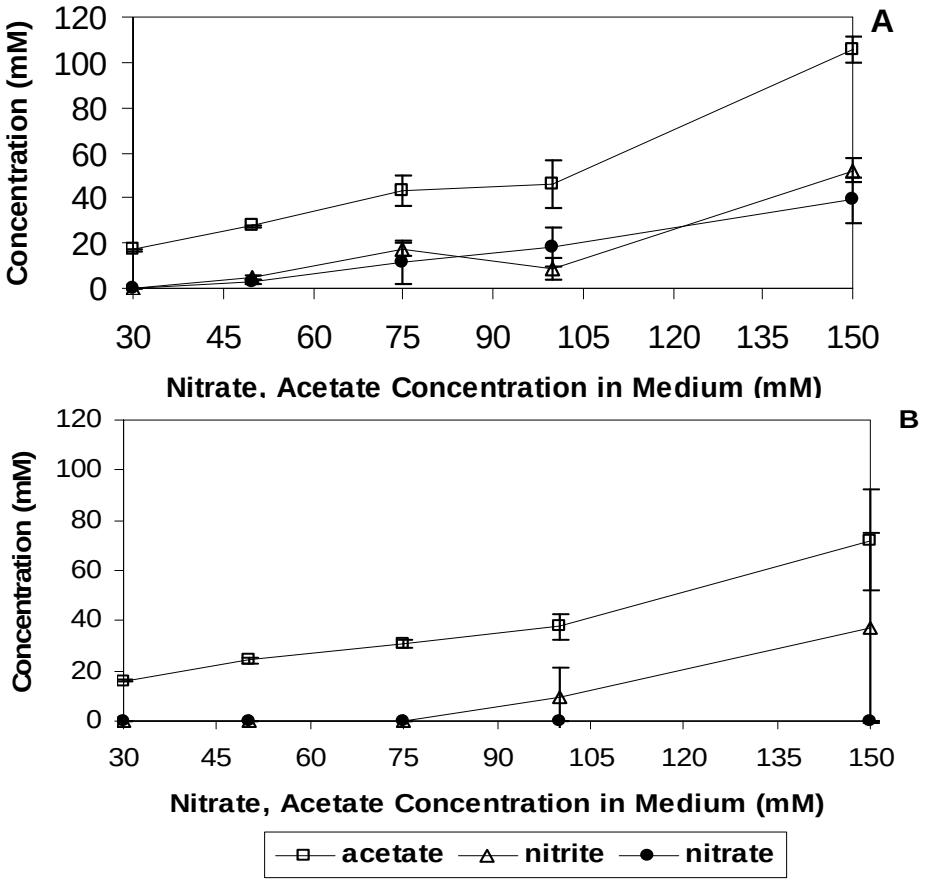

Figure 5-23 Concentration profiles in the biofilm reactor operated with CSB medium containing 30, 50, 75, 100, 150 mM nitrate and acetate at a constant flow rate (A: port1; B: port3)

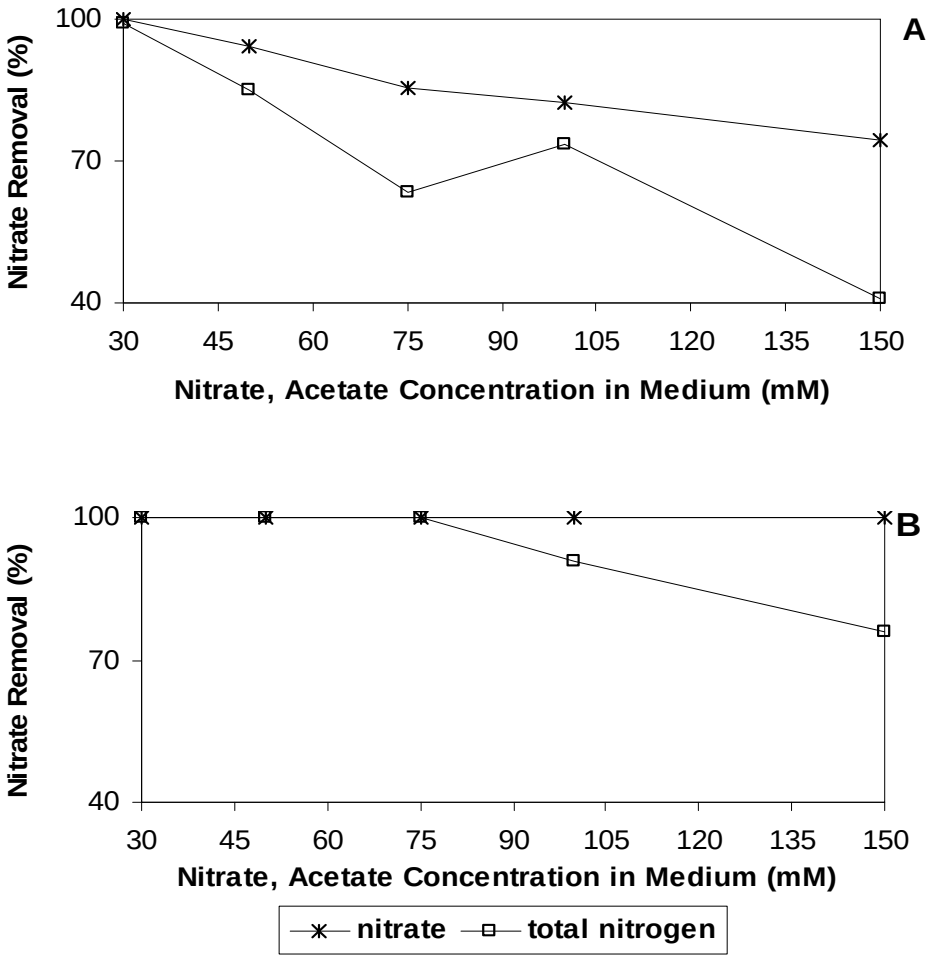

Figure 5-24 Nitrate removal percentages in the biofilm reactor operated with CSB medium containing 30, 50, 75, 100, 150 mM nitrate and acetate at a constant flow rate (A: port1; B: port3)

The performance of the biofilm reactor operated with increasing feed flow rate and constant feed concentration was compared with the reactor operating with increasing feed concentration and constant flow rate in Figure 5-25. For low nitrate loading rates (lower than 124±28 mM h^{-1}), the nitrate and total nitrogen removal rates increased at the same rate as volumetric loading rate of nitrate increased. Nitrate removal percentage and total nitrogen removal percentage were both 100%. At higher nitrate loading (as high as 185 mM h^{-1}), nitrate removal percentage was still 100%, but total nitrogen removal percentage decreased to 76% and nitrite accumulated existed. The nitrate loading rate 124 mM h^{-1} was the critical value for complete denitrification (100% removal of nitrate with no residual nitrite generated) in the biofilm reactor.

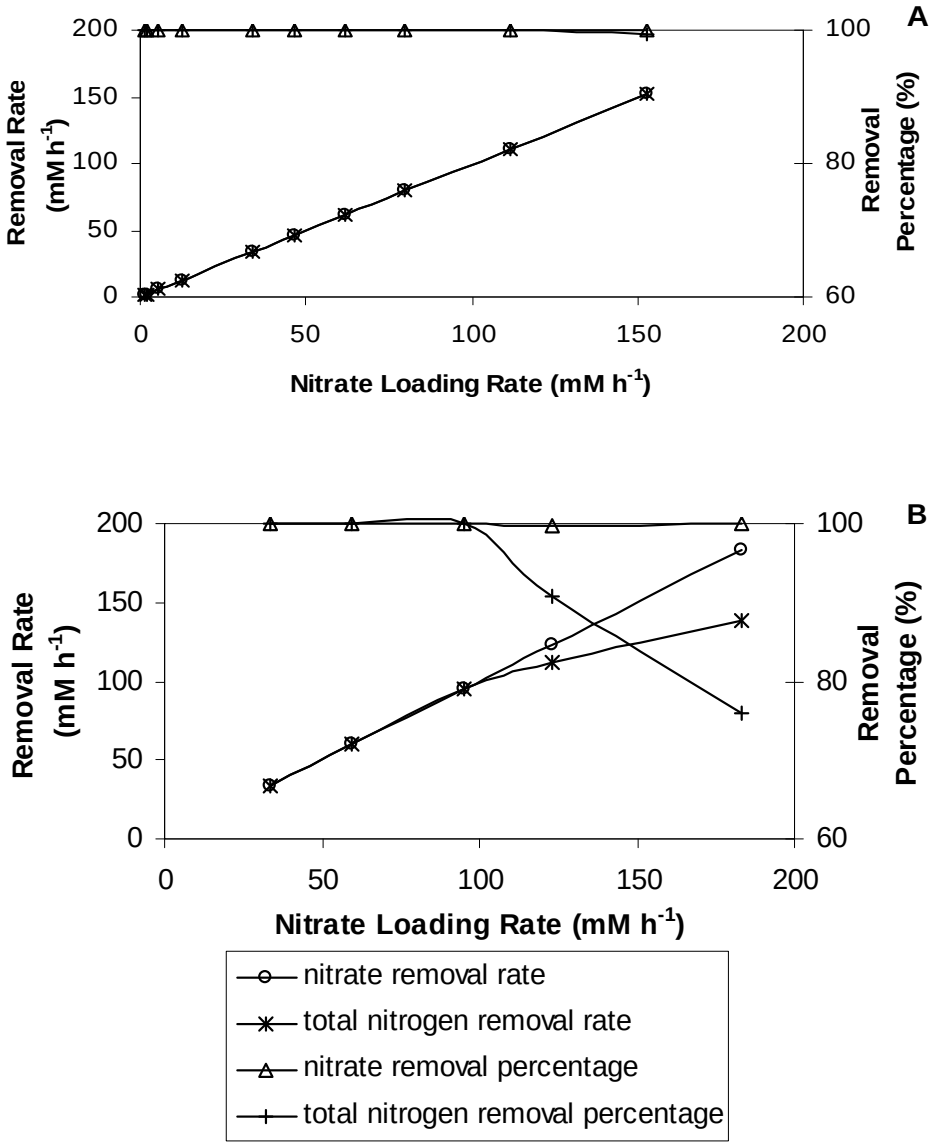

Figure 5-25 Nitrate removal rate and nitrate removal percentage in the biofilm reactor (A: CSB medium with 15 mM nitrate and 30 mM acetate and increasing feed flow rate; B: CSB medium with 30, 50, 75, 100, 150 mM nitrate and acetate and constant flow rate)

5.3 Comparison of Autotrophic and Heterotrophic Denitrification Kinetics in CSTB Systems and Continuous Biofilm Reactor

5.3.1 Autotrophic and Heterotrophic Denitrification in CSTB

Nitrate removal rate as a function of nitrate loading rate in the autotrophic denitrification (7.5 mM nitrate) and heterotrophic denitrification (10 and 30 mM nitrate) CSTB systems are compared in Figure 5-26.

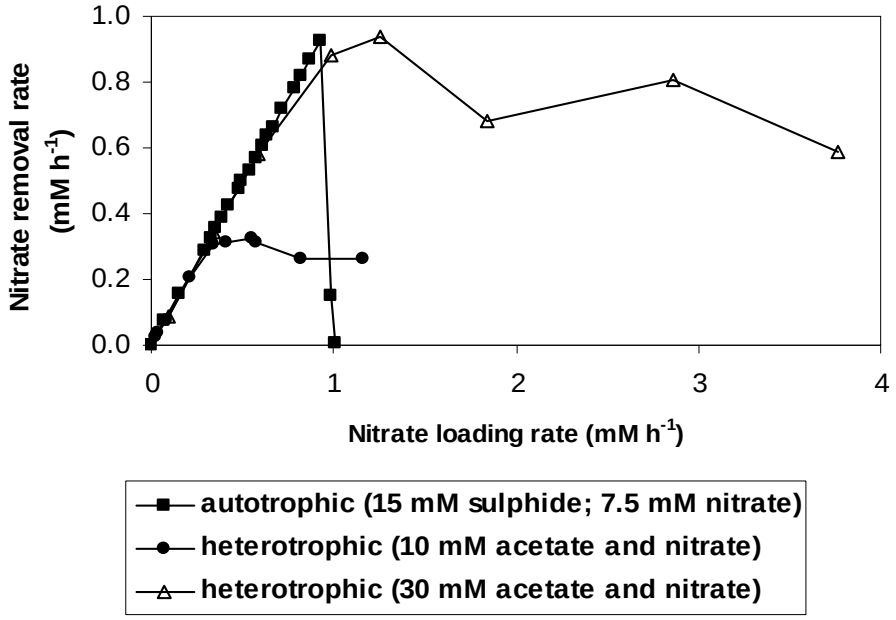

Figure 5-26 Nitrate removal rate as a function of nitrate loading rate in CSTB operated under autotrophic and heterotrophic conditions

Initially in all three cases the same linear relationship between nitrate removal rate and nitrate loading rate was observed and there was no obvious difference between autotrophic and heterotrophic processes as far as the effects of nitrate loading rate on nitrate removal rate was concerned. In the CSTB operated under autotrophic denitrification the maximum nitrate removal rate of 0.93 mM h^{-1} was achieved at a loading rate of 0.93 mM h^{-1} and a slight increase in loading rate (from 0.93 to 1.00 mM h^{-1}) caused cell washout happen and nitrate removal rate dropped to zero. The reason was the culture used in this research was very sensitive to the high sulphide loading rate as described in the earlier parts (Section 5.1). The situation was quite different in CSTBs operated under heterotrophic conditions (both 10 and 30 mM nitrate in the feed). The linear relationship between nitrate removal rate and nitrate loading rate was observed for nitrate loading rates up to 0.42 and 1.26 mM h^{-1} at which maximum nitrate removal rate of 0.31 and 0.94 mM h^{-1} were observed for systems with 10 and 30 mM nitrate respectively. Further increase in loading rate led to a slight decrease in nitrate removal rate. It appears that with acetate as electron donor (instead of sulphide) microbial culture was not that sensitive to increases of nitrate and acetate loading rates and the existing balance between cell growth and removal of cell by the effluent prevent the sudden cell wash out and decrease in removal rate of nitrate which was observed in the bioreactor operated under autotrophic conditions.

Nitrate removal percentage as a function of nitrate loading rate in the bioreactors operated under autotrophic and heterotrophic conditions are compared in Figure 5-27. In the CSTB run under autotrophic conditions, 100 percent nitrate removal was achieved with nitrate loading rates up to 0.93 mM h^{-1}. Nitrate removal percentage decreased to zero with a small increase of loading rate from 0.93 to 1.00 mM h^{-1} as a result of cell washout. In the CSTB operated under heterotrophic conditions with 10 mM of each nitrate and acetate in the feed, 100 percent nitrate removal was achieved with nitrate loading rate up to 0.34 mM h^{-1}. The nitrate removal percentage slowly decreased to 22.76% when loading rate increased from 0.34 to 1.17 mM h^{-1}. Similar results were achieved in other CSTB system operated with 30 mM of each nitrate and acetate in the feed. 100 percent nitrate removal was achieved for nitrate loading rates up to 0.58 mM h^{-1} and then nitrate removal percentage decreased continuously to the lowest value of 15.56% at a nitrate loading rate of 3.76 mM h^{-1}.

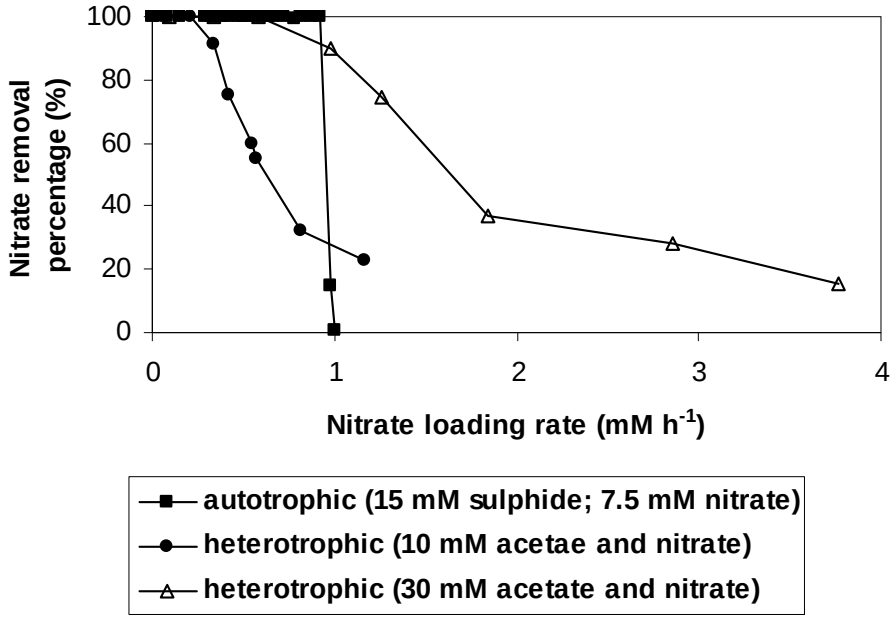

Figure 5-27 Nitrate removal percentage of autotrophic and heterotrophic denitrification in CSTB systems

5.3.2 Comparison of Autotrophic and Heterotrophic Denitrification in Continuous Biofilm Reactor

The effects of nitrate loading rate on the nitrate removal rate in continuous biofilm reactors operated under autotrophic and heterotrophic conditions are compared in Figure 5-28. It should be pointed out that the data for autotrophic denitrification was taken from an earlier work in our laboratory (Tang, 2008). A same linear relationship between nitrate loading rate

and nitrate removal rate was observed in both bioreactors. The maximum nitrate removal rate achieved in the heterotrophic and autotrophic bioreactors were 153 and 24.4 mM h^{-1}, respectively. The lower nitrate removal rate in the autotrophic bioreactor could be due to the lower range of applied nitrate loading rates (up to 24.4 mM h^{-1}). One can speculate that higher nitrate removal rates could be achieved if higher nitrate loading rates were applied in the autotrophic biofilm reactor.

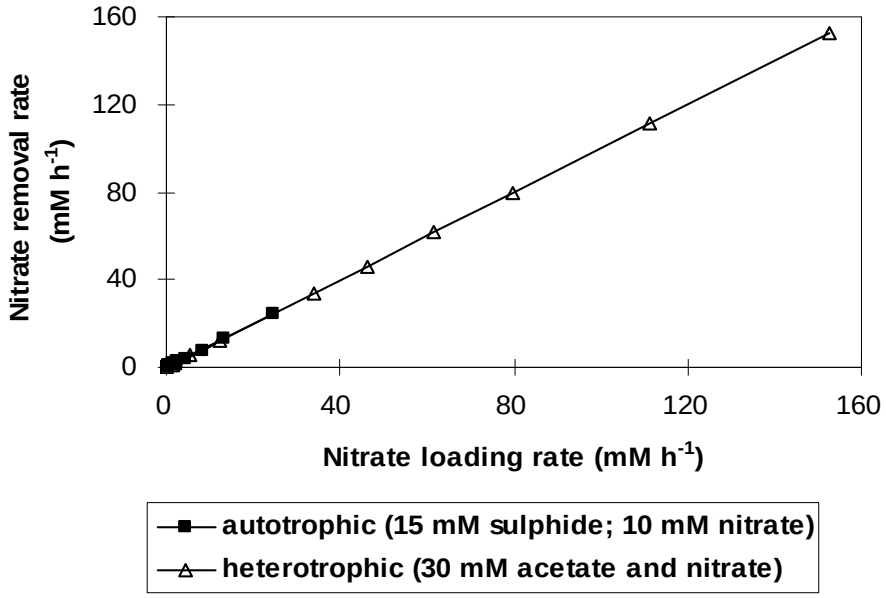

Figure 5-28 Nitrate removal rate as a function of nitrate loading rate in the continuous biofilm reactors operated under autotrophic and heterotrophic conditions (Data for autotrophic denitrification was taken from Tang, 2008)

The effects of nitrate loading rate on the nitrate removal percentage in the autotrophic and heterotrophic biofilm reactors are shown in Figure 5-29. Almost 100 percent nitrate removal was achieved for the entire range of applied loading rates in both reactors, with the highest tested nitrate loading rates for autotrophic and heterotrophic reactors being 24.4 and 153 mM h^{-1}, respectively). It should be pointed out that in the autotrophic reactor a lower nitrate removal percentage of 83% was observed for the nitrate loading rates of 4.6 and 8.3 mM h^{-1} which could be due to a technical problem in operation of the reactor.

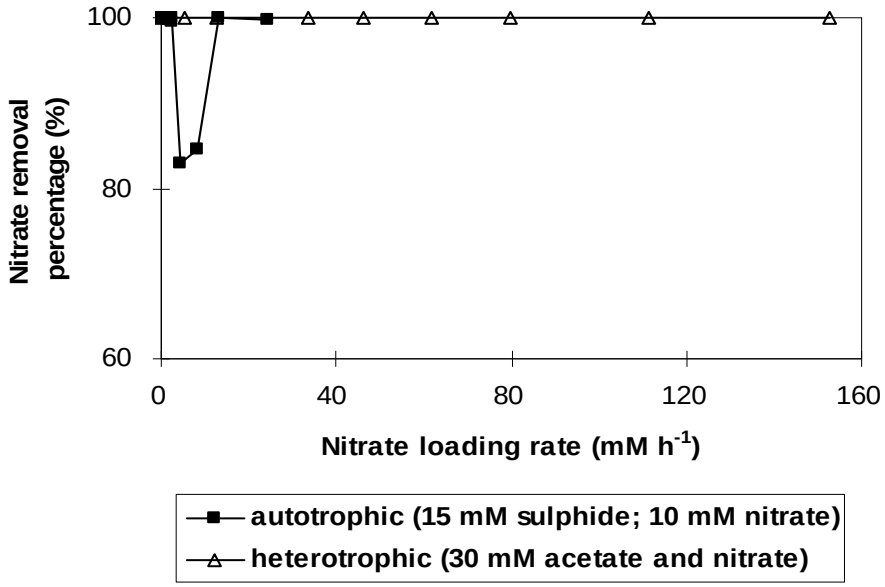

Figure 5-29 Nitrate removal percentage as a function of nitrate loading rate observed in continuous biofilm reactors operated under autotrophic and heterotrophic conditions (Data for autotrophic denitrification was taken from Tang, 2008)

5.3.3 Comparison of Heterotrophic Denitrification in CSTB and Continuous Biofilm Reactor

Figure 5-30 shows the effect of nitrate loading rate on its removal rate in CSTB system and continuous biofilm reactor operated under heterotrophic conditions. A similar linear relationship between nitrate loading rate and nitrate removal rate was achieved in two CSTB systems and biofilm reactor in a certain range of nitrate loading rate. In CSTB systems operated with 10 and 30 mM of each acetate and nitrate maximum nitrate removal rate of 0.32 mM h^{-1} and 0.94 mM h^{-1} were obtained at loading rates of 0.55 mM h^{-1} and 1.26 mM h^{-1}, respectively. The linear relationship between nitrate loading rate and nitrate removal rate did not hold when loading rates were increased above these levels (0.55 mM h^{-1} and 1.26 mM h^{-1}) and nitrate removal rate decreased gradually with the increase of nitrate loading rate. This situation was very different with that observed in the CSTB system operated under autotrophic conditions (as mentioned earlier in Section 5.1) in which increase in loading rate above a critical value led to a sudden decrease in removal rate and cell washout. The possible explanation for this observed difference could be due to inhibitory effect of sulphide which in combination with high loading rates caused sudden deterioration of the reactor performance.

In the continuous biofilm reactor, the linear relationship between nitrate loading rate and nitrate removal rate existed for the entire range of tested nitrate loading rate, with the

maximum loading rate being 153 mM h^{-1}. The maximum nitrate removal rate of 153 mM h^{-1} was also observed at this loading rate. One could speculate that nitrate removal rate would eventually start to decrease with application of higher nitrate loading rates. We could not apply higher loading rates above 153 mM h^{-1} as application of the highest tested flow rate 200 mL h^{-1} caused the carry over of the sand (matrix used for biofilm formation).

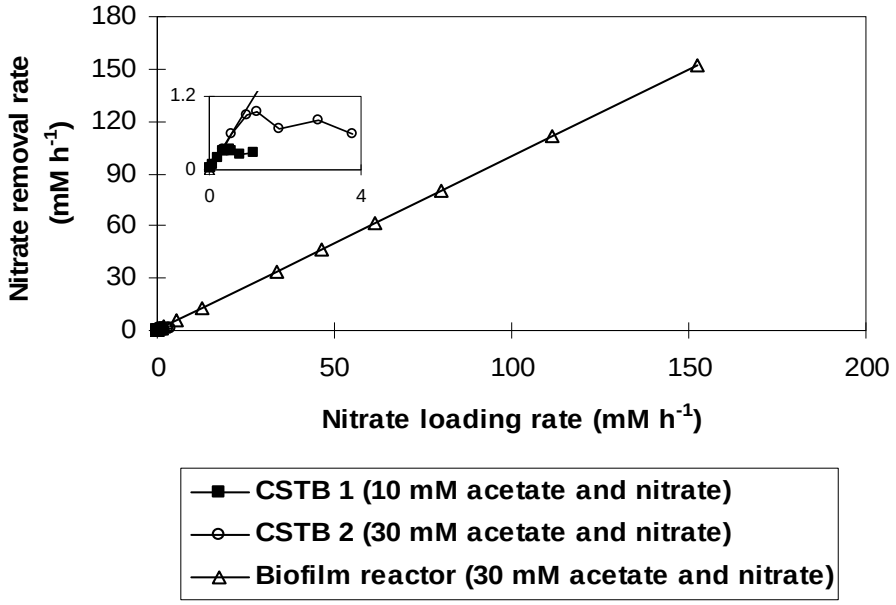

Figure 5-30 Nitrate removal rate as a function of nitrate loading rate in CSTB system and continuous biofilm reactor operated under heterotrophic conditions

Figure 5-31 shows the effects of nitrate loading rate on the nitrate removal percentage during heterotrophic denitrification in CSTB system and continuous biofilm reactor. In biofilm reactor, 100 percent nitrate removal was achieved for the entire range of tested nitrate loading rate (up to 153 mM h^{-1}). For CSTB system, 100 percent nitrate removal percentage was achieved when the nitrate loading rate was lower than 0.21 mM h^{-1} and 0.58 mM h^{-1} for CSTBs operated with 10 mM and 30 mM (both acetate and nitrate), respectively. Nitrate removal percentage started to decrease when loading rate was increased above those given values. At the highest tested nitrate loading rate of 1.17 mM h^{-1} and 3.76 mM h^{-1} in CSTB operated with10 mM and 30 mM of each ion, the nitrate removal percentages were 22.8% and 15.5%.

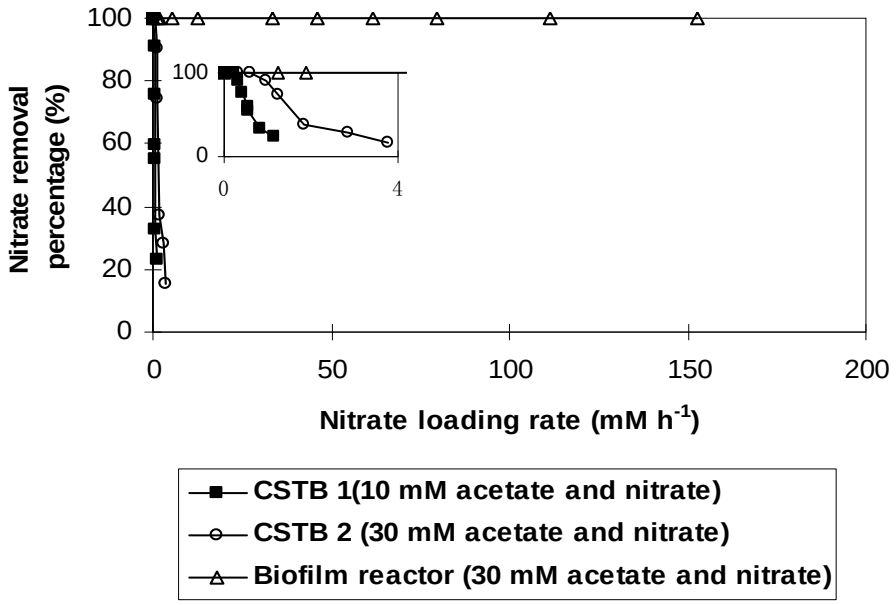

Figure 5-31 Nitrate removal percentage of heterotrophic denitrification in CSTB system and continuous biofilm reactor

CHAPTER 6
CONCLUSIONS ANS RECOMMENDATIONS

6.1 Conclusions

The processes of chemolithotrophic desulphurization (autotrophic denitrification) and heterotrophic denitrification were investigated in batch, continuous bioreactors with free cells and biofilm reactor and kinetics of sulphide and nitrate removals were assessed and compared in these systems.

Studying chemolithotrophic desulphurizaton in continuous bioreactors fed with different initial sulphide concentrations of 10, 15 or 20 mM (corresponding nitrate concentrations of 5, 7.5 and 10 mM), a linear relationship between loading rate and removal rates for sulphide and nitrate was observed regardless of the initial sulphide concentration. The highest sulphide and nitrate removal rates of 1.79 and 0.93 mM h^{-1} were achieved in CSTB operated with 15 mM in the feed. Increase of the sulphide concentration in the feed caused the cell wash out occur at lower loading rates. Studying the effects of sulphide to nitrate loading ratio in bioreactors operated at constant residence times (13.3 and 66.7 h^{-1}, respectively) indicated that conversion of sulphide to sulphate decreased with increases of this ratio and sulphur became the dominant end product. The conversion of sulphide to sulphate decreased from 40% to 6% with the increases of sulphide to nitrate loading ratio from 0.1 to 3.4 due to unavailability of sufficient nitrate (as electron acceptor) in the CSTB with the retention time 66.7 h^{-1}. In the CSTB with retention time 13.3 h^{-1}, a similar result was achieved that the conversion of sulphide to sulphate decreases from 30% to 3% with the increases of sulphide to nitrate loading ratio from 0.1 to 1.9. Kinetic studies in the continuous systems also indicated that at a constant sulphide to nitrate loading ratio application of higher loading rates (shorter residence times) also favored the formation of sulphur as the end product, while at lower loading rates (longer residence times) sulphate became dominant. These results imply that sulphide loading rate (residence time) and ratio of sulphide to nitrate loading ratio could be used effectively to control the composition of sulphide biooxidation toward the desired end product whether it is sulphur or sulphate.

Batch experiment results indicated that during the heterotrophic denitrification nitrate was reduced to nitrite which was followed by reduction of nitrite to other nitrogenous compounds possibly nitrogen. Nitrate itself did not influence the denitrification process but when concentration of produced nitrite reached to or was higher than 35 mM an inhibitory effect

was observed. The microbial growth rate of denitrifying bacteria was increased significantly with the increases of temperature in the test range of 15 to 35ºC. The calculated specific growth rate was increased from 0.011 to 0.039 h^{-1} when temperature was increased from 15 to 35ºC range and the average biomass yield was 0.05 g (biomass) (g nitrate)$^{-1}$ in these batch systems. The activation energy for bacterial growth was calculated to be 60.57 KJ mol^{-1}.

The heterotrophic denitrification was also investigated in continuous systems with free cell (CSTB) fed with a medium containing either 10 or 30 mM of each initial nitrate and acetate. Cell wash-out happened at a dilution rate of 0.11 h^{-1} in both systems. The highest nitrate removal rate was 0.94 mM h^{-1} which obtained at a loading rate 1.26 mM h^{-1} in the CSTB fed with 30 mM of each acetate and nitrate. Using the experimental data bio-kinetic coefficients including μ_m, K_s, m_s, $Y^M_{X/S}$ and k_d for initial concentrations of 10 and 30 mM CSTB systems were calculated as 0.087 and 0.082 h^{-1}, 2.01 and 5.27 mM (NO_3^-), 1.441 and 1.096 mM (NO_3^-) (g biomass)$^{-1}$ h^{-1}, 0.011 and 0.013 g (biomass) (mM NO_3^-)$^{-1}$, and 0.016 and 0.014 h^{-1} respectively.

In the biofilm system used for heterotrophic denitrification the linear dependency between nitrate loading and removal rate was observed and for the tested range removal rate increased with the increases in nitrate loading rate, regardless of the approach used to control the loading rate (increasing feeds flow rate or initial concentrations). The highest nitrate removal rate in biofilm reactor was 183 mM h^{-1} and achieved at a nitrate loading rate of 183 mM h^{-1}. This removal rate was 194 times higher than that achieved in the CSTB system.

A comparison of the autotrophic and heterotrophic denitrification processes studied in the CSTB system indicated that in case of autotrophic denitrification wash-out occurred suddenly and at a much lower nitrate loading rate of 0.93 mM h^{-1} (CSTB with 7.5 mM nitrate and 15 mM sulphide), while in case of heterotrophic denitrification increase of loading rate did not have such a drastic effect and removal rate of nitrate decreased slowly with the increases of nitrate loading rate. Moreover, the maximum nitrate removal rate obtained in the heterotrophic system with 10 mM nitrate and acetate (0.94 mM h^{-1}) was slightly higher than that obtained in the autotrophic system with 7.5 mM nitrate and 15 mM sulphide (0.93 mM h^{-1}).

6.2 Recommendations for future works

The work presented in this thesis has focused on the processes of bio-desulphurization and bio-denitrification in the liquid phase. Gaseous streams contaminated with H_2S are produced

in petroleum industry and other situations such as production of biogas as a by product during anaerobic digestion. Treatment of gaseous stream containing H_2S prior to use is essential. Therefore, a comprehensive study on desulphurization of gaseous streams is recommended for future. This study should be conducted both in the systems with free cells as well as biofilm. The performances of other immobilization supports suitable for gaseous applications should be investigated.

In this study nitrate was used as an electron acceptor for the bio-desulphurization process. However, our results indicated that nitrite could also be used as an electron acceptor. Therefore, studying simultaneous removal of sulphide and nitrite, and heterotrophic removal of nitrite with acetate is recommended for future works. This will allow understanding the potential inhibitory effect of nitrite on both desulphurization and denitrification processes.

Finally the majority of earlier work on the process of denitrification has focused either on sulphide or acetate as potential electron donors. However, this process could also be carried out using elemental sulphur as an electron donor. Therefore, studying the processes of denitrification with sulphur which could also shed light on some of the intermediary reactions occurring during the oxidation of sulphide is recommended.

Finally mathematical modeling of the biofilm reactor used for autotrophic or heterotrophic denitrification should be carried out,

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LIST OF RESEARCH CONTRIBUTIONS

Paper Published / Submitted

1. An, S., Tang, K., Nemati, M., 2010. Simultaneous biodesulphurization and denitrification using an oil reservoir microbial culture: effects of sulphide loading rate and sulphide to nitrate loading ratio. *Water Research* 44 (5): 1531-1541.

2. Tang, K., An, S., Nemati, M., 2010. Evaluation of autotrophic and heterotrophic processes in biofilm reactors used for removal of sulphide, nitrate and COD. Submitted to *Bioresource Technology* (Revised version resubmitted).

Presentation / Paper in Conferences

1. An, S., Tang, K., Nemati, M., 2008. Biological removal of H_2S and nitrate in continuous bioreactors: a comparison of the freely suspended cells and immobilized cells. *58th Canadian Chemical Engineering Conference* No. 0317 Ottawa, Canada, 19-22 October.

2. An, S., Tang, K., Nemati, M., 2009. Biooxidation of sulphide under denitrification conditions. *8th World Congress of Chemical Engineering* No. 516a Montreal, Canada, 23-27 August.

APPENDIX A
SAMPLES CALCULATIONS

A.1 Autotrophic Denitrification with Sulphide Experiments

1) Void volume (V) for CSTB was measured as 230 mL at the beginning of experiments by filling the reactor with pure medium at the beginning.

2) Hydraulic retention time (HRT) and dilution rate (D) were calculated as following equations.

$$HRT = \frac{V}{Flowrate} \quad \text{(A-1)}$$

$$D = \frac{1}{HRT} \quad \text{(A-2)}$$

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

$$LR = Ci \times D \quad \text{(A-3)}$$

Where,

Ci: inlet medium concentration (sulphide or nitrate).

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

$$RR = (Ci - Co) \times D \quad \text{(A-4)}$$

Where,

Ci : inlet medium concentration (sulphide or nitrate);

Co: outlet medium concentration (sulphide or nitrate).

5) Removal percentage (RP) was calculated for both sulphide and nitrate. The calculation equation was as follows.

$$RP = \frac{RR}{LR} \times 100\% \quad \text{(A-5)}$$

Where,

RR: removal rate;

LR: loading rate.

6) Conversion (Con) of sulphide to sulphate was calculated as following equation.

$$Con = \frac{C'o - C'i}{Ci - Co} \times 100\% \qquad \text{(A-6)}$$

Where,

C'o: sulphate concentration in outlet medium;

C'i : sulphate concentration in inlet medium;

C i : sulphide concentration in inlet medium;

C o: sulphide concentration in outlet medium.

7) Sulphide to nitrate ratio in inlet medium was calculated as that nitrate inlet medium concentration was divided by sulphide inlet medium concentration.

A.2 Heterotrophic Denitrification Batch Experiments

1) Specific growth rate was calculated according to the following equation.

$$\ln \frac{X}{Xo} = \mu \times t \qquad \text{(A-7)}$$

Where,

X : biomass concentration in time t;

Xo: biomass concentration in time 0;

μ : specific growth rate;

t : time.

To calculate the specific growth rate, the biomass concentration data in the exponential growth phase were chosen and the value of ln (X/Xo) was calculated first. Then, ln (X/Xo) vs t was plotted and a straight trend line was passed. Finally, specific growth rate was calculated as the slope of the trend line.

Six samples of specific growth rate calculation process were shown as following figures (Figure A-1 to Figure A-6); other calculation figures were not included in this thesis due to page limitation.

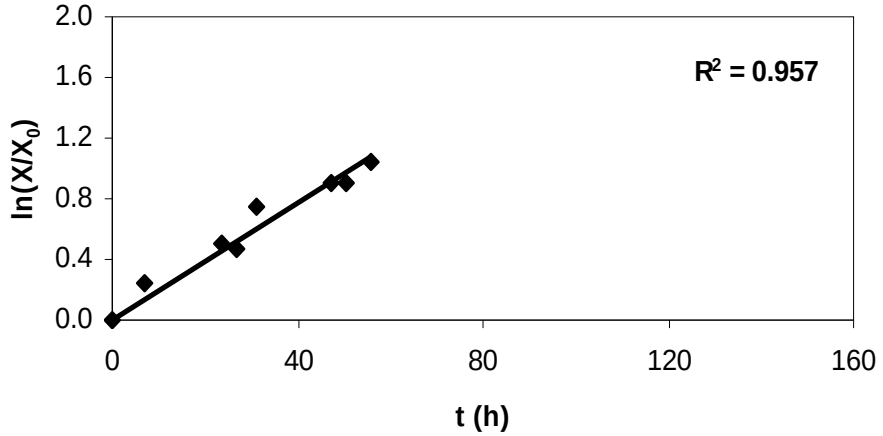

Figure A-1 Specific growth rate calculation in the bacth experiment with nitrate 50 mM and acetate 5 mM at 25ºC

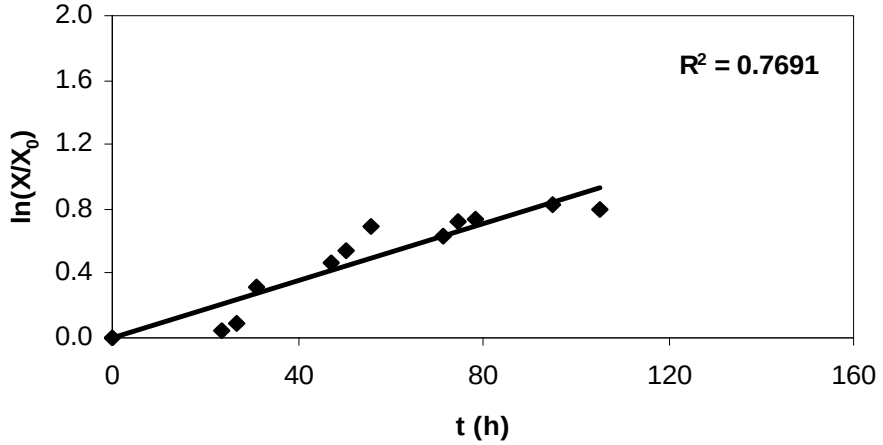

Figure A-2 Specific growth rate calculation in the bacth experiment with nitrate 50 mM and acetate 10 mM at 25ºC

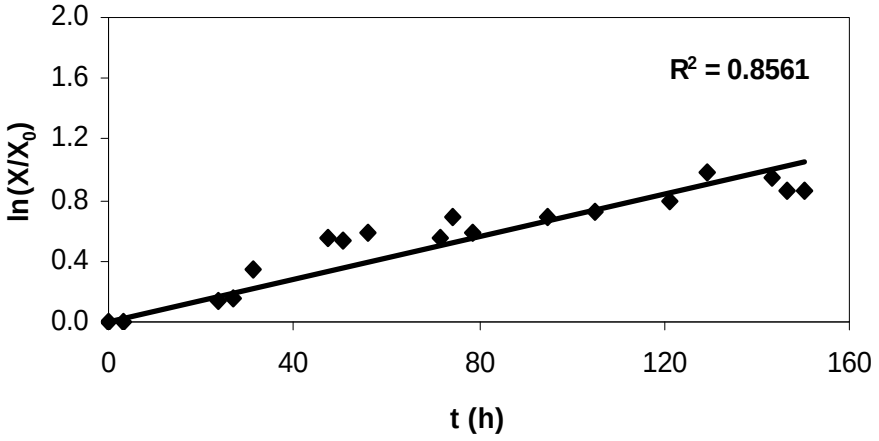

Figure A-3 Specific growth rate calculation in the bacth experiment with nitrate 50 mM and acetate 20 mM at 25ºC

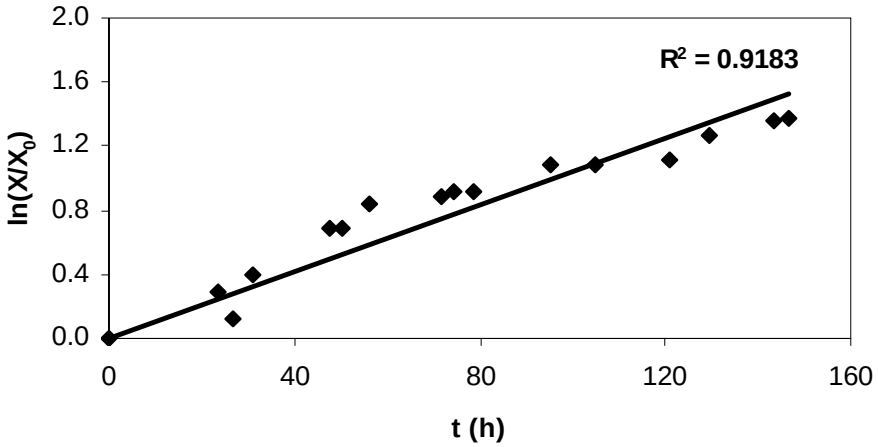

Figure A-4 Specific growth rate calculation in the bacth experiment with nitrate 50 mM and acetate 30 mM at 25ºC

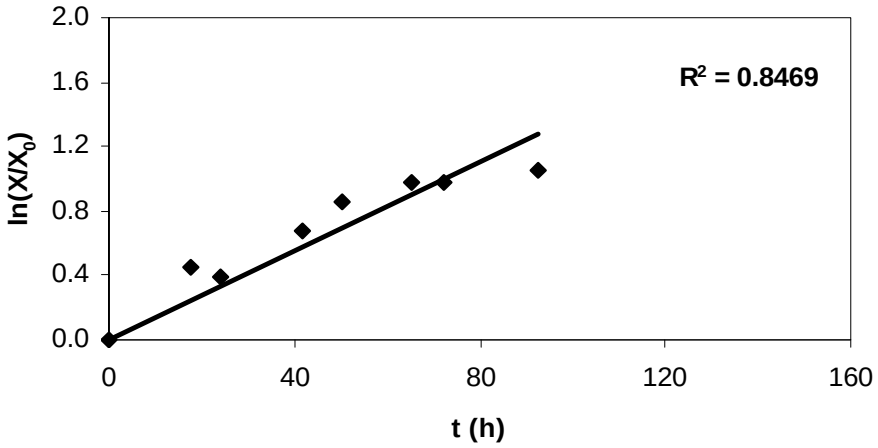

Figure A-5 Specific growth rate calculation in the batch experiment with nitrate 20 mM and acetate 30 mM at 15ºC

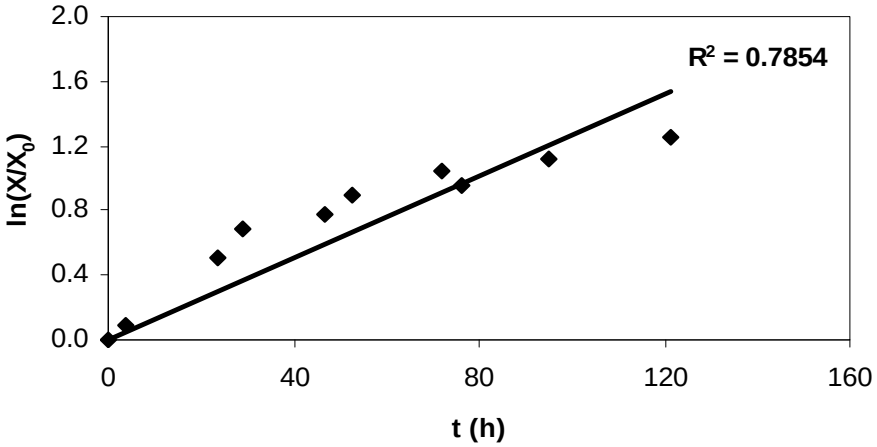

Figure A-6 Specific growth rate calculation in the bacth experiment with nitrate 20 mM and acetate 30 mM at 20ºC

2) Biomass yield was calculated as following equations.

$$Y = -\frac{Xo - Xi}{Co - Ci} \quad \text{(A-8)}$$

Where,

Xo: biomass concentration in time t;

Xi : biomass concentration in time 0;

Co: nitrate concentration in time t;

Ci : nitrate concentration in time 0.

3) Nitrate removal percentage (RP) was calculated as following equation.

$$RP = \frac{Ci - Co}{Ci} \times 100\% \quad \text{(A-9)}$$

Where,

Ci : nitrate inlet medium concentration;

Co : nitrate outlet medium concentration.

A.3 Heterotrophic Denitrification CSTB Experiments

1) Void volume, hydraulic retention time, dilution rate, nitrate loading rate, nitrate removal rate and nitrate removal percentage was determined as that described in Section A.1 part.

2) Monod equation kinetic parameters, μ_m and k_s, were calculated according to the following equation.

$$\frac{1}{D} = \frac{k_s}{\mu_m} \times \frac{1}{S} + \frac{1}{\mu_m} \quad \text{(A-10)}$$

Where,

D: dilution rate;

S : nitrate concentration in outlet medium.

The values of 1/D and 1/S were calculated according to the sample at steady state and the figures (1/D vs 1/S) were plotted as shown in Figure A-7 and Figure A-8.

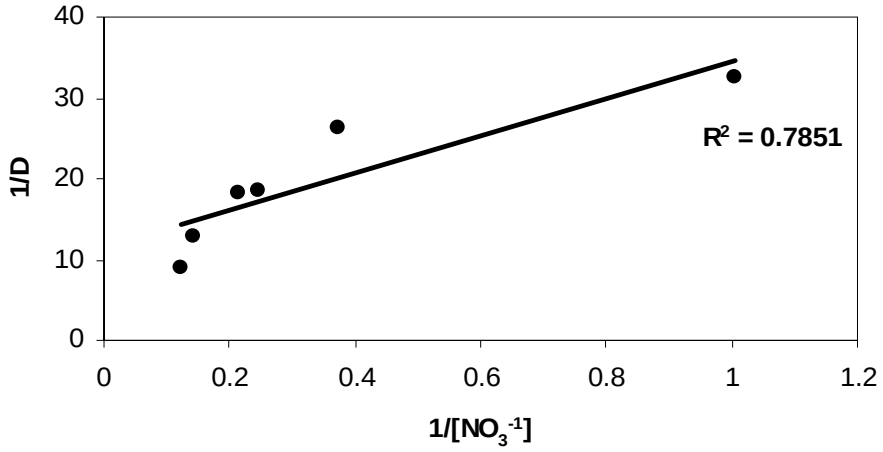

Figure A-7 Kinetics parameters, μ_m and k_s, calculation in the CSTB with nitrate 10 mM and acetate 10 mM

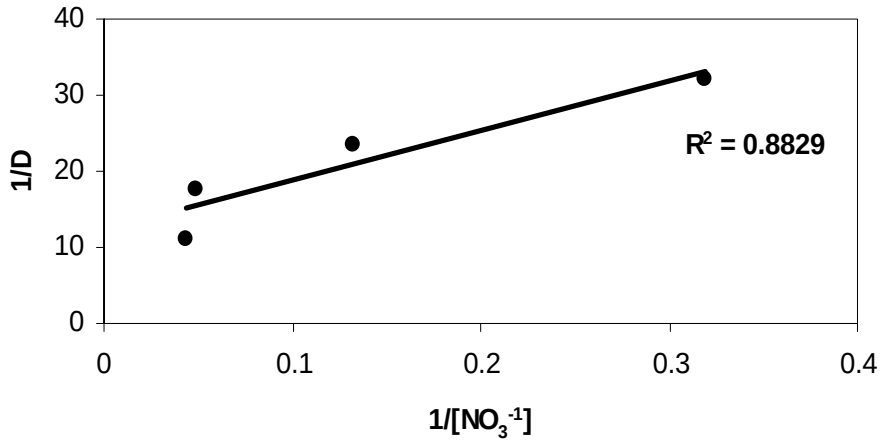

Figure A-8 Kinetics parameters, μ_m and k_s, calculation in the CSTB with nitrate 30 mM and acetate 30 mM

3) Kinetics parameters of $Y^{M}_{X/S}$, m_s and k_d were calculated according to the following equations.

$$\frac{1}{Y^{AP}_{X/S}} = \frac{1}{Y^{M}_{X/S}} + \frac{m_s}{D} \qquad \text{(A-11)}$$

$$m_s = \frac{k_d}{Y^{M}_{X/S}} \qquad \text{(A-12)}$$

$$Y^{AP}_{X/S} = -\frac{Xo - Xi}{Co - Ci} \qquad \text{(A-13)}$$

The figures of $1/(Y^{AP}_{X/S})$ vs 1/D were shown in Figure A-9 and Figure A-10.

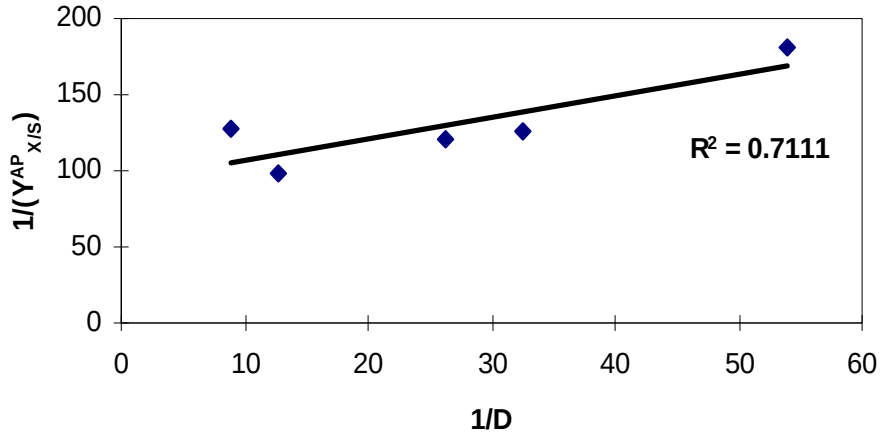

Figure A-9 Kinetics parameters, $Y^{M}_{X/S}$, m_s and k_d, calculation in the CSTB with nitrate 10 mM and acetate 10 mM

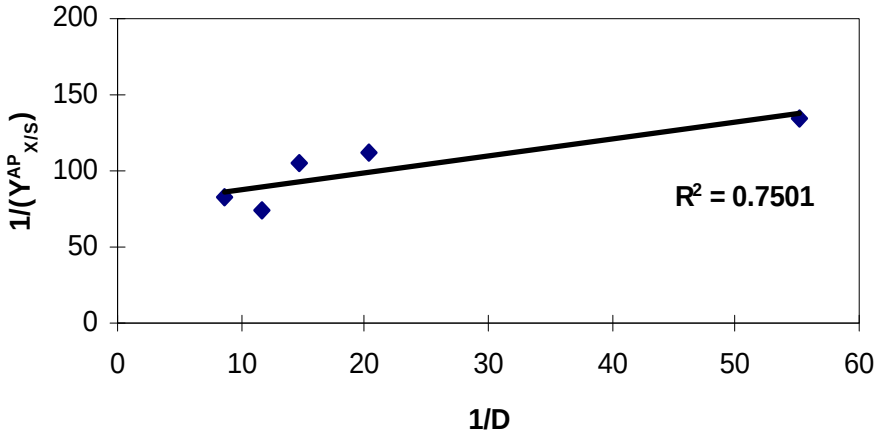

Figure A-10 Kinetics parameters, $Y^{M}_{X/S}$, m_s and k_d, calculation in the CSTB with nitrate 30 mM and acetate 30 mM

A.4 Heterotrophic Denitrification Biofilm Reactor Experiments

1) The working volume of biofilm reactor was determined by the average value 41.8 mL as the real working volume was variable during the experiments. Prior to beginning the experiments, the void volume was measured as 60 mL and at the end of the experiment, the void volume was measured as 23.6 mL. To measure the void volume, sterilized medium was filled into the reactor and then, the liquid medium was allowed to drain completely (one week) from the bioreactor. The volume of liquid was measured as void volume of biofilm reactor.

2) Hydraulic retention time, dilution rate, nitrate loading rate, nitrate removal rate and nitrate removal percentage was determined as that described in Section A.1 part.

APPENDIX B
CALIBRATION CURVES FOR ANALYTICAL METHODS

B.1 Calibration Curve for Sulphide Measurement

The calibration curve for the measurement of sulphide concentration was shown in Figure B-1. At each sulphide concentration, two samples have been prepared and measured for optical density at 480 nm. The average value was used to plot the figure and standard deviation of these two samples was used as error bar.

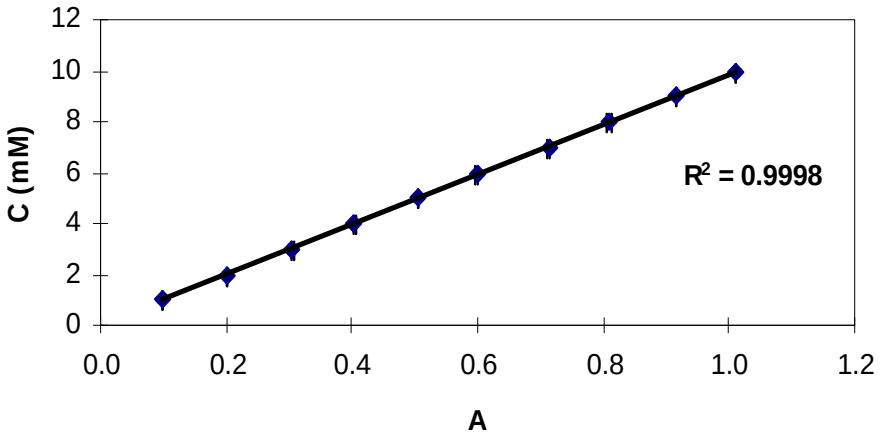

Figure B-1 Calibration curve for sulphide concentration measurement

The equation used to calculate sulphide concentration from optical density was as following.

$$C = 9.88 \times A \qquad \text{(B-1)}$$

Where,

C : sulphide concentration;

A : optical density at 480 nm.

B.2 Calibration Curve for Protein Measurement

The calibration curve for the measurement of protein concentration was shown in Figure B-2. At each protein concentration, three samples have been prepared and measured for optical density at 595 nm. The average value was used to plot the figure and standard deviation of these three samples was used as error bar.

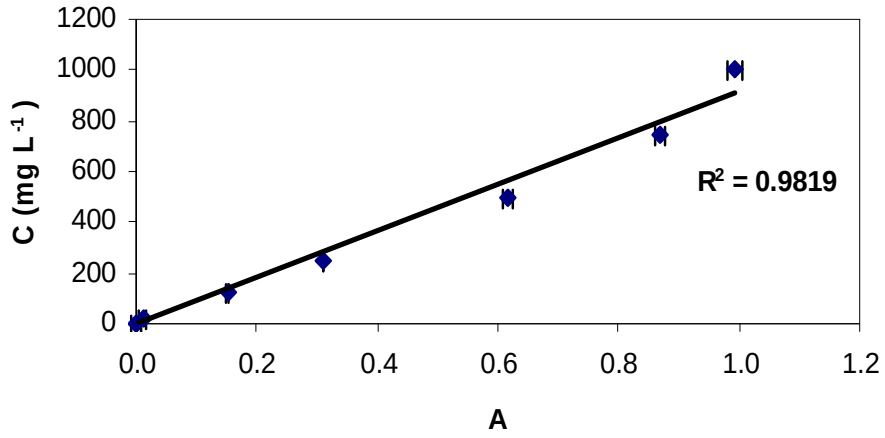

Figure B-2 Calibration curve for protein concentration measurement

The equation used to calculate protein concentration from optical density was as following.

$$C = 914.5 \times A \qquad \text{(B-2)}$$

Where,

C : protein concentration;

A : optical density at 595 nm.

B.3 Calibration Curve for Biomass Measurement

The calibration curve for the measurement of biomass concentration was shown in Figure B-3. A concentrated biomass sample was prepared in a three days old batch culture with 30 mM acetate and 50 mM nitrate, and the biomass concentration was determined as 0.286 g L^{-1} by dry weight methods. Then, this concentrated sample was diluted with millipore water at following ratios, 1:0; 3:1; 1:1; 1:2 and 1:3. The biomass concentrations in these five samples were calculated as 0.286; 0.215; 0.143; 0.095 and 0.072 g L^{-1} respectively. At each biomass concentration, eight samples have been prepared and measured for optical density at 620 nm. The average value was used to plot the figure and standard deviation of these eight samples was used as error bar.

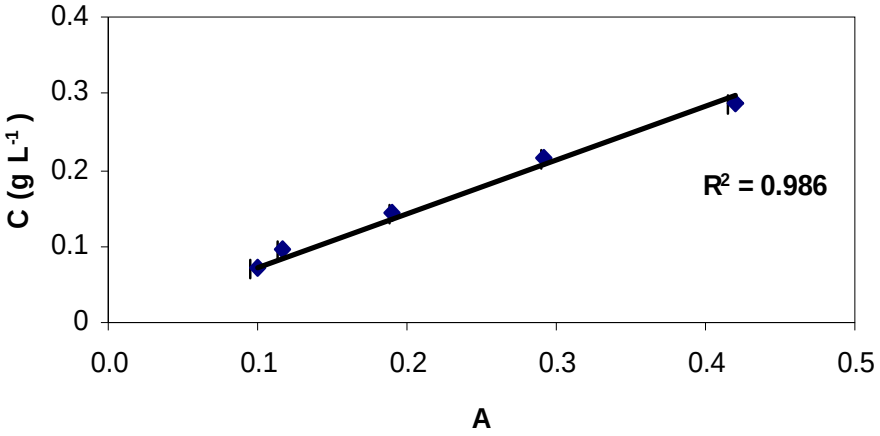

Figure B-3 Calibration curve for biomass concentration measurement

The equation used to calculate protein concentration from optical density was as following.

$$C = 0.6595 \times A + 0.0147 \quad \text{(B-3)}$$

Where,

C : biomass concentration;

A : optical density at 620 nm.