

HYDROGEN-DRIVEN DENITRIFICATION OF NITRATE CONTAMINATED STREAMS

By

Babak Rezania

**A Thesis
Submitted to the Faculty of Graduate Studies
in partial fulfillment of the requirements
for the degree of**

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**Department of Civil Engineering
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ABSTRACT

The goal of this research is to use hydrogenotrophic denitrifying bacteria and hydrogen as exogenous electron donor to stimulate denitrification in nitrate contaminated streams.

The particular nitrate contaminated streams utilized in this study include nitrate contaminated groundwater and tertiary wastewater. The thesis is described in five chapters and covers both engineering and microbiological aspects of implementing hydrogenotrophic denitrification. It explores microbial ecology and kinetics of hydrogenotrophic denitrifiers, which led to the development of a new methodology for studying microbial cultures. The microbiological experiments were initiated to explore if the biological conversion of nitrate to nitrogen gas is a single stage reaction and if is carried out by only autotrophic bacteria. The possibility of presence of acetogenic bacteria in the hydrogenotrophic culture was studied. The kinetics of hydrogenotrophic denitrification was experimentally studied at different pH and temperatures. The hydrogenotrophic culture was further studied to determine the hydrogenotrophic biomass constituents including active biomass, EPS and cell debris. An experimental method was developed for quantifying the active biomass concentration in steady state biomass.

From the engineering perspective, the research is focused on developing anaerobic submerged membrane bioreactors to reclaim water from nitrate contaminated streams including groundwater and wastewater. Technical feasibility of removing nitrate from groundwater and wastewater to produce reusable water using different membrane bioreactor configurations has been experimentally studied. The challenge of effective hydrogen delivery has been addressed by developing a novel bubble-less hydrogen delivery system, which was coupled with the biological treatment unit. Finally, the potentials application of the developed technology and the costs associated with implementing the technology has been discussed and compared with different alternatives.

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GLOSSARY

b_A	decay coefficient (d^{-1})
b_H	Decay coefficients for heterotrophs
BAP	Hydrolyzed soluble EPS ($mg\ EPS\ l^{-1}$)
EPS	Extracellular polymeric substances ($mg\ l^{-1}$)
fd	Biodegradable fraction of active biomass
K_{NO_3}	Half-saturation coefficient for NO_3-N ($mgNO_3-N\ l^{-1}$)
K_{O_2}	Half-saturation coefficient for SO_2 ($mgSO_2\ l^{-1}$)
K_{BAP}	Half-saturation coefficient on hydrolyzed EPS (BAP) ($mg\ EPS\ l^{-1}$)
S_{NO_3}	Concentration of NO_3-N ($mg\ l^{-1}$)
S_{NO_2}	Concentration of NO_2-N ($mg\ l^{-1}$)
q_{BAP}	Maximum specific BAP utilization rate (d^{-1})
SO_2	Dissolved oxygen concentration ($mg\ l^{-1}$)
UAP	utilization-associated products ($mg\ l^{-1}$)
t	time (d)
X_A	Active biomass concentration ($mg\ l^{-1}$)
X_i	Cell debris concentration ($mg\ l^{-1}$)
θ_x	Solids retention time SRT (d)
K_s	Half saturation coefficients ($mg\ l^{-1}$)
SMP	Soluble microbial by-products ($mg\ l^{-1}$)
HRT	Hydraulic retention time (d)
SRT	Solids retentions time (d)
VSS	Volatile suspended solids ($mg\ l^{-1}$)
TSS	Total suspended solids ($mg\ l^{-1}$)
COD	Chemical oxygen demand ($mg\ l^{-1}$)
BOD	Biological oxygen demand ($mg\ l^{-1}$)
TMP	Transmembrane pressure
SEM	Scanning electron microscopy
EDAX	Energy dispersive X-ray spectroscopy
XRD	X-ray diffraction
BER	Biofilm electrode reactor
MBR	Membrane bioreactor

CHAPTER 1

1.1 Introduction

Nitrate is primary pollution in groundwater as it is highly mobile and thermodynamically stable. Nitrate, not only can cause eutrophication in natural environments, but also is harmful to humans and animals. In this section, the mechanism of nitrate transport to groundwater is introduced and adverse effects of nitrate on health are discussed.

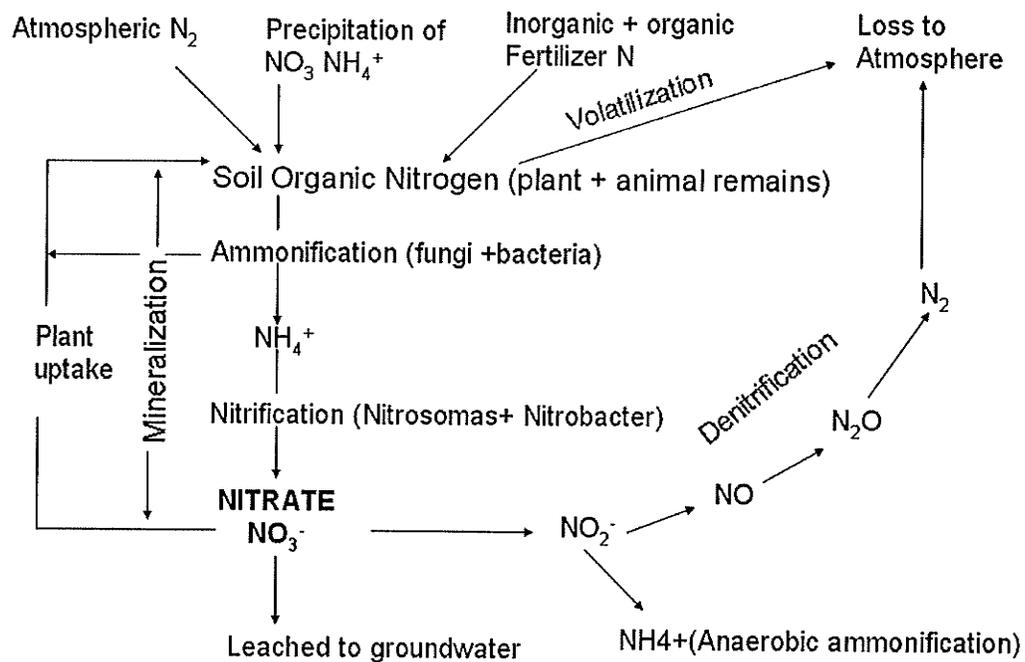
1.1.1 Nitrate transport to groundwater

Nitrogen comprises 79% of earth's atmosphere. In the natural environment, nitrogen is recycled through a series of chemical and biological processes as shown in Figure 1.1. Nitrogen in the atmosphere must be fixed to nitrate and ammonia in order to enter the living systems. A certain amount of atmospheric nitrogen is fixed by lightning and by some *cyanobacteria* (blue-green algae). The great bulk of nitrogen fixation is performed by soil bacteria of two kinds. Those that live free in the soil and those that live enclosed in nodules in the roots of certain plants. Bacteria that live in the roots of legumes are of the genus *Rhizobium*. As free-living microorganisms, heterotrophs in the genera of *Azotobacter* and *Clostridium* are the best known of nitrogen fixers. The fixed nitrogen (Ammonia) is oxidized aerobically to nitrite and eventually to nitrate by *Nitrosomas* and *Nitrobacter*. In the nitrogen cycle, denitrification represents the loss of nitrogen by which the produced nitrate is reduced to nitrogen gas (Leea et al., 2002).

Human activity annually fixes vast amounts of nitrogen, which might upset the natural nitrogen cycle in the biosphere. The main nitrogen sources include the waste produced by humans and animals and fertilizers. Nitrate from human waste originates mostly from

individual septic systems or municipal wastewater treatment facilities. Typically, effluent from such septic systems is in the order of 30 to 60 mg l⁻¹ total nitrogen, with ammonia making up the majority of the nitrogen (MacQuarrie and Sudicky, 2001). The nitrogen content of this effluent varies widely depending upon the condition of the individual system and the type of waste being treated. Another source of nitrogen comprises fertilisers in the forms of inorganic fertilizers and animal waste. Extensive usage of fertilizers is another shock to nitrogen cycle that causes leaching of nitrate to the groundwater.

Figure 1.1 Nitrogen cycle (Leea et al., 2002)



1.1.2 Nitrate: adverse effects on health and environment

1.1.2.1 Human health effects

In human infants nitrate can be reduced to nitrite in the gastrointestinal tract, which may be absorbed into the blood stream and react with haemoglobin to form methemoglobin causing methemoglobinemia, which is also known as Blue Baby Syndrome. This process blocks the oxygen carrying capacity of the blood. When the concentration of methemoglobin becomes too high, the infant becomes cyanotic and can die because of asphyxiation. This condition especially occurs in infants below the age of six month, when the water containing high concentration of nitrate (higher than $10 \text{ mg l}^{-1} \text{ NO}_3\text{-N}$) is mixed with infant's formula. Not only infants, but children and adults suffering from maladies and low levels of stomach acid are also vulnerable to methemoglobinemia.

In 1984, an infant in Laurel, Nebraska was treated for Blue Baby Syndrome. The water used to mix her formula was shown to contain 66 to 80 mg l^{-1} nitrate as nitrogen (Mulvay, 1986). In 1986, an infant in South Dakota died because of ingesting water from a farm well containing approximately 150 mg l^{-1} nitrate as nitrogen (Meyer, 1994). In 1992, a six-week-old Wisconsin infant was diagnosed with methemoglobinemia on the second hospital admission. The contamination was traced to a shallow water supply well contaminated with 40 to 60 mg l^{-1} nitrate as nitrogen and up to 7.8 mg l^{-1} copper. It was concluded that the symptoms were caused by a synergistic effect of the nitrate and copper (Centers for Disease Control and Prevention, 1993). At least two cases of methemoglobinemia have been documented in New Mexico. One case occurred in an area of widespread septic-tank contamination in 1961. The other case occurred in an area contaminated by agricultural fertilization in 1980 (McQuillan, 1997). Two cases of

methemoglobinemia from nitrate contaminated private wells have been documented in South Dakota in 1981 and 1986 (Meyer,1994). A 1982 survey of doctors in the Big Sioux River basin of South Dakota reported the occurrence of approximately 80 cases during the previous 30 years (Meyer, 1994).

1.1.2.2 Animal Health Effects

Poor water quality may cause health problems for Livestock. Nitrate-contaminated water consumed by livestock has resulted in nitrate poisoning. At high enough nitrate concentrations ($> 300 \text{ mg l}^{-1}$), nitrate poisoning may result in animal death. At lower concentrations, nitrate poisoning can increase the incidence of stillborn calves, abortions, retained placenta, cystic ovaries, lower milk production, reduced weight gains, and vitamin A deficiency. Livestock may be harmed at nitrate-nitrogen concentrations between 100 to 300 mg $\text{NO}_3\text{-N l}^{-1}$, and nitrate poisoning in cattle, sheep, and horses may occur at concentrations $> 300 \text{ mg NO}_3\text{-N}$. Recommended limits of nitrate in drinking water for livestock and poultry should not exceed 100 mg $\text{NO}_3\text{-N l}^{-1}$. The accurate assessment of the source of nitrate poisoning is difficult because if the diet includes crops prone to nitrate accumulation, nitrite accumulation in the animal may occur (Kvasnicka and Krysl, 1990).

1.1.3 The Water Quality Guideline

The effects of nitrate on health has provided enough evidence for World Health Organization to (WHO) to set the acceptable limit for nitrate in drinking water at 10 mg $\text{NO}_3\text{-N l}^{-1}$.

1.2. Nitrate removal methods

This section introduces different methods for nitrate removal from contaminated water. Alternatives including, physical, chemical and biological methods are described.

1.2.1 Physical methods

1.2.1.1 Ion exchange (IX)

The ion exchange process comprises the passage of nitrate contaminated water through a resin bed containing strong base anion resin, on which nitrate is exchanged with chloride or bicarbonate ions. The exhausted resin is regenerated using either sodium carbonate or sodium chloride salt. Typically, the order of ion selectivity for IX resin is bicarbonate, chloride, sulphate and then nitrate. Treatment of high sulphate water reduces the capacity of nitrate removal. For instance, an increase in sulphate concentration from 42.5 to 310 mg l⁻¹, decreased nitrate breakthrough time from 400 to 180 bed volumes (BV) (Kapoor et al., 1998). The main limitations of IX include the high cost for regeneration of exhausted resin, production of highly concentrated brines, and organic fouling when dissolved organic matter in the water is relatively high.

1.2.1.2 Electrodialysis

ED involves the transformation of ions through an ion permeable membrane from less concentrated to a concentrated solution by aid of direct electric charge. An electrodialysis unit requires pressurized water (50-70 Psi), a membrane stack and a DC power supply. Nitrate removal is very fast but can only be used for treating soft waters due to scaling problem. Similar to ion exchange, electrodialysis produces waste brine,

which needs to be handled properly. Electrodialysis is an expensive process. The cost of nitrate removal is similar to that by reverse osmosis.

1.2.1.3 Reverse osmosis (RO)

In RO process nitrate is separated from water by forcing the water across a semi-permeable membrane by applying a pressure higher than osmotic pressure. Pressure ranging from 300 to 1500 Psi is applied to reverse normal osmotic flow of water. The RO membranes do not show any preference to remove any specific ion but the degree of rejection is dependent on the valance of ion. RO generally reduces the mineral content of water and is subject to problem such as fouling, compaction and deterioration over time.

1.2.2 Chemical denitrification

Chemical denitrification of drinking water can be achieved using the reaction of either Fe^{2+} or Aluminium powder with nitrate. Both reactions result in reduction of nitrate to ammonia under basic conditions. The end product, which is ammonia, needs to be stripped out by air. These chemical processes are very expensive and produce high quantity of sludge that needs to be properly handled (Kapoor et al., 1998).

Another recently developed method is catalytic reduction of nitrate in the presence of hydrogen, using palladium-alumina as the catalyst. The nitrate removal rate is $3.13 \text{ mg NO}_3^- \text{ min}^{-1} \text{ g}^{-1} \text{ catalyst}$ (Horold et al., 1993). This process is limited to small water treatment systems due to the low denitrification rate and requires further research to assess long-term performance.

1.2.3 Biological Denitrification

Biological denitrification comprises reduction of nitrate to nitrogen gas by different types of bacteria.

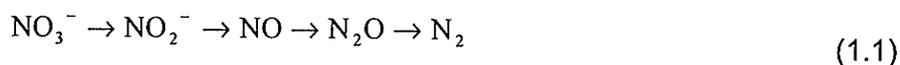
1.2.3.1 Diversity of Denitrifiers

There are two biological pathways for nitrate in nature. A certain few obligate anaerobes such as *Clostridium* reduce nitrate to nitrite and eventually to ammonia. The second pathway, which is called denitrification, comprises dissimilatory reduction of nitrate to nitrogen gas by certain bacteria.

Figure 1.2 Species having denitrifying genera (Lee et al., 2002)

<i>Achromobacter</i>	Gm(-) rods	<i>Moraxella</i>	Gm(-) coccoid
<i>Acintobacter</i>	Gm(-) rods	<i>Neisseria</i>	Gm(-) cocci
<i>Agrobacterium</i>	Gm(-) rods	<i>Paracoccus</i>	Gm(-) coccoid
<i>Alcaligenes</i>	Gm(-) rods	<i>Propionibacterium</i>	Gm(+) rods
<i>Arthrobacter</i>	G(variable) pleomorphic	<i>Pseudomonas</i>	Gm(-) rods
<i>Azospirillum</i>	Gm(-) rods	<i>Rhizobium</i>	Gm(-) rods
<i>Bacillus</i>	Gm(+) rods	<i>Rhodopseudomonas</i>	Gm(-) rods
<i>Chromobacterium</i>	Gm(-) rods	<i>Spirillum</i>	Gm(-) spirals
<i>Corynebacterium</i>	Gm(+) rods	<i>Thermotrix</i>	Gm(-) filaments
<i>Cytophaga</i>	Gm(-) rods	<i>Thiobacillus</i>	Gm(-) rods
<i>Flavobacterium</i>	Gm(-) rods	<i>Thiomicrospira</i>	Gm(-) spirals
<i>Hyphomicrobium</i>	Gm(+) swarmer cells	<i>Vibrio</i>	Gm(-) bent rods
<i>Kingella</i>	Gm(-) coccoid		

The denitrification process involves the formation of a number of nitrogen intermediates that eventuates to nitrogen gas.



Most denitrifiers are heterotrophic gram-negative rods, but few are autotrophic denitrifiers. Autotrophic denitrifiers can use inorganic carbon as the carbon source and either hydrogen or reduced sulphur compounds as the energy source. *Thiobacillus denitrificans* and *Thiomicrospira denitrificans* utilize elemental sulphur and thiosulfate as

electron donor for denitrification. *Alcaligenes eutrophus*, *Paracoccus denitrificans* and several *Pseudomonas* species can grow autotrophically by oxidizing hydrogen.

1.2.3.2 Heterotrophic Denitrification

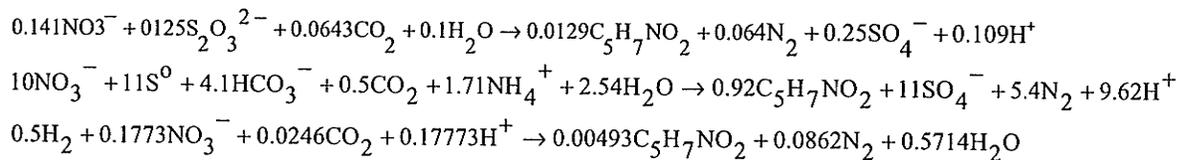
In heterotrophic denitrification, the nitrate is the terminal electron acceptor and variety of organic carbon sources (methanol, ethanol acetic acid etc.) can be used for cell and energy synthesis. In order to drive denitrification in nitrate-contaminated groundwater, an exogenous carbon source must be added, as groundwater often has low organic carbon content.

There are several parameters affecting the choice of organic carbon including cost, low biomass yield and half saturation coefficient (K_s). The half saturation coefficient (K_s) of chosen organic carbon should preferably be low. Half saturation constant is an important factor in biological treatment processes, as the minimum substrate concentration in the effluent at steady state is directly proportional to the half saturation coefficient. Therefore, the lower the K_s value the lower the concentration of substrate in the effluent at steady state condition. For instance for methanol, K_s values of 0.1 mg $\text{NO}_3\text{-N l}^{-1}$ to 67 mg $\text{NO}_3\text{-N l}^{-1}$ and as high as 72 mg $\text{NO}_3\text{-N l}^{-1}$ have been reported (U.S EPA, 1993). Although methanol is considered a cheap organic carbon source, the high K_s of methanol results methanol carry-over to the effluent leading to bio-instability of the treated water and high post treatment costs.

Problems associated with high cost, organic carbon carry-over, high sludge production may be minimized by autotrophic denitrification.

1.2.3.3 Autotrophic denitrification

Autotrophic denitrification is a biological process that uses inorganic carbon to derive denitrification. Different electron donors can be used for autotrophic denitrification. These donors include hydrogen, elemental sulphur, and thiosulfate. The stoichiometric equations for different donors driving autotrophic denitrification are shown as follow (Mateju et al., 1992).



The autotrophic denitrification of water using sulfur compounds carries several disadvantages over hydrogenotrophic denitrification, including:

- Sulfate production as by-product, which requires further treatment.
- Higher alkalinity consumption per unit of $\text{NO}_3\text{-N}$.
- Higher biomass yield.
- Sensitive to pH.

Autotrophic denitrification using hydrogen is an attractive option for removing nitrate from groundwater. The advantages are that (Gantzer, 1995):

- The residual hydrogen can easily be removed from the treated water.
- It produces less biomass compared to heterotrophic denitrification.
- It has lower cost of electron donor per unit of nitrate removed compared to heterotrophic denitrification.

Hydrogen also carries the disadvantages of:

- Low solubility in water.
- Explosivity when it is mixed with air.

- o Delivery efficiency

1.3. A critical review on hydrogen-dependent denitrification systems

This section critically reviews different designs and reactor configurations used for hydrogenotrophic removal of nitrate from groundwater. Advantages and constraints of each system are discussed. The discussions are followed with recommendations for improving the systems.

1.3.1 Fixed film reactors

The early designs for removing nitrate by means of hydrogen as electron donor comprised attached growth reactors. Using hydrogen-oxidizing bacteria for autotrophic denitrification date back to 1987, when (Kurt et al., 1987) used a fluidized bed sand bioreactor for denitrification of drinking water. An external bubble absorption tank was used for saturating the feed. The adsorption tank was coupled with the fluidized bed with an internal recycle loop. In this study $25 \text{ mg NO}_3\text{-N l}^{-1}$ of nitrate was removed within a 4.5 hour hydraulic residence time in a reactor with the volume of 5.1 l, where pH and temperature were kept at 7.5 and 30°C . Volumetric and surface removal rates of $0.13 \text{ kg N m}^{-3}\text{d}^{-1}$ and $0.3 \text{ g N m}^{-2} \text{ d}^{-1}$ were obtained, respectively. At hydraulic retention times (HRT) less than 4.5 hours, nitrite accumulation was observed. In batch experiments, nitrite accumulation was consistently observed. The nitrite accumulation in the batch test could be the result of lower hydrogen mass transfer rates into the biofilm in compare to continuous operation. In the continuous operation higher mass transfer

rates are expected as hydrogen was already dissolved in the feed and the feed was circulating.

In 1988, Gros and his coworkers developed a full-scale plant for denitrification of 100m³/h nitrate contaminated groundwater with nitrate concentration of 75 mg l⁻¹. They incorporated four fixed bed reactors in series, flocculent addition, a two layer sand filter, and UV disinfection. Hydrogen, CO₂ and groundwater were mixed and injected to the first reactor. During five years operation, the plant was able to reduce nitrate concentration from 75 mg l⁻¹ to less than 1 mg l⁻¹ nitrate with the overall denitrification rate of 0.15-0.5 kg N m⁻³.d⁻¹. The actual consumption of hydrogen was 11 gram for 100 gram of nitrate removed. The total organic carbon in the feed was between 0.4 to 0.7 mg l⁻¹. After denitrification the organic carbon increased to 1 -1.1 mg l⁻¹ TOC. TOC was reduced to 0.7- 0.8 mg l⁻¹ after flocculation and sand filtration (Gros et al., 1988).

Dries et al., (1988) used two column reactors filled with polyurethane, in which nitrate was removed in the first reactor, and nitrite and excess hydrogen were oxidized in the second reactor. In this research, water containing 15 mg NO₃-N l⁻¹ was denitrified with the volumetric removal rate of 0.2 kg m⁻³.d⁻¹ and surface removal rate of 0.28 g NO₃-N m⁻².d⁻¹. In 1999, Cheng et al. immobilized *Alcaligenes eutrophus* in polyacrylamide and alginate copolymer in a fluidized bed system. Maximum denitrification of 0.6-0.7 kg N m⁻³.d⁻¹ was achieved. Reduction of nitrite to nitrogen gas was inhibited at dissolved hydrogen concentrations of below 0.2 mg l⁻¹, while reduction of nitrate was inhibited at dissolved hydrogen concentration below 0.1 mg l⁻¹. As it will be discussed later, the half saturation coefficient for hydrogen is very small (less than 1% of saturation). Therefore,

the dramatic decrease in denitrification rate might be inferred from the diffusion limitation of dissolved hydrogen from the bulk into the polymer.

Table 1. 1 A comparison between different fixed film systems for hydrogenotrophic denitrification of water

Reactor type	Temp.°C	Volumetric Removal (kg Nm ⁻³ d ⁻¹)	Surface Removal (g N m ⁻² .d ⁻¹)	Reference
Fixed film	10.5	0.4	-	Gros et al.,(1988)
Fluidized bed	30	0.13	0.3	Kurt et al.,(1987)
Fixed film	12-20	0.5	0.28	Dries et al.,(1988)
Fixed film	15	0.31-0.34	-	GINOCCHIO et al.,(1984)
Fluidized bed	30	0.6-0.7	-	Cheng et al.,(1998)

Fixed film reactors carry the disadvantage of poor hydrogen delivery and possible tendency for clogging. Despite the fact that hydrogenotrophic denitrifiers has been considered very slow growing (Gros et al., 1988), kinetics study by different researchers show that the growth rate of hydrogenotrophic denitrifiers is in the range of heterotrophic denitrification. Therefore clogging might be an issue.

In hydrogenotrophic fixed film systems the operating pH should be relatively low, although the optimum denitrification rates are obtained in the pH range of 8.5 to 9.5 (Rezania et al., 2004). High pH increases the precipitation rate of calcium and magnesium ions with carbonate and phosphate (Lee & Rittmann, 2003), which might accumulate in the reactor.

1.3.2 Hydrogen-dependent denitrification using a biofilm electrode reactor

The limitation of fixed film reactors including, poor mass transfer and loss of hydrogen to the effluent, and need for separate hydrogen production was minimized by using a

biofilm electrode reactor (BER). The BER is an electrochemical cell, which is composed of a cathode, an anode, a cation permeable membrane and a DC power supply. The hydrogen is produced on the surface of cathode and oxygen is produced on the surface of anode. The hydrogen produced on the surface of cathode allows formation of hydrogenotrophic denitrifiers. The produced oxygen on the anode can increase the dissolved oxygen in the bulk, which can inhibit the denitrification. Therefore, a permeable membrane separates the anode and cathode allowing the transfer of ions while limiting the oxygen dissolution to the bulk.

By applying an electric current to the electrodes, the following reactions occur.

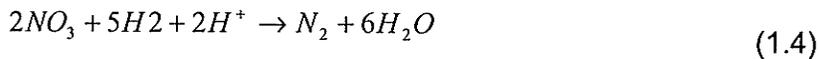
The cathodic reaction:



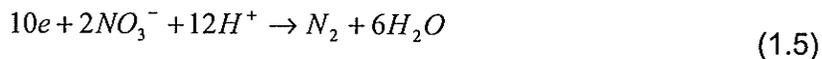
The anodic reaction:



The biological denitrification using hydrogen (Kurt et al., 1987)



Combining equations (1.2) and (1.4):



In the case that there is no separation between anode and cathode the net reaction is:

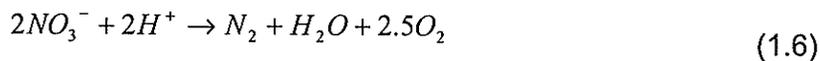


Table 1.2 summarises the performance of BER systems used for denitrification of nitrate contaminated water.

Table 1. 2 Performance of BER systems used for denitrification of nitrate contaminated water.

Reactor Type	Cathode	Anode	Temp.	Current mA	NO ₃ -N	Denitrification Rate (kg N.m ⁻³ .d ⁻¹)	Reference
Batch	graphite	graphite	-		8.6	0.019	Sakakibara & Kuroda, 1993
Contentious	Carbon	Carbon	Room	0-100	20	0.035	Islam et al., 1998
Batch	-	-	-	-	26	0.0064	Sakakibara et al., 1995
Batch	Stainless steel & Graphite	Stainless steel & Graphite	7	12	20	0.0067	Cast & Flora, 1998
Contentious	Stainless steel	Carbon	20-30	5	20	0.06	Fleke et al., 1998
Contentious	Titanium	Metal coated pt	25±3		10	0.12	Sakakibara & Nakayama, 2001
Contentious	SS+GAC	Pt coated with Ti	Room	40-300	5.4	0.393	Prosnansky & Sakakibara, 2002
Contentious	graphite	Ti coated pt	26±1	20-80	23	0.45	Kiss et al., 2000

In some cases (Feleke et al., 1998 ; Islam et al., 1998) low nitrate removal efficiencies per surface area of cathode was observed. These low surface removal rates can be due to the fact that the cathode and anode were not separated and dissolved oxygen in the bulk might have been high.

In future studies, nitrate removal efficiency was improved by increasing the cathode surface area and separating the cathode and anode. Materials such as granular activated carbon (GAC) have been used as a cathode due their high porosity and large surface area (Sakaribara and Nakayama, 2001; Kiss et al., 2000). In the case that GAC is used, the efficiency of the systems can be further increased by using multi-electrode systems. In a multi-electrode system, a series of porous metal sheets are connected in series, which are in contact with GACs (Prosnansky & Sakakibara, 2002). The metal sheets reduce the resistance, which results in easier current passage.

The main drawback of BER systems is the gradual scale formation on the cathode surface. The precipitation rate in BER reactors is expected to be higher compared to the other hydrogen dependent denitrification systems. Comparing equation (1.4) and equation (1.5) shows that in BER systems 6 moles of proton is consumed per 1 mole of $\text{NO}_3\text{-N}$ reduced, which is 6 times higher than non-electrically aided denitrification systems. Higher hydroxyl (OH^-) production rate on the cathode side shifts the pH faster, causing precipitation of calcium and magnesium ions and scale formation.

Good performance of BER reactors relies on different factors such as:

1. High surface area of cathode and reduced current passage resistance by use of multi-electrode systems
2. High oxidation resistance of the anode material, otherwise, the corrosion of the anode leads to the contamination of the treated. However the corrosion resistance materials such as Ti coated with platinum are expensive.
3. The anode and cathode should be separated by means of an ion permeable membrane to limit the diffusion of dissolved oxygen to biofilm.
4. The current density should be controlled; high currents higher than 60 mA can reduce the ORP to very low values causing methane production by methanogens (Islam et al, 1998).
5. The optimum current density is $4\text{-}5 \text{ A m}^{-2}$ (Prosnansky & Sakakibara, 2002).
6. Scale formation on the surface of electrodes might be controlled by reversing the current every few minutes, which is a common method for scale controlling in electro dialysis systems.

1.3.3 Hydrogenotrophic denitrification with a membrane biofilm reactor

With developing membrane technology, the bubble-less dissolution of hydrogen into water was made possible by using microporous membrane diffusers. These

membranes can be made of different hydrophobic materials including polypropylene, poly tetrafluorethylene (PTFE), polypropylene, Teflon™ and Gortex™. The pore diameter of these membranes usually ranges from 0.02 to 0.05 μm. Different membrane configurations have been used for bubble-less gas diffusion including, tubular (Hirasa et al., 1991), plate and frame (Kniebusch et al., 1990) and hollow fibre (Ergas & Ruess, 2001). The hollow fibre module is the most common configuration due to its higher packing density. The internal diameter of the fibres ranges from 200 to 400 μm, depending upon the application.

A membrane biofilm reactor is simply fabricated by immersing the membrane gas diffuser in the reactor. The membrane acts as a support medium for the biofilm, while introducing the gas to the developed biofilm. In hydrogenotrophic denitrification, hydrogen (electron donor) is provided through the membrane while nitrate diffuses from the bulk liquid to the biofilm.

Mass transfer is facilitated by diffusion through gas filled pores, where the mass transfer coefficient is directly proportional to the porosity of the membrane. The hydrogen flux to the bulk liquid is directly proportional to hydrogen pressure on the lumen side of the membrane and can be calculated from the following equation (Ahmed & Semmens, 1992).

$$N = K \left(\frac{P_{H_2}}{H} - C_L \right) \quad (1.7)$$

N is the hydrogen flux ($\text{g m}^{-2} \text{s}^{-1}$), K is the overall mass transfer coefficient (m s^{-1}), P_{H_2} is the partial pressure of H_2 (atm) H is Henry's law constant ($\text{atm.m}^{-3} \text{g}^{-1}$), and C_L is the dissolved hydrogen concentration in the bulk liquid (g m^{-3}). The hydrogen flux from the membrane to the biofilm is a function of the distance from the membrane. The mass

transfer inside the biofilm varies as a function of the concentration profile within the biofilm.

Higher hydrogen flux to the biofilm will result in higher denitrification rates. (Gantzer, 1995) reported that increasing the pressure in the fibre from 1 to 2 atm improved the denitrification rate from 1.4 to 2 g NO₃-N m⁻².d⁻¹ but resulted in an increase in dissolved hydrogen concentration from 0.17 to 0.66 mg H₂ l⁻¹. A similar trend was observed by Lee and Rittmann (2000), as increasing the hydrogen pressure from 0.31 to 0.42 atm increased the denitrification rate from 1.5 to 2.1 g NO₃-N .m².day, also raising the effluent dissolved hydrogen from 0.009 to 0.07 mg l⁻¹.

Table 1.3 The performance of membrane biofilm reactors

Reference	HRT (Hour)	%Removal	Surface Removal (g NO ₃ -N m ⁻² .d ⁻¹)	Volumetric Removal(kg NO ₃ -N m ⁻³ .d ⁻¹)
Lee & Rittmann, 2002	0.7	76-92	0.8-1	0.25-0.552
Ergas& Reuss,2001	30	90	2.5	0.77
Pierkiel, 2002	1.75	93	0.32	0.312
Benedict, 1996	6	42.7	1	2.63

1.3.3.1 The operation mode

Hydrogen can be introduced to the membrane module in two ways. The membranes can be operated in flow-through mode or dead-end mode. In the flow-through mode the introduced gas is vented after passing through the membrane module, while in the dead-end mode all of introduced gas is forced to the biofilm. In flow-through operation, venting the gas results in hydrogen loss and potential explosion problems. The dead-end mode of operation is advantageous due to the fact that higher hydrogen transfer efficiencies can be obtained up to 100%. It also carries some disadvantages. At the membrane-biofilm interface, the back diffusion of nitrogen gas to the membrane fibre

decreases the hydrogen pressure along the length of the fibres (Ahmed & Semmens, 1992). The decrease in hydrogen partial pressure reduces mass transfer rate and also causes formation of an uneven biofilm along the fibre. The back diffusion of nitrogen can be reduced by increasing the hydrogen pressure on the lumen side. Membrane gas diffusers are also subject to condensation. Due to the high mass transfer coefficient of water vapour, the water vapour will saturate the feed gas within the fibre length. During the dissolution of the gas, its volume reduces and it will become supersaturated with water, and water condensation might occur (Ma et al., 2003). Condensation may take place at the liquid membrane interface, inside the fibre on the membrane surface, in the bulk gas or within the pores. Water condensation inside the pores and at the phase inside the membrane reduces mass transfer efficiency. In the flow through mode the condensed water in the gas phase or on the membrane surface inside the module might be removed from the module. However water condensation can cause significant problems in dead-end modules. The diffused water vapour can carry ions resulting in inorganic membrane fouling. There are different challenges in the application of membrane biofilm reactors in large scale including:

1. The membrane gas diffusers are delicate and any physical damage to the fibre will cause bubble release and inefficient hydrogen delivery.
2. Biofilm thickness control. An internal recycle was used by some researchers to remove the excess biomass, but it requires high energy (Ergas & Reuss, 2001).
3. The precipitation of calcium and magnesium ions with phosphate and carbonate ions makes the biofilm hard to shear off.

4. Porous membrane diffusers are subject to condensation of water vapour in the pores.

Suggestions:

- Non-porous membrane diffusers might minimize the problems associated with condensation. However lower mass transfer rate can be expected, which could be compensated by providing large surface area.
- Precipitation of ions in the biofilm might be reduced by introducing mixture of carbon dioxide and hydrogen through the diffuser. Carbon dioxide, which will be used as carbon source, decrease the pH inside the biofilm leading to less precipitation.

1.3.4 Hydrogenotrophic denitrification in extractive membrane bioreactors

One of the limitations of using hydrogen for denitrification of water is the release of soluble microbial products to the water, which appears in the effluent as soluble COD. Soluble microbial products (SMP) can be classified as utilization-associated products (UAP) and biomass associated products (BAP). UAP are associated with substrate metabolism and biomass growth and are produced at a rate proportional to the denitrification rate. BAP are associated with biomass decay and are produced at a rate proportional to biomass concentration (Barker and Stuckey, 1999). Soluble microbial products are slowly biodegradable and their degradation kinetics has been studied in aerobic membrane bioreactors (Lu et al., 2002).

One approach for preventing contamination of treated water with soluble microbial products is separating the microbial culture from the feed. In this case the suspended denitrifying culture is separated from nitrate contaminated water by means of a

membrane with average pore size of 0.02 μm (Mansell and Schroeder, 1999; Mansell and Schroeder, 2002). The mechanism of nitrate removal is based on diffusion of nitrate through the membrane to the denitrifying culture, which requires addition of an electron donor. Mansell and Schroeder (1999) used methanol as the energy source. A nitrate removal efficiency of 90% with a flux of 4 g $\text{NO}_3\text{-N m}^{-2} \text{d}^{-1}$ was achieved with an influent concentration of 20 mg l^{-1} $\text{NO}_3\text{-N}$ at an HRT of 66 min. The reverse diffusion of methanol to the treated water was the main limitation.

The problem with methanol contamination was eliminated with the use of hydrogen as electron donor (Mansell and Schroeder, 2002). By using a hydrogenotrophic culture, removal efficiencies ranging from 96% to 92% with the rate of 2.7-5.3 g $\text{NO}_3\text{-N m}^{-2} \text{d}^{-1}$ were achieved at influent concentrations ranging from 20 to 40 $\text{NO}_3\text{-N mg l}^{-1}$.

CHAPTER 2: HYDROGEN-DEPENDENT DENITRIFICATION OF WATER IN AN ALTERNATING ANOXIC-AEROBIC MEMBRANE BIOREACTOR

2.1 Objectives

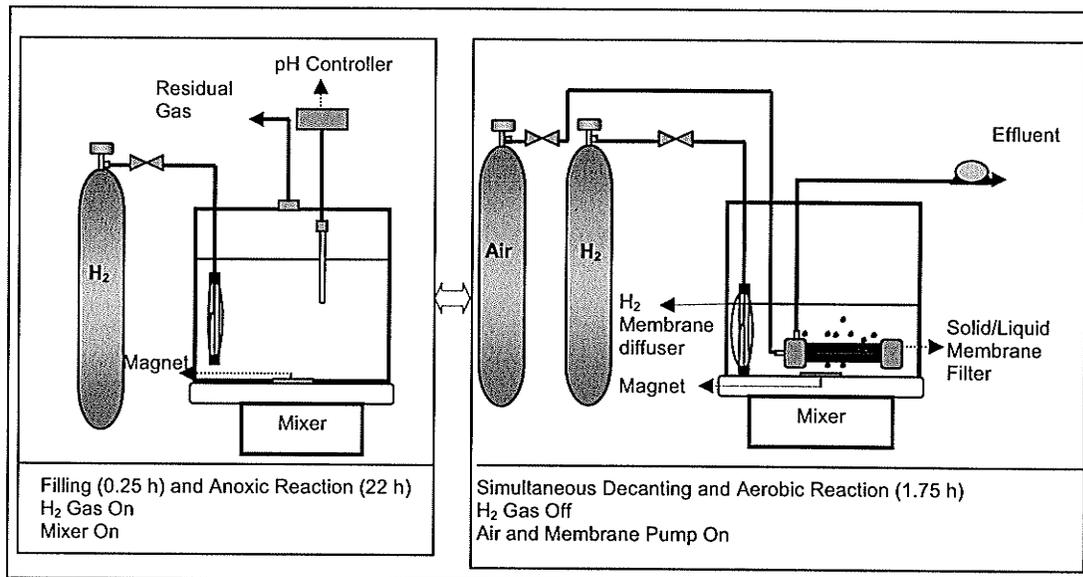
A hydrogenotrophic denitrification system, which consisted of a sequencing batch membrane bioreactor, was evaluated for simultaneous removal of nitrate and soluble microbial products (SMP) from a synthetic groundwater feed. The main goals of this research were as follow:

- Removal of nitrate from synthetic groundwater in a suspended culture membrane bioreactor using hydrogen as electron donor.
- Minimizing the concentration of soluble microbial products (SMP) in the effluent.
- Studying the hydrogenotrophic denitrification rate at different hydrogen pressures applied to the membrane diffuser.

2.2 Experimental set-up

The experimental system used for denitrification of highly nitrate contaminated groundwater is illustrated in Figure 2. 1. The system consisted of a cylindrical Plexiglas reactor with the total volume of 10.1 L and working volume of 8.1 L, a hollow fibre membrane gas diffuser with the total surface area of 0.0056 m², a hollow fibre membrane water filter with the total surface area of 0.047 m², and a pH controller. The hollow fibre membrane diffuser (constructed from Celgard® gas contacting fibres X30-240) was used for bubble-less diffusion of hydrogen gas into the reactor and the membrane filter (ZeeWeed® ZW-1) with a nominal pore size of 0.04 micron was used for separating biomass from treated water.

Figure 2. 1 Schematic of the system used for hydrogenotrophic denitrification of groundwater



2.2.1 Reactor start up

The reactor was initially seeded with acclimated hydrogenotrophic biomass and fed highly nitrate contaminated synthetic groundwater. The synthetic feed was composed of $750 \text{ mg l}^{-1} \text{ NaHCO}_3$, $20 \text{ mg l}^{-1} \text{ K}_2\text{HPO}_4$, $5 \text{ mg l}^{-1} \text{ CaCl}_2$, $20 \text{ mg l}^{-1} \text{ MgSO}_4 \cdot 7\text{H}_2\text{O}$ and $1 \text{ mg l}^{-1} \text{ FeSO}_4$. A concentrated nitrate stock solution was added to the feed so that the concentration of $\text{NO}_3\text{-N}$ in the reactor (ultimately maintained at $330 \text{ mg l}^{-1} \text{ NO}_3\text{-N}$) was not dependent on the hydraulic retention time (HRT).

2.2.2 Operating conditions

The reactor was operated in a one fill and draw cycle per day. After filling the reactor, the hydrogen gas was introduced to the reactor for 22 hours anoxic period. After finishing the anoxic period, the hydrogen was stopped and air was injected for 1.75

hours through the central manifold of the membrane for both membrane scouring and aerobic biological degradation of SMP produced during anoxic period. The reactor was operated with an SRT of 20 days and an HRT of 3 days. The operating temperature and pH were maintained constant at 10-12 °C and 8±0.2, respectively.

2.2.3 Membrane maintenance and cleaning

In order to provide sufficient hydrogen for autotrophic denitrification, hydrogen pressure and flow-rate to the diffuser were maintained at 0.55 atm and 20-40 ml min⁻¹ respectively. Every three weeks of operation, the membrane diffuser was first soaked in 20% solution of sodium hydroxide and rinsed with deionised water (for removing the biological fouling), followed by a soak in a 5% phosphoric acid solution and rinse with deionised water (for removing the precipitated inorganics). During the draw cycle, the ZW-1 membrane was used for biomass separation at an average flow-rate of 20 ml min⁻¹. Compressed air was pushed through the central manifold of the membrane module to facilitate scouring and introduce dissolved oxygen during the aerobic draw cycle. The membrane was cleaned every two weeks by soaking it in a 200 ppm sodium hypochlorite solution for 5 hours.

2.2.4 Analytical methods

pH was controlled using a pH controller (OAKTON Instruments, Alpha 100 Series 1/8 DIN). Effluent nitrate and nitrite were analyzed using an automated Technicon nitrogen analyzer following Standard Methods 4500-NO₃-F (APHA, 1998). Mixed liquor suspended solids (TSS and VSS) were analyzed using Standard Methods 2540D and 2540E (APHA, 1998), and the soluble chemical oxygen demand (SCOD) was measured

using the Hach Digestions Vials and Hach spectrophotometer (Hach, USA) after filtration of the samples through the 0.45 μm membrane filter.

2.3 Results and Discussions

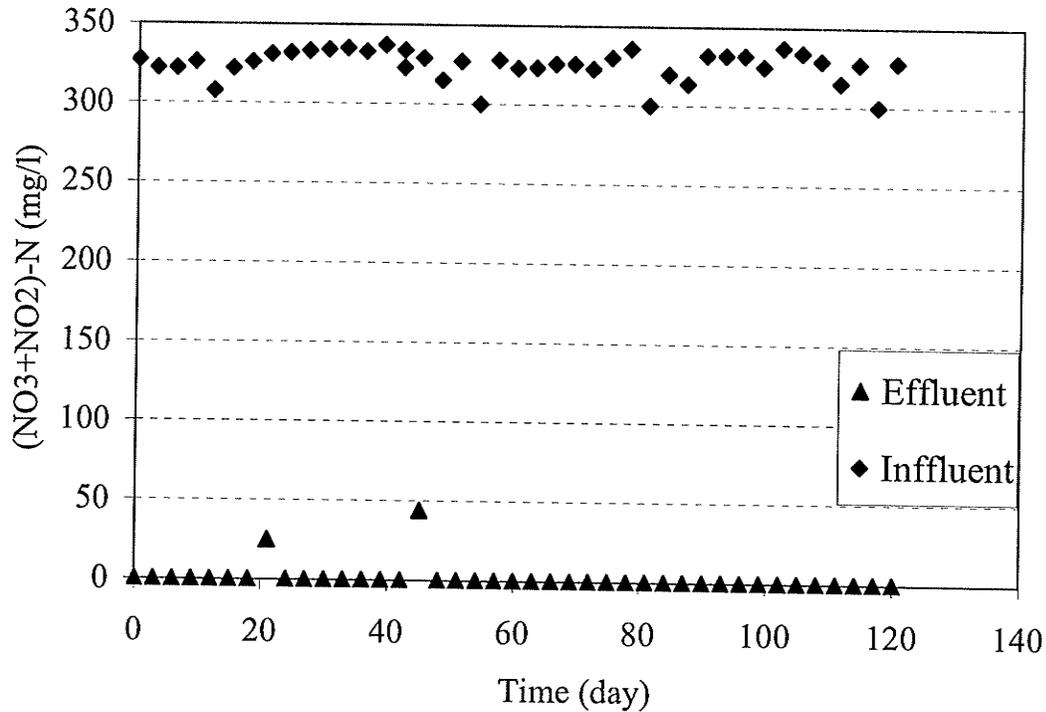
2.3.1 Nitrate removal

As shown in Figure 2. 2, the SBR-membrane bioreactor system was effective in complete nitrate removal from the synthetic feed, when the nitrate concentration in the reactor was kept 330 mg l^{-1} $\text{NO}_3\text{-N}$.

However, nitrite accumulation was observed a couple of times during the experiment. Nitrite accumulation was found to be the result of unexpected membrane diffuser fouling. The fibres used in the construction of the hydrogen membrane diffuser were made from polypropylene, which is naturally hydrophobic, to avoid water from passing through the membrane wall. Therefore, membrane fouling was expected to occur only because of particle deposition on the surface of the membrane. However, the initial measure of physical cleaning of the membrane surface was found to be insufficient for fully restore hydrogen diffusion, resulting in incomplete denitrification. When chemical cleaning was instituted every 3 weeks, nitrite accumulation was not observed.

In the previous research studies on hydrogen dependent denitrification of ground water, nitrogen removal rates in the range of 0.13 - 0.7 $\text{kg N m}^{-3} \text{d}^{-1}$ (Table 1.1) were achieved. In the conducted study the denitrification rate of 0.8 $\text{kg N m}^{-3} \text{d}^{-1}$ was obtained, which is comparable with the other studies (Table 1. 1).

Figure 2. 2 Nitrogen removal at steady-state condition at the load of $0.328 \text{ kg N m}^{-3} \text{ d}^{-1}$.

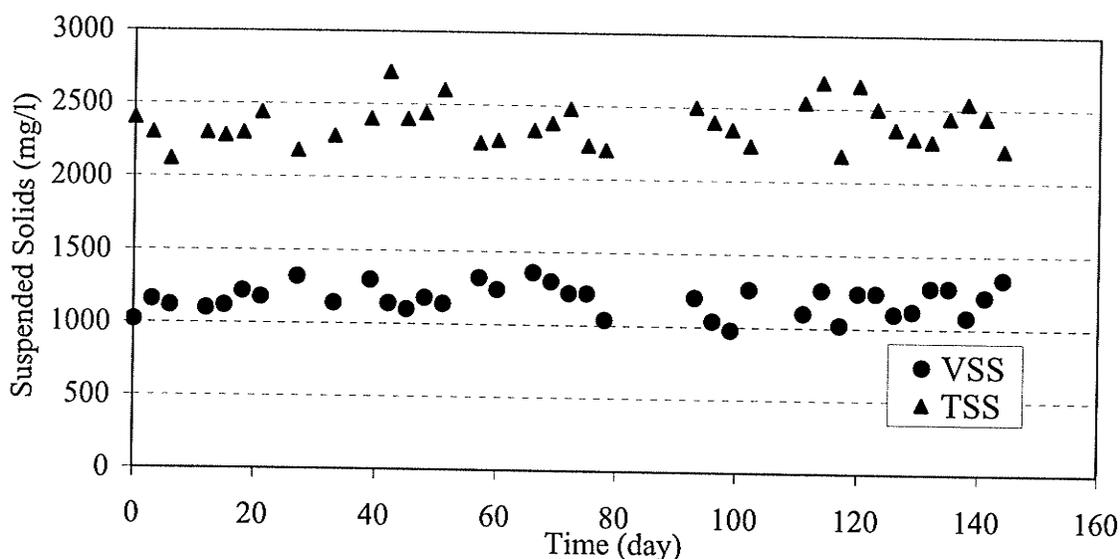


2.3.2 Biomass concentration

Figure 2.3 shows the concentration of volatile and suspended solids at steady state condition, with average values of 1162.6 mg l^{-1} for VSS and 2322.5 mg l^{-1} for TSS. The inorganic fraction of TSS can be attributed to the precipitation of Ca^{2+} with phosphate or carbonate ions, creating calcium-phosphate or calcium-carbonate solids. Precipitation seems to be one of the major reasons for fouling of hydrogen diffusers, as was reported by previous researchers (Ergas and Reuss, 2001). Precipitation of mineral solids was found to have a negative impact on the performance of hydrogen diffuser membranes as build-up inside microbial aggregates and on the surface of membranes was reported (Lee and Rittman, 2002). Cations in water, such as Ca^{2+} and Mg^{2+} , can precipitate basic

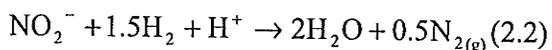
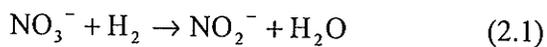
anions, such as hydroxide, carbonate, phosphate, mono-hydrogen phosphate and di-hydrogen phosphate. Minerals with higher pK_{s0} , such as $Ca_5(PO_4)_3OH$, $Ca_3(PO_4)_2$ and $CaCO_3$ have lower solubility, and are therefore expected to be the major contributors to the inorganic fraction of TSS. It should be noted that solubility of the precipitated materials is pH dependent, as higher precipitation of inorganic compounds is expected at higher pH.

Figure 2. 3 Total and volatile suspended solid concentration at steady state conditions.



2.3.3 Effect of hydrogen partial pressure on denitrification rate

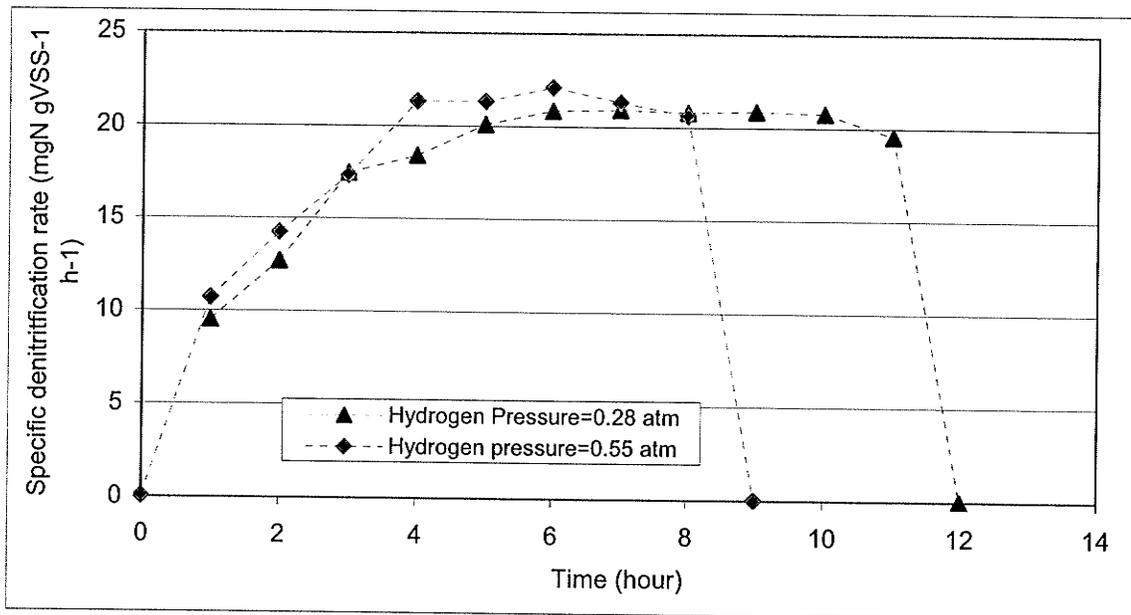
Biological denitrification involves the reduction of NO_3^- to NO_2^- , NO , N_2O and ultimately to nitrogen (N_2) gas. The stoichiometry of denitrification reactions can be summarized in two main reactions as follows:



Nitrate and nitrite consumption along with overall denitrification rate were evaluated at two different hydrogen pressures (0.28 and 0.55 atm). Samples were taken at 1 hour

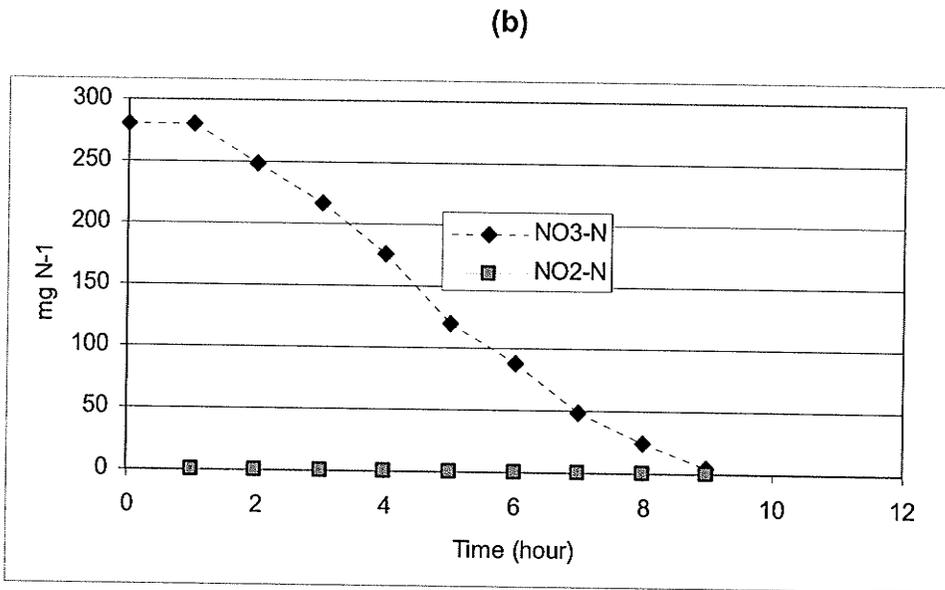
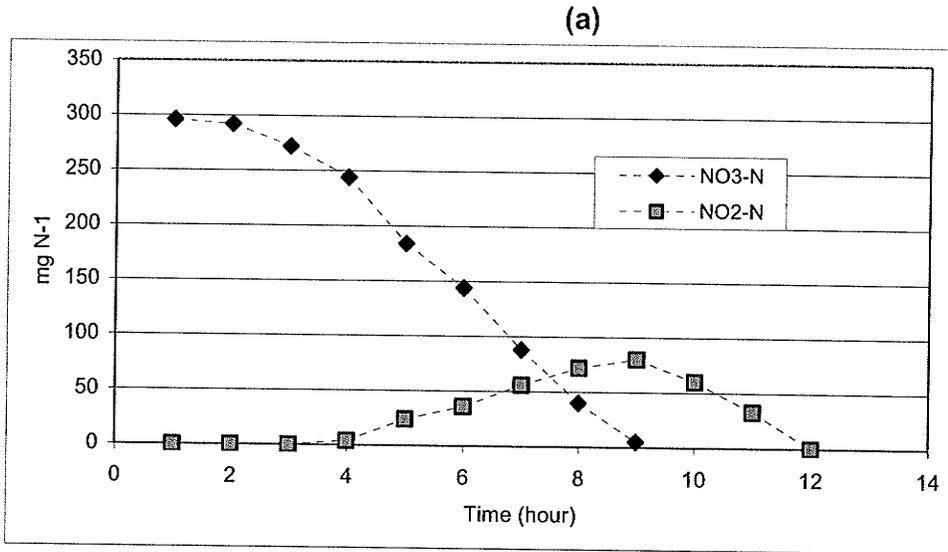
intervals after feeding the reactor and analyzed for nitrate and nitrite concentrations. Figure 2.4 shows the specific denitrification rate in the reactor for the two different hydrogen pressures.

Figure 2. 4 Effect of hydrogen pressure on denitrification rate at steady state condition.



At start-up, a lag phase was observed due to the residual dissolved oxygen present in the reactor after the aerobic cycle. This was followed by steady denitrification rates. Despite the 22 hour anoxic period, all of the nitrate was removed after 9 and 12 hours for hydrogen pressures of 0.55 and 0.28 atm, respectively. This indicates that the reactor was fairly resistant to shock loadings of nitrate. According to equations 1 and 2, 1 mole of hydrogen is consumed per 1 mole of nitrate, whereas 1.5 mole of hydrogen is consumed per 1 mole of nitrite. Therefore, the accumulation of nitrite might occur if there is not sufficient hydrogen available for the biomass. The effect of hydrogen on accumulation of nitrite is shown in Figure 2.5.

Figure 2. 5 Nitrate and nitrite concentrations in the reactor at different hydrogen pressures (a) 0.28 atm, (b) 0.55 atm



While no nitrite was detected at a hydrogen pressure of 0.55 atm, nitrite build-up and gradual disappearance was observed at hydrogen pressure of 0.28 atm. When the hydrogen pressure was lowered below 0.2 atm, reaction 2 was much slower than reaction 1 and nitrite accumulation was observed in the effluent.

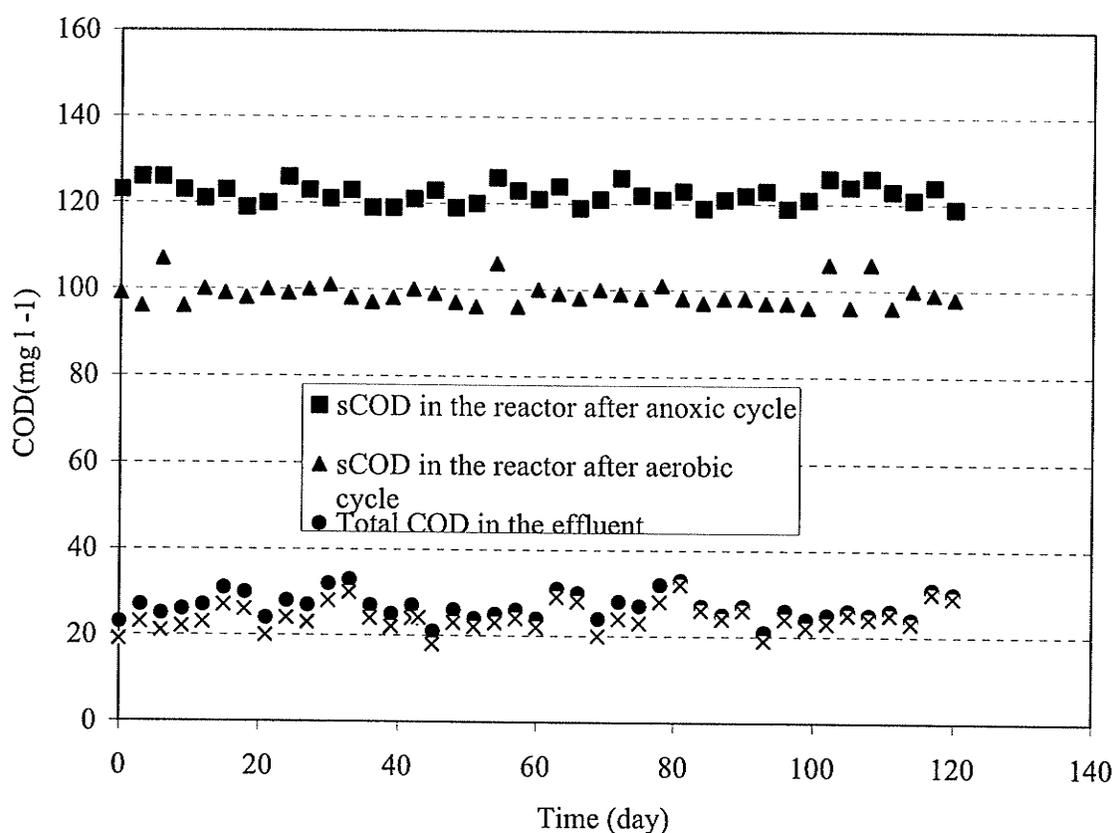
This study showed that the kinetics of hydrogenotrophic denitrification is comparable with heterotrophic denitrification as the denitrification rate of 17- 20 mg N gVSS⁻¹ h⁻¹ was obtained at pH of 8±0.2 and temperature of 10 °C. Heterotrophic denitrification rate varies based on the type of organic carbon used for denitrification. Foglar and Briski, (2003) used methanol as the carbon source for denitrification of water at 25 °C, and denitrification rate of 17.88 mg N gVSS⁻¹ h⁻¹ at biomass concentration of 1.2 g l⁻¹ was achieved. In another study (Glass and Silverstein, 1998) acetic acid was used as a carbon source for denitrification of highly contaminated water and a denitrification rate of 23 - 25 mg N gVSS⁻¹ h⁻¹ was obtained at pH range between 7.5 to 8.5 and operating temperature of 25 °C.

2.3.4 Removal of soluble microbial products

Soluble microbial products (SMP) can be classified as utilization-associated products (UAP) and biomass associated products (BAP). UAP are associated with substrate metabolism and biomass growth and are produced at a rate proportional to substrate utilization, which is the denitrification rate in this study. BAP are associated with biomass decay and are produced at a rate proportional to biomass concentration (Barker and Stuckey, 1999). The production of SMP can affect the system in two ways. Firstly, SMP will leave the system in the effluent as soluble COD (sCOD), which will necessitate further downstream treatment. Secondly, SMP can act as major bio-foulants of membranes, requiring frequent chemical cleaning and reducing overall operational efficiency (Cicek *et al.*, 2003). SMP are biodegradable, but their removal requires heterotrophic activity. It has been found that all of hydrogen-dependent denitrifiers are mixotrophic as they are able to use inorganic carbon under autotrophic and organic

carbon under heterotrophic conditions (Szekeres *et al.*, 2002). Therefore, in this system, autotrophic denitrification was alternated with heterotrophic SMP removal under aerobic conditions. Soluble COD (measured by filtering through 0.45 μm) was used as an indicator of SMP in the reactor and the effluent. Figure 6 shows the soluble COD in the reactor and in the effluent before and after decanting.

Figure 2. 6 Soluble COD removal during filtration at steady state condition.



As Figure 2.6 shows, after the completion of the anoxic period, a significant portion of the soluble organic matter remains in the reactor (average of 122.2 mg l^{-1}). This is due to the rejection of higher molecular weight SMP by the membrane, as the pore size of the membrane is 0.04 μm . Smaller size SMP passed through the membrane resulting

in an average of 24.19 mg l⁻¹ of soluble COD and 26.8 mg l⁻¹ of total COD in the effluent.

The mass balance of SMP at steady state condition is given as follows:

$$SMP_{\text{Consumed}} = SMP_{\text{In the reactor}} - SMP_{\text{Out}} - SMP_{\text{Rejected}}$$

$$SMP_{\text{Consumed}} = 122.2 (8) - 122.2 (0.4) - 24.19(2) - 99. \quad (2.3)$$

$$SMP_{\text{Consumed}} = 88.34 \text{ mg}$$

According to this mass balance, the amount of SMP consumed during the aerobic period can be calculated by subtracting the SMP rejected by the membrane and removed through waste activated sludge (WAS) and filtrate from total amount of SMP in the reactor produced during the anoxic period. As a result, 81% of SMP was rejected by the membrane, 9% was removed, 5% was passed through the membrane and 5% was wasted in WAS.

2.5 Membrane fouling

In the conducted study for denitrification of nitrate-contaminated water, the chemical cleaning was performed for removing the organic fouling and the inorganic fouling was neglected, as the TMP (Transmembrane pressure) was not monitored.

Fouling of the membrane diffuser was evaluated based on nitrite accumulation, because nitrite accumulates when the culture is hydrogen limited. As the system was operated at a long hydraulic retention time (HRT), lack of nitrite in the effluent can not be assumed as evidence for the performance of the membrane diffuser. Furthermore, the fouling of membrane gas diffusers was observed by other researchers (Ma et al., 2003) used porous polyethylene membrane fibres with the pore size of 0.03 µm (Mitsubishi Rayon EHF390) for hydrogen delivery to groundwater to stimulate PCE dechlorination. The scale formation on the surface of the membrane failed the system. They found that the

precipitate was phosphate rich participate, containing high concentration of calcium and lesser concentration of magnesium, manganese and iron. In other applications, gas permeable membranes were subject to both inorganic and organic fouling. When membranes were used for transferring oxygen, 30% decrease in transfer efficiency was reported due to biofilm growth on the membrane surface (Johnson et al., 1999a ;Johnson et al., 1999b). In another study the oxygen transfer performance dropped off by 40% after 1700 hour of operation (Weiss et al., 1998), which was again attributed to biofilm growth and possible chemical precipitation. As for inorganic fouling, iron fouling caused by ferric hydroxide was the main foulant in aerobic systems (Kinnan and Johnson, 2002; Schwarz at al., 1991).

The main objective of this study was to study the mechanism of inorganic fouling as well as quantification of the foulants.

2.5.1 Methodology

Scanning electron microscopy (SEM), (EDAX) and X-ray diffraction analysis (XRD) were the main tools used for the fouling study.

Two different fibres were harvested from the membrane diffuser module and the membrane filter module, respectively. The fouled membrane fibres were soaked in sodium hypochlorite solution to remove organics from the pores. After cleaning with 200 ppm hypochlorite solution, the fibres were rinsed and dried at room temperature and prepared for the scanning. The harvested membrane from the membrane filter was cut into half along the fibre length, and the cross section was scanned. In addition, the fixed portion of suspended solids was analyzed, using X-ray diffraction analysis.

2.5.2 Results and Discussion

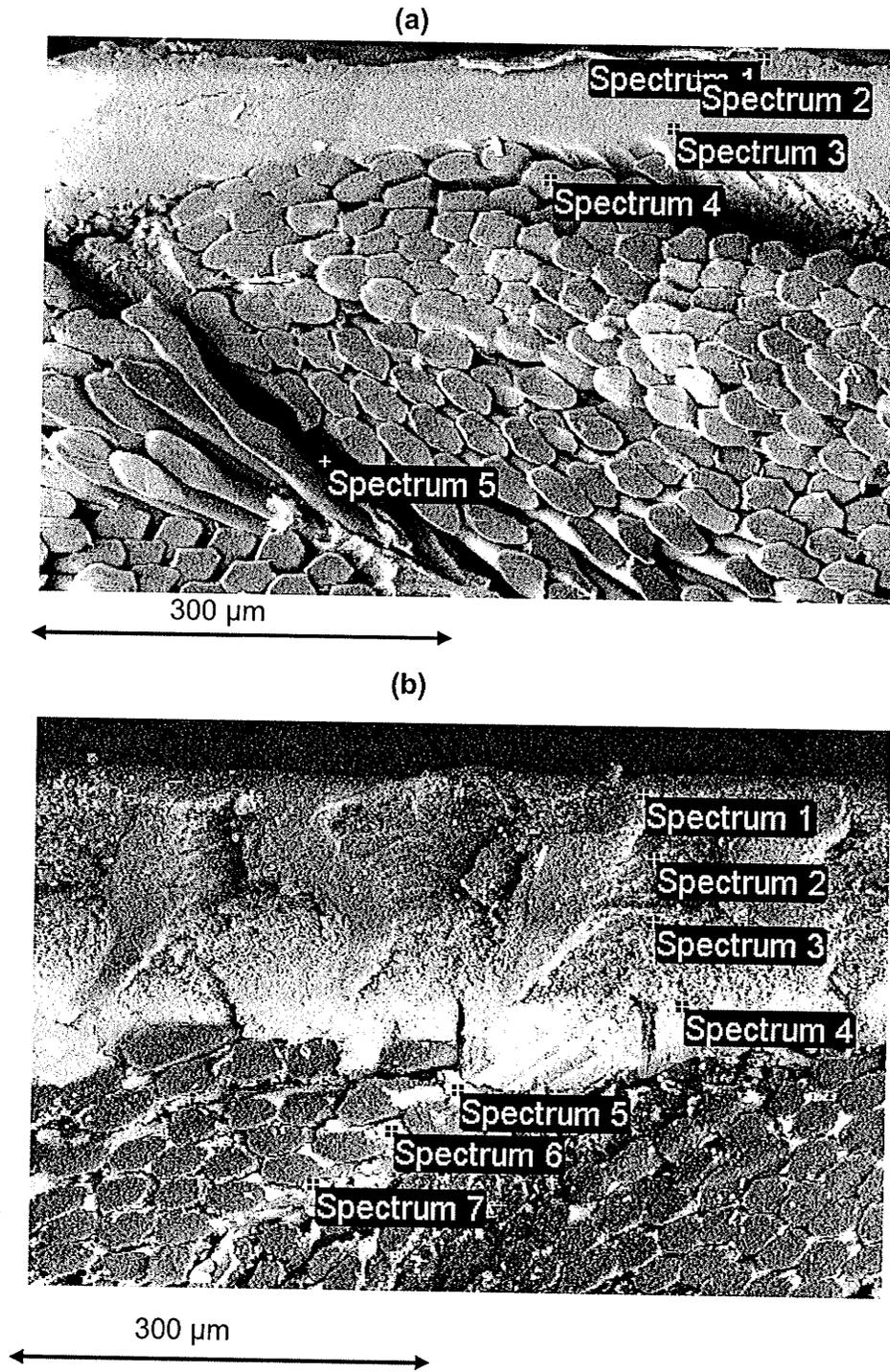
2.5.2.1 Fouling in membrane filter

The cross section scanning electron microscopy of the clean and fouled membrane is shown in Figure 2.7a and b, respectively. The membrane is composed of a support layer and a microporous dense layer on the top. The pores in the dense layer are not visible as the average pore size in the dense layer is only 0.04 micrometer.

Comparing the SEM of the clean and fouled membrane, the presence of foulants in the support layer can be observed.

In order to quantify the elements present in the foulant material, energy dispersive X-ray spectroscopy (EDAX) was used for both clean and fouled membranes. The EDAX results of the clean membrane showed that it is composed of carbon, fluorine, and oxygen. The EDAX machine used in this study was not able to detect hydrogen, however hydrogen must be present in the membrane material as the membrane was a polymeric membrane. In order to quantify the foulants elements, different locations in the dense and support area were chosen and analyzed using (EDAX).

Figure 2. 7 (a) Scanning electron microscopy of the clean and (b) fouled membrane filter

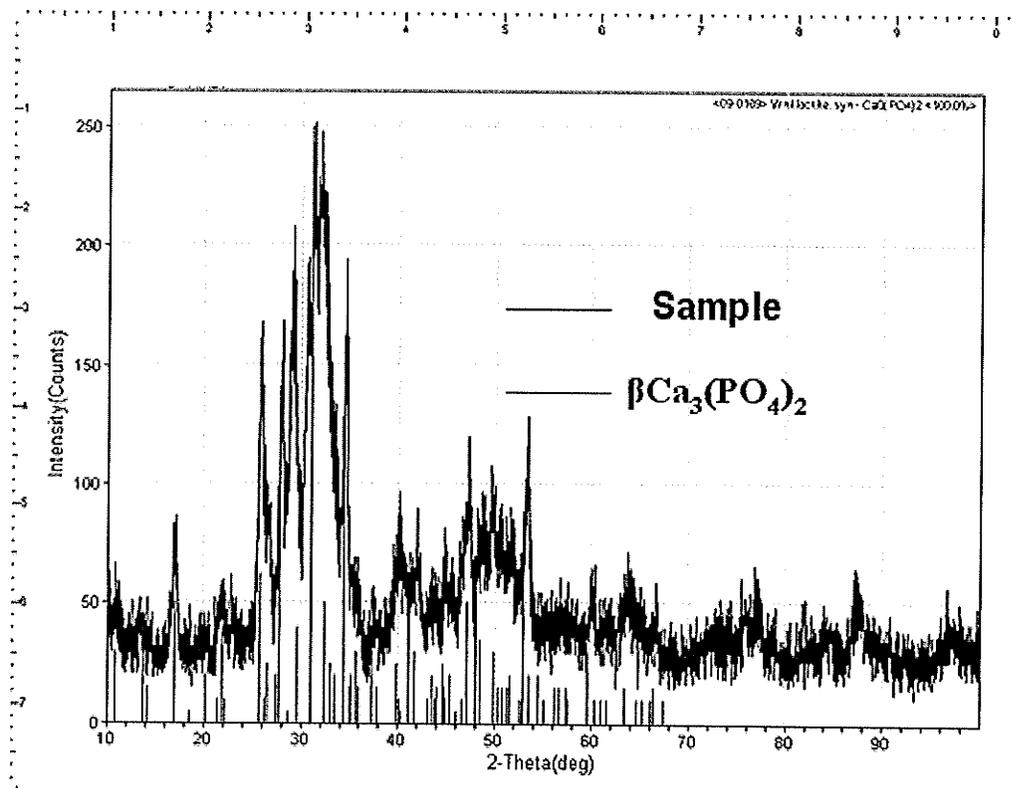


As shown in Table 2.1, several elements were detected in the fouled membrane. Calcium and phosphate were the most abundant elements among those detected. Knowing the elements present in the precipitation, an attempt was made to obtain the composition of the foulants.

Table 2.1 Quantification of elements in the fouled membrane

Spectrum	F	Na	Mg	Al	Si	P	Cl	K	Ca	Total
Spectrum 1	0.00	4.13	0.76	4.18	6.78	1.26	6.79	3.06	73.06	100.00
Spectrum 2	46.47	19.84		1.46	1.64	2.46	14.51	2.02	11.60	100.00
Spectrum 3	15.08		0.37			16.15	0.97		67.43	100.00
Spectrum 4	1.00		0.41			21.57			77.03	100.00
Spectrum 5			1.80			27.22			70.99	100.00
Spectrum 6			2.78			30.92			66.30	100.00
Spectrum 7			3.06			32.33			64.60	100.00

Figure 2. 8 X-ray diffraction of inorganic fraction of suspended solids in the reactor



In order to define the composition of the chemical found in the foulant material, it was assumed that precipitated materials in the membrane are the same as inorganic fraction of TSS. The composition of the inorganic portion of TSS was analyzed using X-ray diffraction. Almost 90 percent of the inorganic fraction of the TSS was found to be beta three calcium phosphate ($\beta\text{Ca}_3(\text{PO}_4)_2$) as shown in the Figure 2. 8.

2.5.2.2 Fouling in the membrane gas diffusers

The fibres used in the construction of the hydrogen membrane diffuser were made from polypropylene, which is naturally hydrophobic. The pores are filled with gas and water is not supposed to pass through the membrane wall. During the operation, the diffuser failed on two occasions. The hypochlorite wash did not fully recover the membrane. After acid cleaning, no nitrite was accumulated in the reactor, suggesting that the inorganic precipitation on the surface of the membrane was causing failure. A more detailed study showed that the foulants materials not only were present on the surface but also inside the fibres. As it is shown in Figure 2.9(a) and (b) different locations on the clean and fouled membrane were analyzed. As expected, carbon, oxygen and hydrogen were the only elements present on the clean fibre, which represent only the membrane material. Several other elements were found both on the surface and inside of the fouled fibre. As shown by Table 2. 2

Figure 2. 9 (a) Scanning electron microscopy of the clean and (b) fouled membrane filter

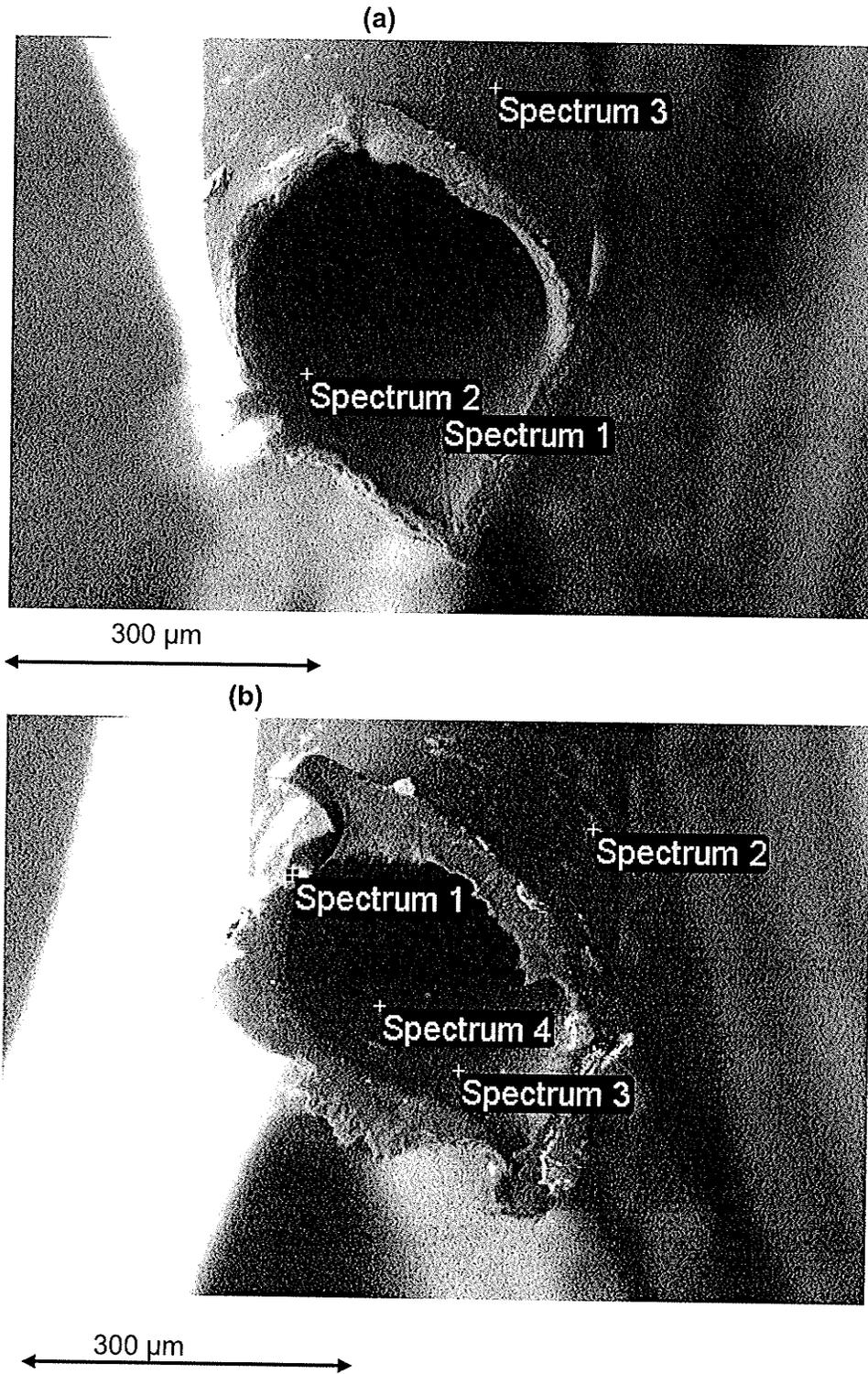


Table 2. 2 Quantification of elements in the fouled membrane

Spectrum	Na	Mg	Al	Si	P	Ca	Fe	Total
Spectrum 1	2.82	2.67			25.41	55.99	13.11	100.00
Spectrum 2			2.58	3.35	16.74	60.40	16.92	100.00
Spectrum 3					17.25	47.83	34.92	100.00
Spectrum 4					16.84	50.66	32.49	100.00

Calcium, phosphate and iron were dominant elements in the composition of the foulant.

The composition of the foulant material in the membrane gas diffuser was different from the membrane filter, as high concentration of iron was found in the foulant. This suggests that the material used in fabrication of the membrane gas diffuser had higher sorption coefficient for iron as the pore size of the membrane filter and membrane gas diffuser are in the same range (0.03-0.04 μM).

Surprisingly, the foulant elements were detected on both surfaces of the membrane and inside the fibres. As the membrane gas diffuser was made of hydrophobic material and the pores were filled with gas no water penetration to the pores was expected. The foulant material found inside the fibre suggests that water has been passed through the pores. The presence of foulants inside the fibre can be explained by water condensation (Fang et al., 2004). Due to large mass transfer coefficient of water vapour, it will saturate the feed gas within the fibre length. During the dissolution of the gas, its volume reduces and it will become supersaturated with water, resulting in water condensation. The condensation may take place at the liquid membrane interface, inside the fibre on the membrane surface, in the bulk gas or within the pores. Finding inorganic chemicals inside the fibre proves that in the present study the condensation occurred inside the fibre and in the pores. Condensation not only makes the membrane

gas diffuser vulnerable to inorganic fouling but also to organic fouling due to reduction in hydrophobicity of the membranes.

2.5.3 Mechanism of Fouling

Precipitation of inorganic minerals due to pH increase in the denitrification reactor can be considered as the main cause for inorganic fouling. As it is shown by the equation.1, during the denitrification process each mole of reduced NO_3^- consumes one acid equivalent (H^+), which converts to alkalinity generation of 3.57 g as CaCO_3 per g NO_3^- -N reduced. Increasing the alkalinity can increase the pH in the system, which can affect denitrification rate or cause precipitation of mineral deposits.

In the present study, the synthetic feed was composed of 750 mg l^{-1} NaHCO_3 , 20 mg l^{-1} K_2HPO_4 , 5 mg l^{-1} CaCl_2 , 20 mg l^{-1} $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 1 mg l^{-1} FeSO_4 . Cations in feed, such as Ca^{2+} and Mg^{2+} , can precipitate basic anions, such as hydroxide, carbonate, phosphate, mono-hydrogen phosphate and di-hydrogen phosphate. Minerals with higher pK_{SO} , such as $\text{Ca}_5(\text{PO}_4)_3\text{OH}$, $\beta\text{Ca}_3(\text{PO}_4)_2$ and CaCO_3 have lower solubility, and are therefore expected to be the major contributors to the inorganic fraction of TSS.

The precipitation kinetics is not only effected by the pH but also the concentration of cations and anions participating in precipitation. In this study, calcium and phosphate were the major contributors to precipitation, as potassium phosphate was used as a buffer. However, groundwater usually contains low concentration of phosphate, which would suggest that calcium carbonate is most likely to precipitate.

2.5.4 How to control precipitation?

As it was mentioned before, the precipitation rate is directly proportional to pH. Therefore, higher precipitation is expected at higher pH. One strategy to reduce pH is to introduce a mixture of carbon dioxide and hydrogen to the membrane gas diffuser. The optimum ratio of carbon dioxide to hydrogen varies upon different factors. These include the alkalinity of the feed, the hydraulic retention time of the system, nitrate concentration in the feed and mass transfer rate of carbon dioxide through the membrane.

It is possible to calculate a carbon dioxide-hydrogen ratio to achieve a specific pH under steady state operation. This can be done through alkalinity mass balance and correlating the alkalinity to CO₂ concentration to obtain the desirable operating pH. However, in practice the concentration of CO₂ to reduce the pH to the desirable operation value can be measured by changing the CO₂ concentration and measuring the pH in the effluent.

2.6 Conclusions

- 100% nitrate removal efficiency was achieved at nitrate loading of 0.328 kg N m⁻³ d⁻¹ with the SMBR system.
- Denitrification rates varied at different hydrogen pressure to the diffuser, ranging from 17 to 20 mg-N VSS⁻¹ h⁻¹ at hydrogen pressure between 0.28-0.55 atm. Compared to other reactor configurations in past research studies, higher denitrification rates were obtained with this system at the low operating temperature of 10-12 °C.

- Conversion rates of nitrate to nitrite and nitrite to nitrogen gas varied at different hydrogen pressures. At hydrogen pressures above 0.55 atm, nitrate was removed within 9 hours and no nitrite was observed in the reactor during the operation. At hydrogen pressure of 0.28 atm, all of the nitrate was removed at 10 hours, while nitrite accumulated and gradually disappeared within 12 hours. At hydrogen pressure below 0.2 atm, residual nitrite was observed in the effluent.
- Precipitation of inorganics was observed, and at the SRT of 20 days, the average TSS and VSS concentrations were 2322.5 mg l⁻¹ and 1162.6 mg l⁻¹, respectively.
- During the aerobic period, 81% of SMP produced within the anoxic phase, was rejected by the membrane, 9% was biodegraded, and 5% was passed through the membrane.
- The precipitation of inorganics affected both the membrane diffuser and the membrane filter. Inorganic fouling was found to be due to the precipitation of calcium and phosphate ions. The chemical composition of foulant is found to be $\beta\text{Ca}_3(\text{PO}_4)_2$

CHAPTER 3: MICROBIAL KINETICS AND ECOLOGY OF HYDROGENOTROPHIC DENITRIFIERS

3.1 Introduction

The interactions of various types of hydrogenotrophic culture, is not well understood. Microbial population in hydrogen-dependent denitrification was studied by a limited number of researchers. Selenka, and Dressler, (1990) isolated the culture from a fixed film reactor treating nitrate contaminated groundwater. They used three types of media for isolating the microorganisms involved in hydrogenotrophic denitrification. Nutrient-reduced peptone- mineral salt agar (P agar) was used for isolation of oligocarbophilic organisms under aerobic and anaerobic incubation conditions. Carbonate-nitrate-mineral-salt agar (Aut agar) was used for isolation of autotrophic nitrate reducing bacteria under hydrogen atmosphere. Finally, lactate-nitrate-mineral-salt agar (N agar) was used as a selective medium for heterotrophic nitrate reducing bacteria. Distribution of isolates from the biomass on different media showed that, in the case of aerobic cultivation, about 50% of the isolates were classified as *Pseudomonas spp*, and the remaining portion was classified as *Alcaligenes* and *Achromobacter spp*. Similar results were obtained were the same media was used under anaerobic condition and hydrogen atmosphere. *Pseudomonas* was the dominant species in both cases of cultivation on Aut agar and N agar under hydrogen atmosphere. All microorganisms isolated from bioreactors and cultivated under hydrogen atmosphere proved to be non-fermentive, gram-negative mobile rods.

In another study on a fluidized bed pilot-plant treating nitrate contaminated groundwater, bacteria that colonize the denitrification under mesophilic and psychotropic conditions

were isolated and identified (Vanbrabant et al., 1993; Liessens et al., 1992). The nitrate and nitrite reducing capacity of the isolates under autohydrogenotrophic and heterotrophic was also studied. Different groups of microorganisms seem to be involved in hydrogenotrophic nitrate reduction. Several isolated strains had no direct use of hydrogen or nitrate (Group A). They had to grow aerobically on organic matter entering with the water or on soluble metabolic products, produced by dominant denitrifiers. The next group of isolated microorganisms (Group B) could use organic matter or hydrogen for nitrate reduction, but they did not go beyond nitrite. These microorganisms were probably responsible for an important part of nitrate to nitrite conversion. Microorganisms (group C) reduced nitrate completely to nitrogen gas when a large supply of organic carbon was provided, but under hydrogenotrophic conditions, incomplete denitrification of nitrate to nitrite was observed. Group D microorganisms are interesting because they behave differently under autotrophic or heterotrophic conditions. This group converted nitrate to nitrite under autohydrogenotrophic conditions, while the presence of organic carbon switched the reaction toward ammonia production. Normally, the concentration of less than 0.1 mg l^{-1} of ammonia was observed, in hydrogenotrophic denitrification systems, suggesting that the group D is not a dominant group.

Table 3. 1 shows the mesophilic isolates from hydrogenotrophic fluidized bed reactor after one year of operation, showing that the approach of pure culture hydrogenotrophic nitrate removal is far from evident under natural process conditions. The presence of *Acinetobacter sp*, which is not capable of reducing nitrate remained might be involved in creating an anoxic environment by consuming the dissolved oxygen in the feed.

Table 3. 1 Strain numbers, isolation condition, Gram staining and nitrate reducing capacity of mesophilic isolates from hydrogenotrophic fluidized bed reactor after one year of operation (Adapted from (Vanbrabant et al., 1993))

Strain nr.(LMG)	Isolation method	Isolation medium	Gram staining	Autohydrogenotrophic nitrate reduction	Heterotrophic Nitrate reduction	Identification	
9753	AR	I	-	NR	NR	<i>Acinetobacter johnsonii</i>	A
9754	AR	I	-	NR	NR	<i>Acinetobacter johnsonii</i>	
9755	AR	I	-	NR	NR	<i>Acinetobacter johnsonii</i>	
10154	R	IV	+	NR	NR	<i>Aureobacterium</i> sap.	
10155	R	IV	+	NR	NR	<i>Aureobacterium</i> sap.	
9771	R	III	+	NR	NR	<i>Bacillus pumilus</i>	
9772	R	II	-	$\text{NO}_3^- \rightarrow \text{NO}_2^-$	$\text{NO}_3^- \rightarrow \text{NO}_2^-$	<i>Aeromonas hydrophila</i>	
9773	AR	II	-	$\text{NO}_3^- \rightarrow \text{NO}_2^-$	$\text{NO}_3^- \rightarrow \text{NO}_2^-$	<i>Comamanos acidovorans</i>	B
9774	R	II	-	$\text{NO}_3^- \rightarrow \text{NO}_2^-$	$\text{NO}_3^- \rightarrow \text{NO}_2^-$	<i>Shewenella putrefaciens</i>	
9776	R	II	-	$\text{NO}_3^- \rightarrow \text{NO}_2^-$	$\text{NO}_3^- \rightarrow \text{NO}_2^-$	<i>Aeromonas hydrophila</i>	
9779	R	IV	-	$\text{NO}_3^- \rightarrow \text{NO}_2^-$	$\text{NO}_3^- \rightarrow \text{NO}_2^-$	<i>Comamanos acidovorans</i>	
9780	AR	II	-	$\text{NO}_3^- \rightarrow \text{NO}_2^-$	$\text{NO}_3^- \rightarrow \text{NO}_2^-$	<i>Aeromonas hydrophila</i>	
9781	AR	II	-	$\text{NO}_3^- \rightarrow \text{NO}_2^-$	$\text{NO}_3^- \rightarrow \text{NO}_2^-$	<i>Pseudomonas</i> sp.	
10156	R	III	+	$\text{NO}_3^- \rightarrow \text{NO}_2^-$	$\text{NO}_3^- \rightarrow \text{NO}_2^-$	<i>Kurthia zopfii</i>	
9751	R	I	-	$\text{NO}_3^- \rightarrow \text{NO}_2^-$	$\text{NO}_3^- \rightarrow \text{N}_2$	<i>Pseudomonas</i> sp.	C
9752	AR	I	-	$\text{NO}_3^- \rightarrow \text{NO}_2^-$	$\text{NO}_3^- \rightarrow \text{N}_2$	<i>Pseudomonas</i> sp.	
9764	R	IV	-	$\text{NO}_3^- \rightarrow \text{NO}_2^-$	$\text{NO}_3^- \rightarrow \text{N}_2$	<i>Pseudomonas</i> sp.	
9765	R	IV	-	$\text{NO}_3^- \rightarrow \text{NO}_2^-$	$\text{NO}_3^- \rightarrow \text{N}_2$	<i>Pseudomonas</i> sp.	
9767	R	III	-	$\text{NO}_3^- \rightarrow \text{NO}_2^-$	$\text{NO}_3^- \rightarrow \text{N}_2$	<i>Pseudomonas</i> sp.	
9770	AR	I	-	$\text{NO}_3^- \rightarrow \text{NO}_2^-$	$\text{NO}_3^- \rightarrow \text{N}_2$	<i>Pseudomonas</i> sp.	
4218	R	I	-	$\text{NO}_3^- \rightarrow \text{NO}_2^-$	$\text{NO}_3^- \rightarrow \text{N}_2$	<i>Paracoccus dinitrificans</i>	
1196	R	I	-	$\text{NO}_3^- \rightarrow \text{NO}_2^-$	$\text{NO}_3^- \rightarrow \text{N}_2$	<i>Alcaligenes eutrophus</i>	D
9757	AR	I	-	$\text{NO}_3^- \rightarrow \text{NO}_2^-$	$\text{NO}_3^- \rightarrow \text{NH}_4^+$	<i>Aeromonas hydrophila</i>	
9758	AR	I	-	$\text{NO}_3^- \rightarrow \text{NO}_2^-$	$\text{NO}_3^- \rightarrow \text{NH}_4^+$	<i>Aeromonas hydrophila</i>	
9759	AR	I	-	NR	$\text{NO}_3^- \rightarrow \text{NH}_4^+$	<i>Serratia</i> sp.	
9760	AR	I	-	NR	$\text{NO}_3^- \rightarrow \text{NH}_4^+$	<i>Serratia</i> sp.	

R; isolated by direct plating, AR: isolated after enrichment for nitrate reducing bacteria. I: Taylor and Hoare medium with acetate; II: Taylor and Hoare medium with bicarbonate; III: Taylor and Hoare medium with glucose; Todd Hewitt broth. NR: no reduction of nitrate.

Due to the fact that, under strict hydrogenotrophic conditions, most of hydrogen-oxidizing bacteria could only reduce nitrate to nitrite, nitrite was found to be a key intermediate in hydrogenotrophic nitrate reduction. The nitrate is converted to nitrite using hydrogen (Group B). Further reduction of nitrite to nitrogen gas required organic carbon and was suggested to be carried out by mixotrophic microorganisms (Group C). The organic carbon necessary for nitrite reduction can be partly provided by decaying cells or soluble microbial products released from the conversion of nitrate to nitrite. However the soluble microbial products are not easily biodegradable and might not be completely responsible for reduction of nitrite to nitrogen gas. Another possibility is the production of acetate by acetogenic bacteria.

3.2 Hypothesis 1: Possible role of acetogenic bacteria in hydrogenotrophic culture

Previous researchers suggested that the conversion of nitrate to nitrogen gas is carried out by two different groups of microorganisms. The first group converts nitrate to nitrite by first group under the autotrophic growth condition, while the second group converts nitrite to nitrogen gas under heterotrophic growth condition. When treating water in hydrogenotrophic reactors, complete conversion of nitrate to nitrogen gas (full denitrification) is observed, despite the lack of organic carbon in the fee. To explain, the source of organic carbon required for conversion of nitrate to nitrogen gas, past researchers suggested that the source organic may be the soluble microbial by products. However, SMP are not readily biodegradable and might not be the answer to complete denitrification.

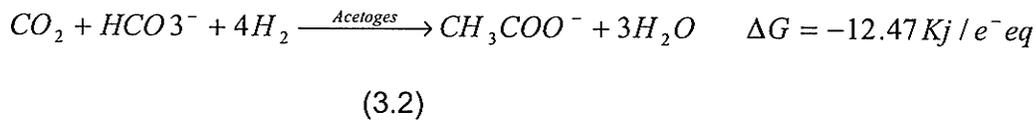
Besides hydrogenotrophic denitrifiers, hydrogen can be used as electron donor by a number of different types of bacteria. Under anaerobic conditions, e.g. at oxidation reduction potential (ORP) below (-) 250 mV, hydrogen can be consumed by methanogenic, sulfate-reducing, and homoacetogenic bacteria. Under anoxic conditions, at higher ORP e.g. above (-) 50 mV, the presence of nitrate limits the activity of methanogens and sulfate-reducing bacteria. The homoacetogenic bacteria are found in anoxic environments such as sewage sludge (Greening and Leedle, 1989) and it is possible that hydrogen dependent denitrification can be selective for the growth of both acetogens and autotrophic denitrifiers. Mixotrophic behaviour of hydrogenotrophic denitrifiers and possible growth of acetogens in the culture can affect the kinetics of hydrogen dependent denitrification. The presence of acetogens in the culture performing autotrophic denitrification would significantly influence observed denitrification rates, as heterotrophic denitrification will overwhelm microbial kinetics due to its thermodynamic advantage.

It is hypothesized that autotrophic nitrate reducers convert nitrate to nitrite, and the produced nitrite is further reduced to nitrogen gas by heterotrophic or mixotrophic nitrite reducers. In the case of heterotrophic nitrite reduction, nitrite is the electron acceptor and acetate is both the electron donor and carbon source. As for mixotrophic nitrite reduction nitrite is the electron acceptor, organic carbon is the carbon source and hydrogen is the electron donor (Equations 3.1 to 3.4).

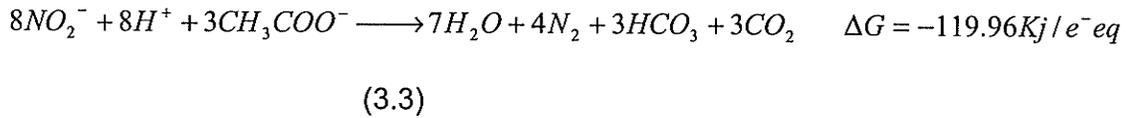
Autotrophic:



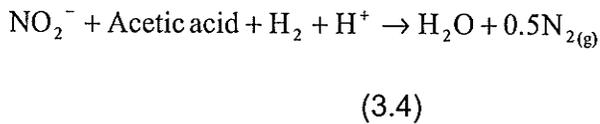
Acetogens:



Heterotrophic:



Mixotrophic:



3.2.1 Experimental investigations related to hypothesis 1

In order to evaluate the activity of acetogenic bacteria in the hydrogenotrophic culture, the steady state biomass from the reactor described in section 5 was taken and diluted by the factors of 10^{-1} , to 10^{-9} using the selective medium for acetogenic bacteria. The medium was composed of 300 mg l^{-1} NaHCO_3 , 1100 mg l^{-1} KH_2PO_4 , 900 mg l^{-1} K_2HPO_4 , 5 mg l^{-1} CaCl_2 , 25 mg l^{-1} $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.4 mg l^{-1} FeSO_4 and 1000 mg l^{-1} NH_4Cl . The prepared tubes were anaerobically cultivated under 20% carbon dioxide and 80% hydrogen at room temperature between 15 to 20 °C. The initial optical densities of the samples were measured using a spectrophotometer and monitored for the following 4 weeks. If the acetogenic bacteria were present in the culture, their growth would result in a change in optical density should change over time.

In addition to cultivating the biomass for measuring biomass activity, the effluent from the hydrogenotrophic reactor described in section 5 was tested for any trace of acetic acid.

3.2.2 Results of hypothesis 1

No acetic acid was detected in the effluent of the hydrogenotrophic reactor treating nitrate contaminated water. The lack of acetic acid by itself does not prove that acetogens are not present in the culture. There is a possibility that acetogens are present in the culture and acetic acid produced by the acetogens is consumed very quickly by heterotrophic nitrate reducers.

Further investigations proved that acetogens were not present in the culture. No activity of acetogenic bacteria was observed in the hydrogenotrophic denitrifying culture, as the initial optical density of the samples did not change during 4 weeks of cultivation. Also in the main reactors, after nitrate depletion no acetate was detected. Despite existing evidence for presence of acetogenic bacteria in heterotrophic anoxic environments, it seems that acetogens do not grow under autotrophic anoxic conditions. There might be two possible reasons for the inhibition of acetogenic activity in hydrogenotrophic culture. First, is the initial dissolved oxygen concentration in the feed, and second, is the possible inhibitory effect of nitrate toward acetogenic activity.

Homoacetogenic bacteria are generally considered to be strict anaerobes. However previous studies on homoacetogenic bacteria showed that they can establish conditions favourable for growth by actively removing oxygen from their environments (Karnholz et al., 2002). This would seem to alleviate concerns about the inhibitory effect of dissolved oxygen on acetogenic bacteria. It is therefore suggested that nitrate has an inhibitory

effect on growth of homoacetogenic bacteria under autotrophic conditions as the elimination of nitrate from the feed could provide a selective medium for growth of all homoacetogenic bacteria. As acetogens were not detected in the hydrogenotrophic culture, further analysis was performed.

Hypothesis 2: The complete denitrification is carried out under autotrophic conditions without heterotrophic activity

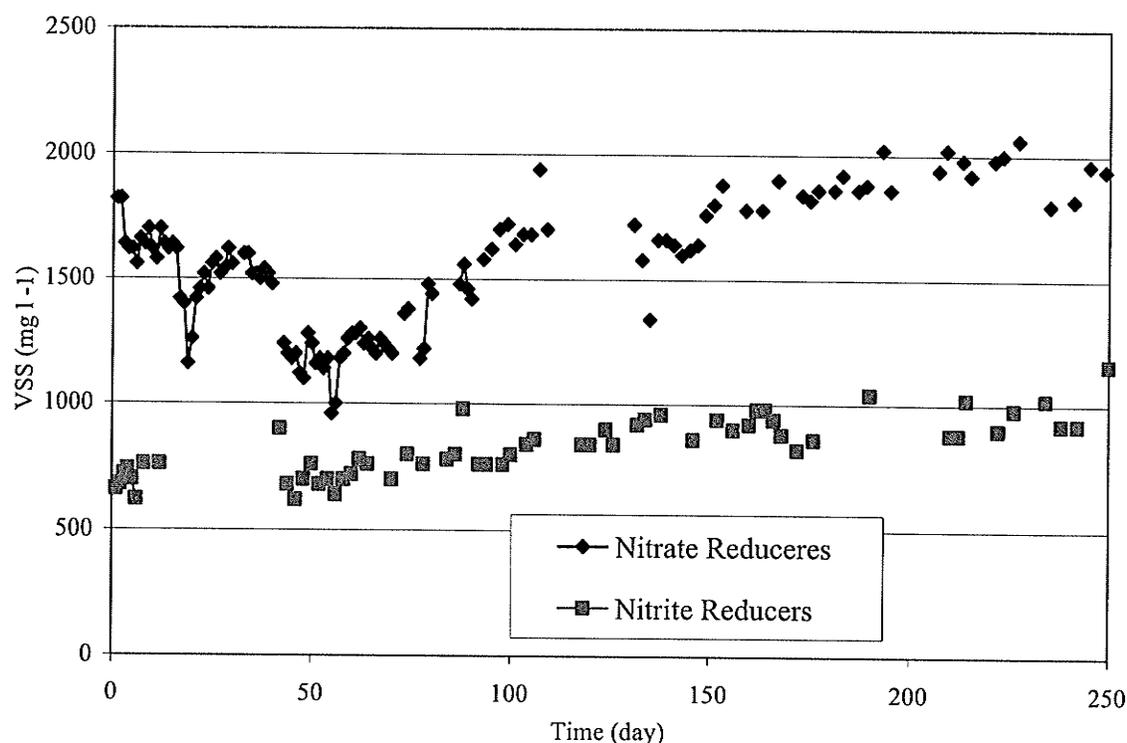
Previous researchers (Vanbrabant, et al., 1993) proposed that the conversion of nitrate to nitrogen gas is carried out by two different groups of bacteria. The reduction of nitrate to nitrite was proposed to be autotrophic despite heterotrophic conversion of nitrite to nitrogen. This is unexpected, as there are some known bacteria with the ability to drive complete denitrification under autohydrogenotrophic conditions. These bacteria include *Paracoccus denitrificans*, *Hydrogenophaga pseudoflava*, and *Alcaligenes eutrophus* (Vanbrabant, et al., 1993). The possibility of complete denitrification under autotrophic conditions was investigated. The possibility of one group of bacteria carrying out the conversion of nitrate to nitrogen gas was also evaluated.

3.3.1 Experimental investigations related to hypothesis 2

The following tests were initiated to observe if two separate groups of bacteria were responsible for denitrification. Two sequencing batch reactors (SBRs) with working volumes 3.5L were set up using the acclimated hydrogenotrophic seed from a steady state denitrification reactor. They were fed synthetic groundwater with the composition of 300 mg l⁻¹ NaHCO₃, 1100 mg l⁻¹ KH₂PO₄, 900 mg l⁻¹ K₂HPO₄, 5 mg l⁻¹ CaCl₂, 25 mg l⁻¹ MgSO₄·7H₂O, and 0.4 mg l⁻¹ FeSO₄. The only difference between the feed used in the two reactors was the electron acceptor. The feed of the first reactor contained nitrate at

loading of $0.94 \text{ g NO}_3\text{-N l}^{-1} \text{ d}^{-1}$, while nitrite was used as the electron acceptor of at a loading of $0.94 \text{ g NO}_2\text{-N l}^{-1} \text{ d}^{-1}$. Both reactors were operated under anoxic conditions as H_2 was delivered using fine bubble diffusers. They were operated at a solids retention time (SRT) of 60 days. The temperature of the reactors was maintained at $12 \pm 1 \text{ }^\circ\text{C}$. the volatile suspended solids concentration in both reactors were measured and monitored at steady state condition(Figure 3.1).

Figure 3. 1 Volatile suspended solids concentration at steady-state condition



According to the hypothesis proposed by (Liessens et al., 1992), the conversion of nitrite to nitrogen gas is carried out by nitrite reducers under heterotrophic mode. However, in the conducted study the nitrite reducers were grown under autotrophic condition as it is shown in Figure 3.1.

In the next step the nitrite reducers were spiked with nitrate. They could reduce nitrate to nitrogen gas, which suggests nitrate and nitrite reducers are the same.

To confirm that, the nitrate reducers were spiked with nitrite and maximum nitrite reduction was measured. The maximum specific nitrite reduction of both reactors was in the same range of 0.25 to 0.3 d⁻¹ at constant pH and temperature conditions.

3.4 Kinetics of hydrogenotrophic denitrifiers

A comprehensive understanding of kinetics involved in hydrogenotrophic denitrification is required to establish optimum reactor design and operating conditions. As it was mentioned before, besides hydrogenotrophic denitrifiers, hydrogen can be used as electron donor by a number of different types of bacteria. In this section kinetics of the main reactions involved in hydrogenotrophic denitrification ($\text{NO}_3 \rightarrow \text{NO}_2 \rightarrow \text{N}_2$) are experimentally evaluated under varying pH and temperature. Additionally, the possible presence of acetogenic bacteria, which would substantially affect denitrification rates, was evaluated.

3.4.1 Model Development

The denitrification process involves the formation of a number of nitrogen intermediates ($\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2$) that eventuates to nitrogen gas. In the case that hydrogen is used as electron donor for denitrification, the sequencing denitrification reactions can be simplified into two steps (Kurt et al; 1987).





The overall reaction is:



The first comprehensive kinetic model of denitrification considering the accumulation of nitrogen oxides was presented by Bleach et al., (1981). According to this model, which follows Monod's kinetics, the individual reduction rates of nitrogen oxides are independent from each other except that the product of one step is the electron acceptor for the next step. Combining this model and activated sludge models (ASM), the following model can be obtained for hydrogenotrophic denitrification:

$$\frac{dS_{\text{NO}_x}}{dt} = - \left(\frac{k_{\text{NO}_x} X S_{\text{NO}_x}}{K_{\text{NO}_x} + S_{\text{NO}_x}} \right) \left(\frac{S_{\text{H}_2}}{S_{\text{H}_2} + K_{\text{H}_2}} \right) \quad (3.8)$$

Where:

S_{NO_x} Concentration of $\text{NO}_3\text{-N}$ or $\text{NO}_2\text{-N}$ (mg l^{-1})

K_{NO_x} Half-saturation coefficient on $\text{NO}_x\text{-N}$ ($\text{mg NO}_x\text{-N l}^{-1}$)

k_{NO_x} Specific NO_x reduction rate of hydrogenotrophic biomass (day^{-1})

X Active biomass concentration (mg l^{-1})

S_{H_2} Dissolved hydrogen concentration (mg l^{-1})

K_{H_2} Half-saturation coefficient of hydrogenotrophic biomass on hydrogen (mg l^{-1})

According to this model, kinetics of nitrate and nitrite reduction is dependent on their concentrations as well as the concentration of dissolved hydrogen.

In previous studies on hydrogenotrophic denitrification saturation coefficients of 0.18 mg N l⁻¹ and 0.16 mg N l⁻¹ were reported for nitrate and nitrite respectively (Kurt et al. 1987). (Smith et al., 1981) isolated hydrogenotrophic denitrifiers and evaluated hydrogen saturation coefficient for each isolate, reporting values ranging from 0.0009 mg H₂ l⁻¹ to 0.0066 mg H₂ l⁻¹. Such low saturation coefficients make the kinetics of nitrate and nitrite reduction independent of nitrate, nitrite and hydrogen concentrations. Therefore, the kinetic model can be simplified as follows:

$$\frac{dS_{NO_3}}{dt} = -k_{NO_3} \cdot X \quad (3.8)$$

$$\frac{dS_{NO_2}}{dt} = -k_{NO_2} \cdot X \quad (3.9)$$

Equations (5) and (6) show the rates of nitrate and nitrate consumption when independent from each other, while equation (7) shows the rate of nitrite accumulation when nitrate is an electron acceptor.

$$\frac{dS_{NO_2}}{dt} = (k_{NO_3} - k_{NO_2}) \cdot X \quad (3.10)$$

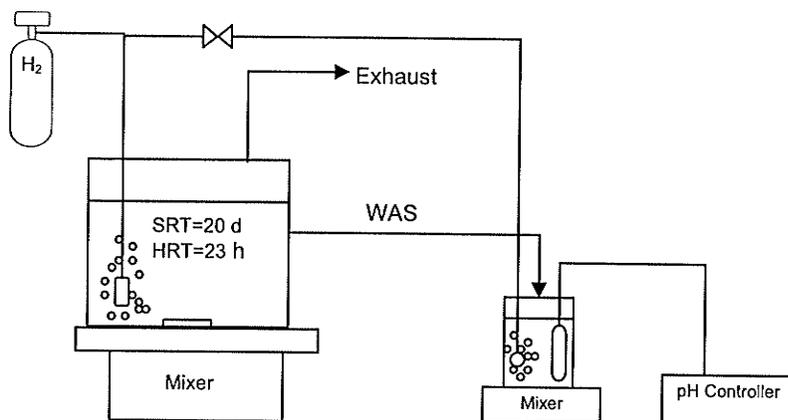
According to these equations, the rate of nitrate and nitrite consumption is only dependent on biomass concentration and maximum specific rates of nitrate and nitrite reduction.

3.4.2 Methodology

Two sequencing batch reactors (SBRs) with working volumes 20L (Figure 3.2) were set up using activated sludge seed from a local non-nitrifying wastewater treatment plant. They were fed synthetic groundwater with the composition of 300 mg l⁻¹ NaHCO₃, 1100 mg l⁻¹ KH₂PO₄, 900 mg l⁻¹ K₂HPO₄, 5 mg l⁻¹ CaCl₂, 25 mg l⁻¹ MgSO₄·7H₂O, and 0.4 mg l⁻¹

FeSO₄. The feed contained 82.3 mg l⁻¹ NO₃-N. Both reactors were operated under anoxic conditions as H₂ was delivered using fine bubble diffusers. They were operated at a solids retention time (SRT) of 20 days and constant loading of 1.65 g NO₃-N d⁻¹. The temperature of the first reactor was maintained at 12±1 °C while the second reactor was operated at room temperature (25±1 °C). The cold temperature was chosen based on the average temperature of groundwater to stimulate the growth of psychrotrophs. And higher temperature was used to allow the growth of mesotrophs.

Figure 3. 2 Schematics of SBR reactors used for denitrification kinetics study.



After the SBR reactors achieved steady state conditions (operating consistently for 5 SRT periods), the waste activated sludge (WAS) was used both for kinetic studies and cultivation of acetogenic bacteria. For the kinetics studies, the WAS was transferred to 3.5L rate-measurement reactors for evaluation of nitrate and nitrite reduction rates at 12°C and 25°C. Due to fluctuations in the biomass (VSS) concentration in the SBRs (650- 750 mg l⁻¹), VSS concentrations were adjusted to 500±10 mg l⁻¹ in the rate measurement reactors to provide reproducibility between tests. Two series of rate measurement reactors were set up. The first series were fed synthetic ground water

containing necessary nutrients (described before), spiked with concentrated nitrite stock solution to obtain an initial nitrite concentration of $25 \text{ mg NO}_2\text{-N l}^{-1}$. The second series of reactors were spiked with concentrated nitrate stock solution to obtain an initial nitrate concentration of $20 \text{ mg NO}_3\text{-N l}^{-1}$. The experiment was started by bubbling hydrogen through the reactors. Each series of reactors was maintained at different pH and temperature. The operating temperature and pH were maintained constant at $12 \pm 1 \text{ }^\circ\text{C}$ and $25 \pm 1 \text{ }^\circ\text{C}$, respectively, by using a temperature-controlled environmental chamber and a pH controller. After 5 minutes operation for removing any trace dissolved oxygen, a 5 ml sample was taken from the rate reactors every 10-15 minutes and analyzed for nitrate and nitrite concentration. The kinetic coefficients regarding nitrate and nitrite consumption were calculated by dividing the slope of the graphs plotting nitrate and nitrite concentration over time by biomass concentration. The procedure for kinetics coefficients were repeated five times to demonstrate reproducibility.

Nitrate and nitrite were analyzed using an automated Technicon nitrogen analyzer following Standard Methods 4500-NO₃--F (APHA, 1998). Mixed liquor suspended solids (TSS and VSS) were analyzed using Standard Methods 2540D and 2540E (APHA, 1998). Digital ChemCadet pH meter/Controller Model 5652-00 was used for pH control and monitoring. Optical density was measured by Biochom Nova spec II.

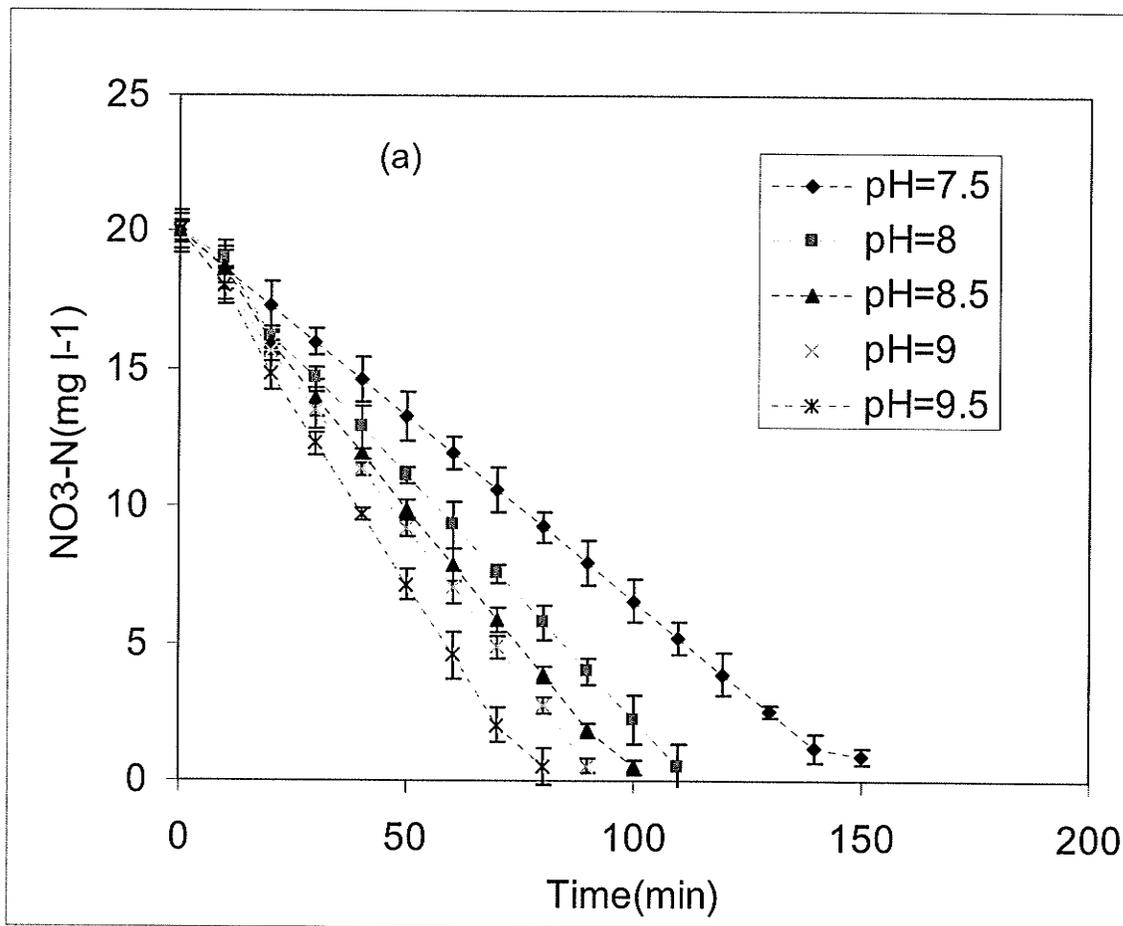
3.4.3 Results and Discussions

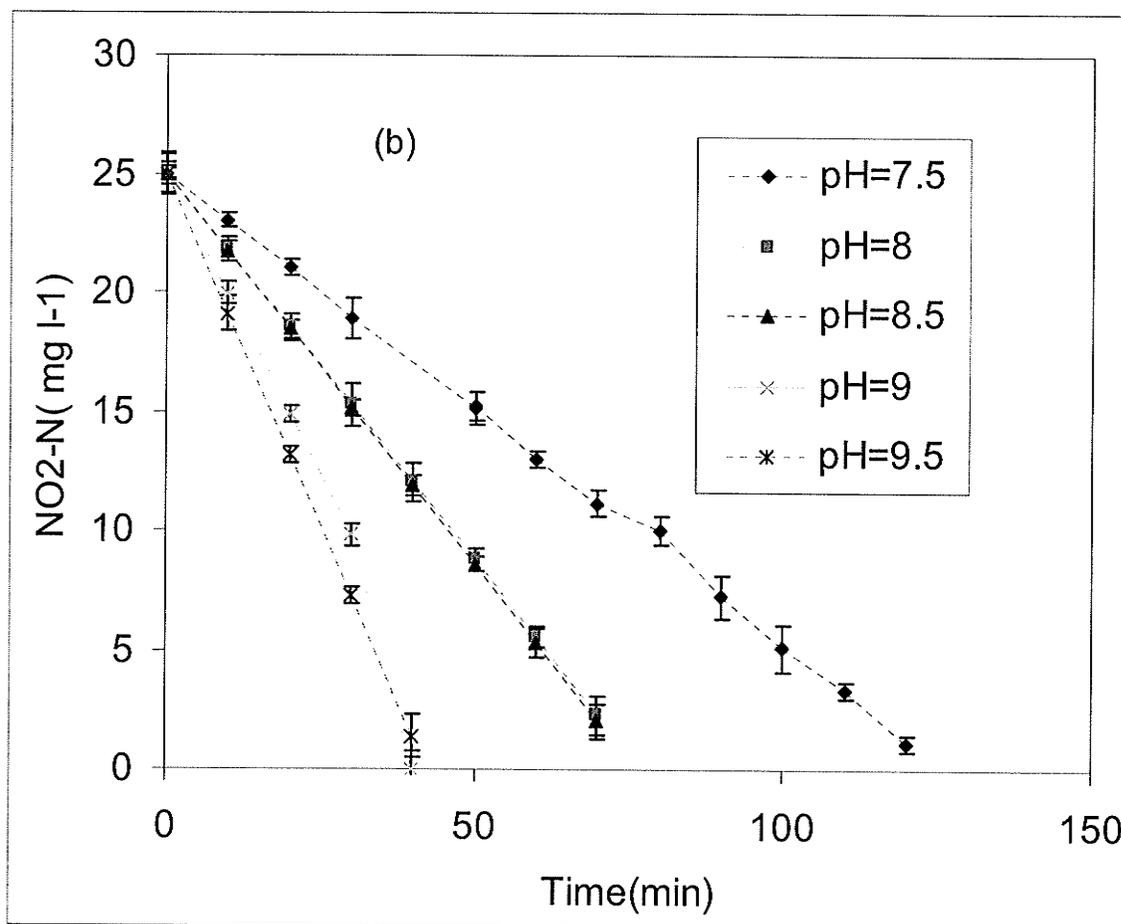
3.4.3.1 Kinetics at $25 \pm 1^\circ\text{C}$:

In Both case when nitrate and nitrite were used separately as electron acceptor, the denitrification was inhibited at pH values below 7 at both temperatures. This inhibition can be due to decomposition of carbonate ions and carbon dioxide stripping, which

leads to carbon limitation. Hydrogenotrophic denitrification at pH as low as 5.4 has been shown feasible, where carbon dioxide was injected to a fixed film reactor (Gros et al, 1988), however high denitrification rates were not observed. Figure 3. 3 (a) and (b) show the nitrate and nitrite concentration variation at different pHs at constant temperature of 25 ± 1 °C at steady state operation.

Figure 3. 3 (a) Nitrate and (b) Nitrite concentration variation over time at different pHs and constant biomass and temperature of 500 ± 10 mg l⁻¹ and 25 ± 1 °C, respectively





When nitrate was used as electron acceptor (Figure 3. 3(a)), no nitrite accumulation was observed during the denitrification at any pH values ranging from 7.5 to 9.5. The reason for the complete denitrification is explained by Figure 3. 3(b), which shows faster nitrite reduction rate than nitrate reduction as pH was increased, however theoretically faster nitrite reduction rates at higher pHs was not expected. The experimental results show that, with increasing the pH, nitrite reduction rate increased. This can be attributed to possible increased hydrolysis of extra-cellular polymeric substances (EPS) at higher pH, where the resulting biodegradable COD is utilized by the mixotrophic bacteria leading to increased rates.

The denitrification kinetics at the pH higher than 9.5 was not studied as the buffer used in the experiment did not allow it. The buffering capacity of the rate measurements reactors increased with increasing pH. The reactor operating at the pH of 7.5 had the most shift in pH and required the highest volume of acid to keep the pH constant. In contrast the reactor operating at pH 9.5 required very small acid addition to keep the pH constant. Higher denitrification rates at higher pHs is advantageous as biological denitrification produces alkalinity and pH control might not be necessary over wide range of pH.

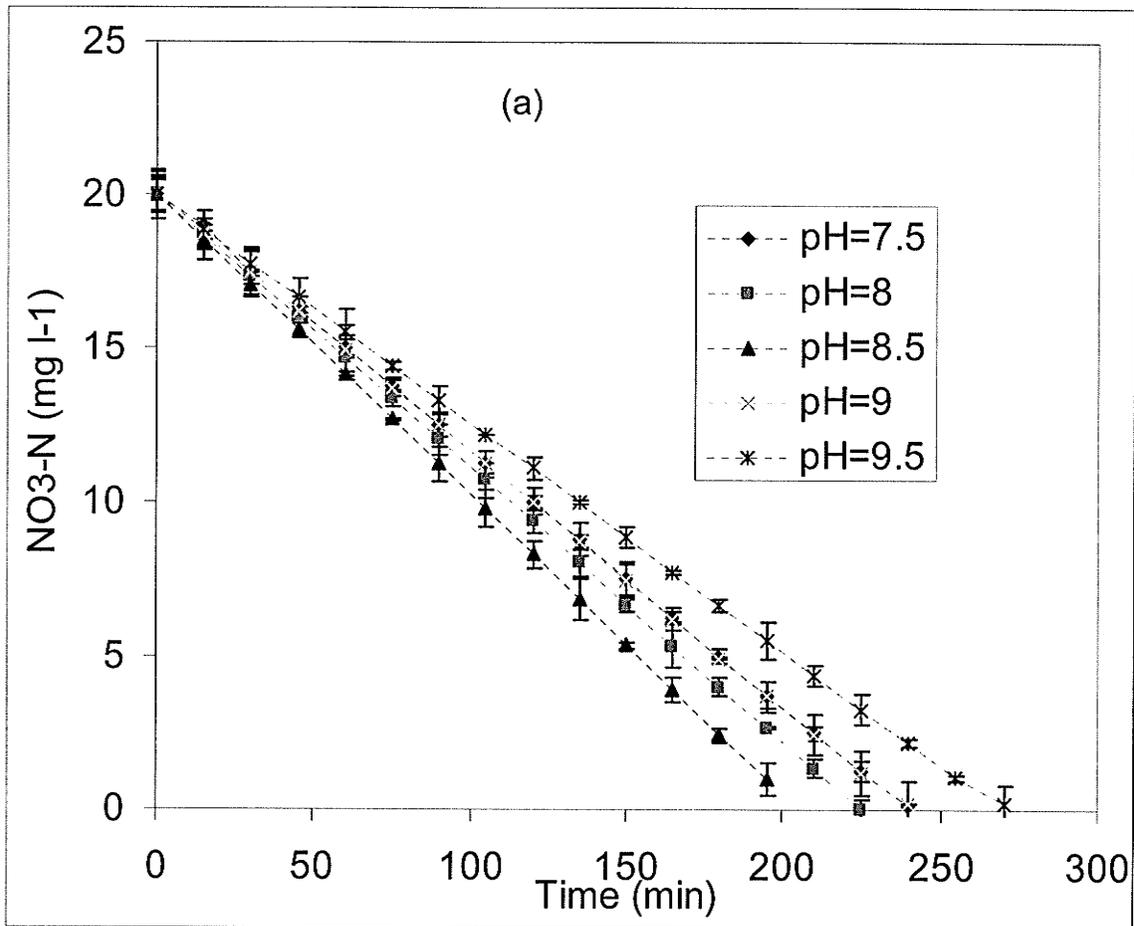
3.4.3.2 Kinetics at 12±1°C

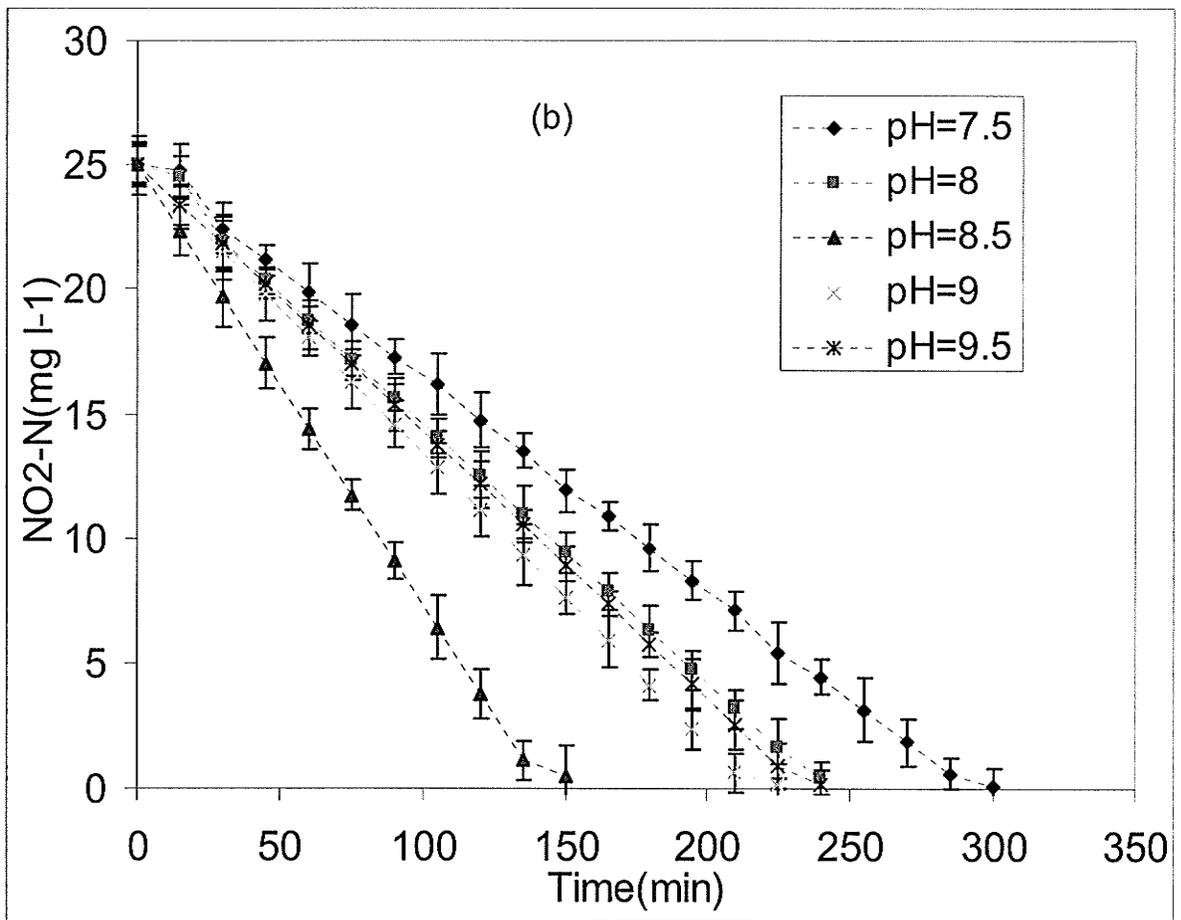
At 12±1°C, the same trend was observed when nitrite was used as electron acceptor (Figure 3. 4b). The rate of nitrite reduction was always faster than nitrate reduction and nitrite accumulation was not expected. However, varying levels of nitrite accumulation were observed at different pHs during nitrate reduction (Figure 3. 4c). This might be the result of slower nitrite reduction rate when nitrate is present. The slower nitrite reduction rates were reported in heterotrophic denitrification using methanol, when nitrate was present (Glass and Silverstein, 1998).

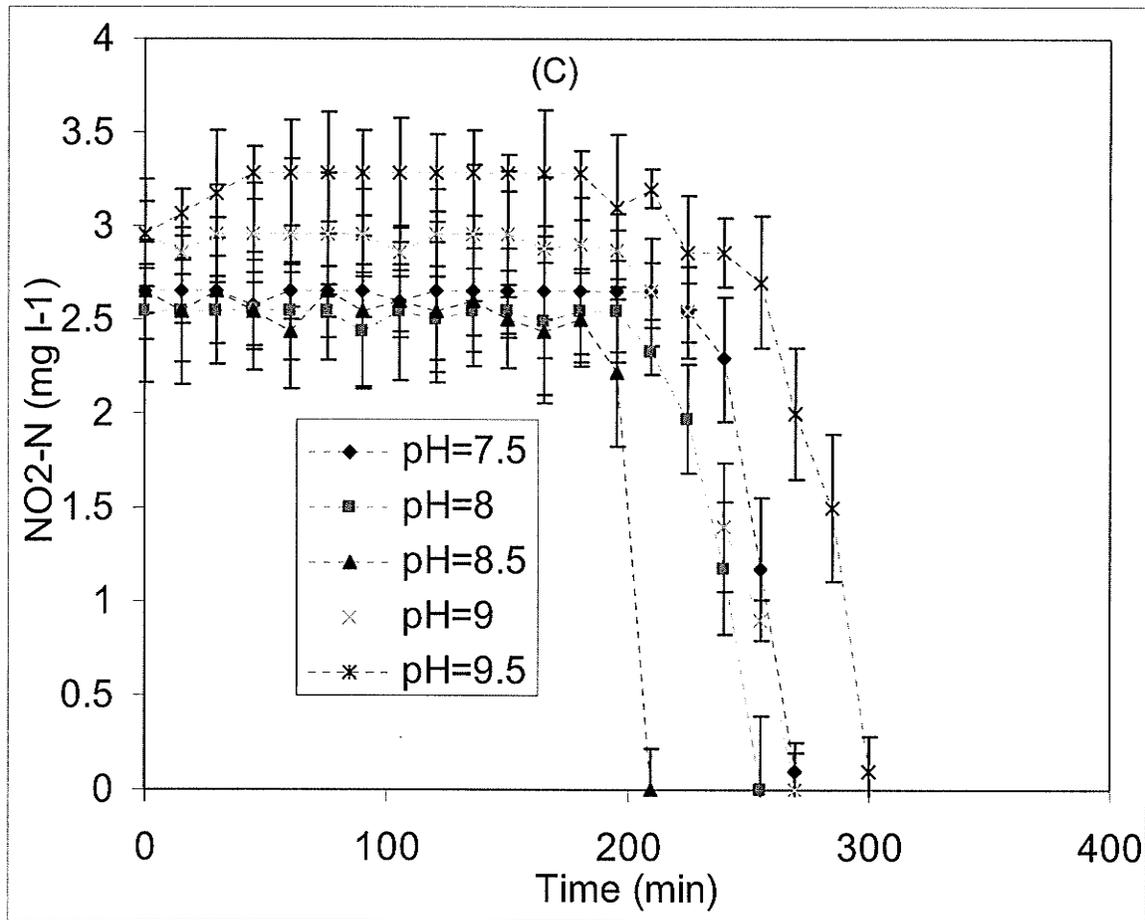
As Figure 3. 4c shows at all pHs the amount of accumulated nitrite was constant over time up to the point that all of nitrate was rapidly consumed. According to the suggested kinetics model for nitrite accumulation (Equation 3.10), the concentration of accumulated nitrite can be constant over time when k_{NO_2} and k_{NO_3} are equal. This suggests that nitrite reduction rate in the case that nitrate is used as electron donor is equal or very close to nitrate reduction rate. In heterotrophic denitrification, nitrite accumulation can inhibit bacteria growth (Almeida et al., 1998). Inhibitory effect of nitrite

also was reported in autotrophic denitrification by thiosulfate (Oh et al., 1989). In hydrogenotrophic denitrification, nitrite inhibition was not observed; in contrast, nitrite appears to be the more favourable electron acceptor.

Figure 3. 4 (a) Nitrate and (b) Nitrite concentration (c) Nitrite accumulation variation over time at different pHs and constant biomass and temperature of $500 \pm 10 \text{ mg l}^{-1}$ and $12 \pm 1^\circ\text{C}$







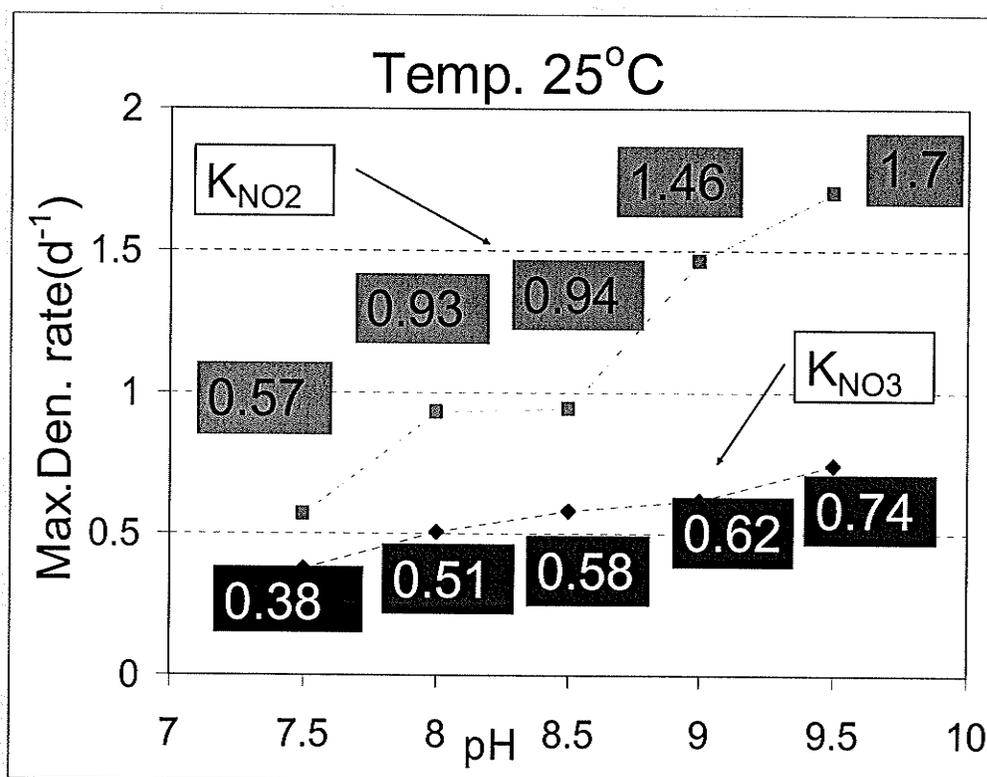
Almost the same trend of pH effect was observed in a membrane biofilm reactor utilizing hydrogenotrophic denitrification. (Lee and Rittmann, 2003) reported the optimum pH for hydrogenotrophic denitrification was in the range of 7.7-8.6. Increasing pH above 8.6 caused nitrite accumulation and a significant decrease in nitrate removal.

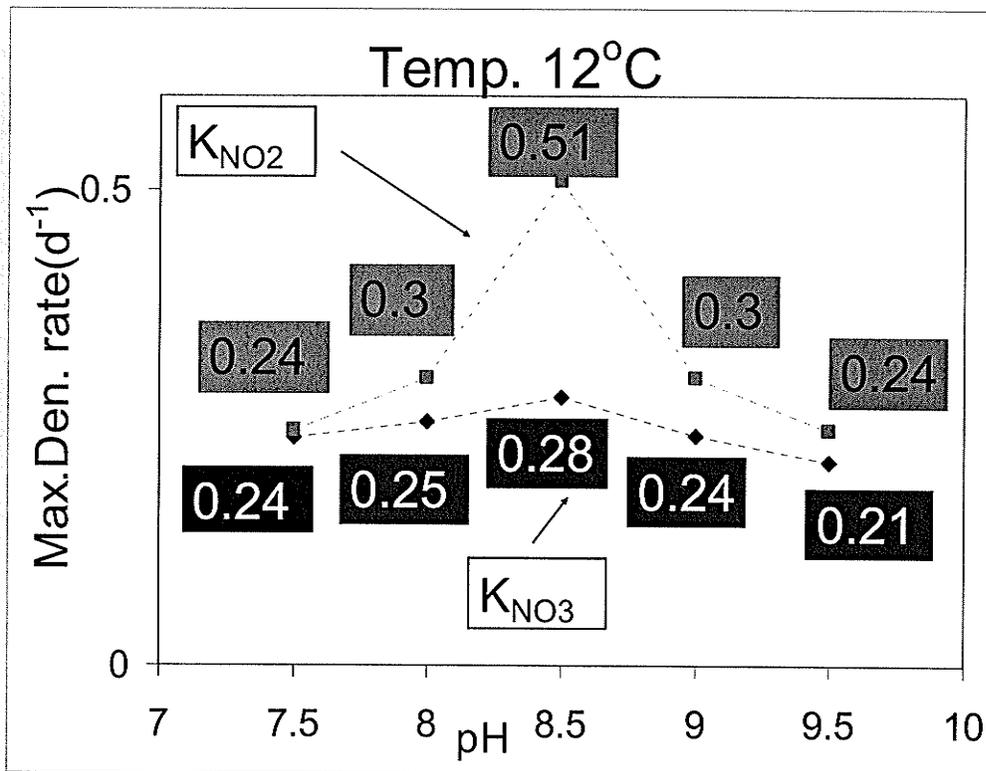
3.4.3.3 Kinetic Coefficients

The zero order kinetics model, suggested for hydrogenotrophic denitrification showed a good correlation with the experimental results, as the square roots (R^2) throughout the experiments were 0.98-0.99. The kinetic coefficients were calculated using the slopes of

the graphs plotting nitrate and nitrite consumption over time divided by biomass concentration. The kinetic coefficients regarding nitrate and nitrite consumption are illustrated in Figure 3. 5.

Figure 3. 5 The specific nitrate and nitrite reduction rate at different pHs and temperature





Linear consumption of nitrate and nitrite in this study agrees with the assumption of low K_s values assumed in this study. Half saturation coefficient is an important factor in biological treatment processes, as the minimum substrate concentration at steady state is directly proportional to half saturation coefficient. Therefore, the lower the K_s value the lower substrate in the effluent at steady state condition.

In case of heterotrophic denitrification the half saturation coefficients is dependent on the type of organic substrate. For instance for methanol the K_s values of $0.1 \text{ mg NO}_3\text{-N l}^{-1}$ to $67 \text{ mg NO}_3\text{-N l}^{-1}$ and as high as $72 \text{ mg NO}_3\text{-N l}^{-1}$ have been reported (U.S EPA, 1993). In autotrophic denitrification depending on the substrate used, the K_s values are different. In denitrification, using elemental sulphur the K_s value was reported as $0.03 \text{ mg NO}_3\text{-N l}^{-1}$ (Batchelor et al., 1989). In the case that thiosulfate was used for denitrification, K_s was reported in the range of $3\text{-}10 \text{ mg NO}_3\text{-N l}^{-1}$ (Oh et al., 1989). The

verification of zero order kinetics in this study demonstrates that Monod kinetics is not applicable for hydrogenotrophic denitrification and half saturation constant are sufficiently low to be assumed negligible. Attempts to fit the experimental results using Monod kinetics for determining Ks values were unsuccessful and constitute further evidence that the zero-order model is valid.

The obtained hydrogenotrophic denitrification rates were comparable with rates of both heterotrophic and autotrophic denitrification in previous studies except for thiosulfate. Although much higher rates were achieved using thiosulfate, it was found that the denitrification was very sensitive to nitrite even at low concentrations. In addition, the responsible bacteria were also sensitive to pH with optimum pH between 6.5-7.5 with significant inhibition at pH of 6 and 9. A comparison between these studies is summarized in Table 3. 2.

Table 3. 2 Comparison between denitrification rates using different substrates

Electron donor	Biomass Con. (g l ⁻¹)	Temp. °C	pH	Nitrate reduction rate (g NO ₃ -N g VSS ⁻¹ d ⁻¹)	Nitrite reduction rate (g NO ₂ -N g VSS ⁻¹ d ⁻¹)	Reference
Methanol	1.2-3.1	25±2	6.8	0.43-0.61	*	Foglar & Briski, (2003)
Thiosulfate	*	33-25	6.5-7.5	7.2-9.6	*	Oh et al., (1989)
Sulfur	1.19-6.61	25	7-8	0.14-0.19	*	Koenig & Liu, (2004)
Hydrogen	0.5	25±1	7.5-9.5	0.38-0.74	0.57-1.70	Current study
Hydrogen	0.5	12±1	7.5-9.5	0.21-0.28	0.25-0.51	Current study

3.4.4 Conclusions

- The proposed zero-order kinetic model for hydrogenotrophic reduction of nitrate and nitrite was highly correlated with the experimental results and kinetic coefficients were calculated at different pH and temperature. Nitrate and nitrite reduction was inhibited at pH 7 at $12\pm 1^\circ\text{C}$ and $25\pm 1^\circ\text{C}$. The inhibition of denitrification was suggested to be due to carbon dioxide stripping.
- At $25\pm 1^\circ\text{C}$ no nitrite accumulation was observed, as nitrate and nitrite reduction rate increased with pH. At this temperature, nitrate and nitrite reduction rates from 0.38 to $0.74(\text{g NO}_3\text{-N g VSS}^{-1} \text{d}^{-1})$ and 0.57 to $1.70(\text{g NO}_2\text{-N g VSS}^{-1} \text{d}^{-1})$, were obtained at pH ranging from 7.5 to 9.5.
- At $12\pm 1^\circ\text{C}$ different levels of nitrite (ranging between 2.5-3.5 mg l^{-1}) accumulated over time under varying pH conditions. Nitrite accumulation increased with pH only after exceeding pH of 8.5. The rate of nitrate and nitrite reduction increased with pH, peaking at 8.5, followed by a decreasing trend at higher pH values - at $12\pm 1^\circ\text{C}$. Nitrate and nitrite reduction rates ranging from 0.21 to $0.28(\text{g NO}_3\text{-N g VSS}^{-1} \text{d}^{-1})$ and 0.25 to $0.51(\text{g NO}_2\text{-N g VSS}^{-1} \text{d}^{-1})$, were obtained at $12\pm 1^\circ\text{C}$. The optimum pH for nitrate and nitrite reduction was 9.5 at $25\pm 1^\circ\text{C}$ and 8.5 at $12\pm 1^\circ\text{C}$.

3.5 Developing a new approach for the measurement of decay, active fraction of biomass and extracellular polymeric substances in hydrogenotrophic denitrification

3.5.1 Introduction and objectives

Microbial kinetics is an essential part of environmental biotechnology. The relationship between the active biomass and substrate concentration is an important factor used for design, operation and modeling of biological treatment processes. In practice, volatile suspended solids concentration is generally assumed to be representative of active bacteria concentration. However, volatile suspended solids represent the summation of active biomass (catalyst), cell debris, extracellular polymeric substances (EPS) and possible trapped organics in the microbial aggregates (flocs). Quantifying the biomass constituents provides essential information for design and modelling of biological treatment processes. In order to develop a model, which can lead to quantifying the inert and viable fraction of steady-state biomass, detailed information on energy transfer during substrate utilization and endogenous respiration is required. Endogenous respiration is particularly important as it leads to production of inert cell debris.

Early researchers suggested that endogenous respiration occurs due to decay of the cells and subsequent consumption of decayed biomass to form new cells (Kountz and Forney, 1959; McKinney, 1960; Washington and Symons, 1962; Grady and Roper, 1974). Although only the biodegradable fraction of the cell is consumed during decay, it was not considered in the calculation of the endogenous respiration rate. The biodegradable fraction was also neglected in the incorporation of endogenous respiration into popular activated sludge models such as ASM1, ASM2. According to these models the rate of endogenous respiration of autotrophs and heterotrophs is dependent on their concentration and decay coefficient. Dold et al. (Dold et al., 1980) observed that after substrate depletion still considerable amount of oxygen was consumed by the bacteria. This suggested that the consumption of the electron

acceptor is involved in the decay process. During the development of the ASM3 activated sludge model, this consumption of electron acceptor was considered as a factor effecting endogenous respiration rates. According to the ASM3 model, the rate of endogenous respiration of both autotrophs and heterotrophs is dependent on their concentration, the concentration of electron acceptor and cell decay rate. Recently, Laspidou and Rittmann, (2002) proposed a unified theory, where the active biomass is separated from the extracellular polymeric substance (EPS) surrounding the cell. They proposed that the consumption of the electron acceptor is as a result of both endogenous respiration and degradation of hydrolyzed EPS. The non-biodegradable fraction of cells produces inert biomass, and hydrolyzed EPS is used as an energy source by bacteria.

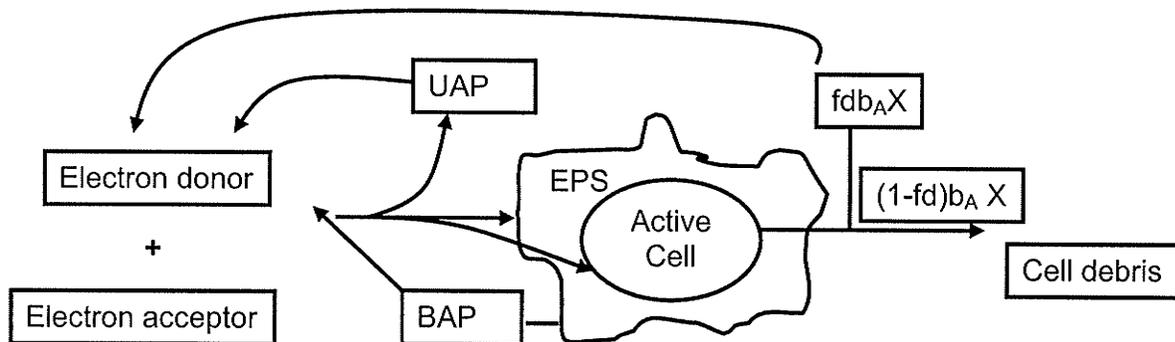
Hydrogenotrophic denitrifiers have been considered autotrophic bacteria that use hydrogen as electron donor, carbon dioxide as carbon source, and nitrate as electron acceptor. It has been shown that they are mixotrophs – i.e. they possess the ability to reduce nitrate under both autotrophic and heterotrophic conditions (Selenka and Dressler, 1990; Liessens et al., 1992; Vanbrabant et al., 1993; Szekeres et al., 2002).

Since no external carbon source is present, hydrogenotrophic denitrifiers serve as an ideal culture to examine measurement approaches for decay rate and endogenous respiration. In this study, a method for measuring decay, active biomass and extracellular polymeric substances (EPS) is proposed by combining the unified theory (as proposed by Laspidou and Rittmann) and ASM3. Using the developed method the decay coefficient, active fraction of biomass, concentration of EPS, and the true yield of hydrogenotrophic denitrifiers were obtained.

3.5.2 Model development

According to the model proposed by Lapsidou and Rittmann, the potential energy from substrate utilization goes to three pathways (Figure 3. 6). Some portion is wasted as soluble microbial products associated with substrate utilization (UAP), the rest goes to synthesis of extracellular polymeric substances and active cell. Some portion of bond EPS, hydrolyses and produces soluble EPS, which is referred to as biomass-associated products (BAP). The active cell (excluding the EPS) by itself is composed of biodegradable part (fd) and non-biodegradable part. The energy required for maintenance is obtained from oxidation of biodegradable part of active cell during endogenous respiration. The particulate inert biomass is produced during endogenous respiration from non-biodegradable part of active cell.

Figure 3. 6 The model proposed by Lapsidou and Rittmann, 2002



A methodology for simultaneous measurement of decay coefficient and active biomass concentration as well as calculating extracellular polymeric substances in steady –state biomass was proposed. In this method, the unified theory by Lapsidou and Rittmann is combined with the ASM3 activated sludge model to describe the consumption of the electron acceptor in a reactor under starvation conditions. This yields the following equation:

$$\frac{dNO_3}{dt} = -b_A \cdot f d \cdot X \left(\frac{NO_3}{K_{NO_3} + NO_3} \right) \left(\frac{K_{O_2}}{K_{O_2} + S_{O_2}} \right) - \left(\frac{q_{BAP} X \cdot BAP}{K_{BAP} + BAP} \right) \left(\frac{NO_3}{K_{NO_3} + NO_3} \right) \left(\frac{K_{O_2}}{K_{O_2} + S_{O_2}} \right) \quad (3.11)$$

The consumption of nitrate during starvation is due to endogenous respiration (Term A) and consumption of hydrolyzed EPS by the heterotrophic fraction of biomass (Term B). In this particular study the mixotrophic behaviour of hydrogenotrophic denitrifiers allows the whole culture to use the hydrolyzed EPS.

With the following assumptions, equation 1 can be simplified.

- There is no dissolved oxygen in the starved reactor.
- The concentration of electron acceptor is high and the half saturation coefficient can be neglected.
- The hydrolyzed EPS can be used only by the heterotrophic fraction of the biomass.
- Nitrate consumption rate due to endogenous respiration is constant only after biodegradable EPS (BAP) are fully consumed. This assumption is directly related to the biodegradability of EPS and is supported by the work of Zhang and Bishop (2003).

This yields the following relationship:

$$\frac{dNO_3}{dt} = -b_A \cdot f d \cdot X_A \quad (3.12)$$

The concentration of active biomass in the decay reactor is decreasing due to biomass decay. The concentration of active biomass as a function of time is described by equation 3.13, which follows a first-order decay mechanism (Avcioglu et al., 1998).

$$X_t = X_{A0} e^{-b_A t} \quad (3.13)$$

Considering the change in biomass concentration, and substituting equation 3.13 into equation 3.12, the following expressions can be obtained:

$$\frac{dNO_3}{dt} = -(b_A \cdot fd \cdot X_{A0}) e^{-b_A t} \quad (3.14)$$

$$\ln\left(\frac{dNO_3}{dt}\right) = -\ln((b_A \cdot fd \cdot X_{A0}) e^{-b_A t}) \quad (3.15)$$

$$-\ln\left(\frac{dNO_3}{dt}\right) = \ln(b_A \cdot fd \cdot X_{A0}) - b_A t \quad (3.16)$$

By plotting nitrogen uptake rate for the starvation period, both the decay rate and the active fraction of biomass can be calculated. The active fraction of biomass (XA) is defined as the concentration of viable bacteria excluding EPS. The slope gives the decay coefficient whereas the active fraction of biomass can be obtained from the intercept. The biodegradable fraction of biomass (fd) is needed, however, in order to calculate the active biomass concentration.

The biodegradable fraction of active biomass can be calculated using the following equation.

$$\frac{dX}{dt} = -b_A \cdot fd \cdot X \quad (3.17)$$

In equation (3.17), X can be substituted by total COD. The total COD in the starved reactors is decreasing due to endogenous respiration and consumption of biodegradable fraction of biomass. Therefore, keeping track of total COD decline in the starved reactor yields fd.

3.5.3 Methodology

A SBR reactor with the working volume of 20 l was set up using activated sludge seed from a local non-nitrifying wastewater treatment plant. It was fed artificial groundwater with the composition of 300 mg l⁻¹ NaHCO₃, 1100 mg l⁻¹ KH₂PO₄, 900 mg l⁻¹ K₂HPO₄, 5 mg l⁻¹ CaCl₂, 25 mg l⁻¹ MgSO₄·7H₂O, and 0.4 mg l⁻¹ FeSO₄. The feed contained 82.3 mg l⁻¹ NO₃-N. The reactor was operated under anoxic conditions as H₂ was delivered using fine bubble diffusers. The reactor was operated at a solids retention time (SRT) of 20 days and constant loading rate of 0.08 g NO₃-N d⁻¹ l⁻¹. After achieving steady-state, the reactor was operated for 5 SRTs (100 days), before kinetic testing was conducted. This was done to ensure no pure heterotrophic organisms were present. The temperature of the reactor was maintained at 12±1 °C. The suspended solid concentration at steady state condition as well as reactor performance regarding nitrate removal was monitored to obtain the observed yield. As for the decay measurements the following procedures were used in order to measure the decay coefficient, biodegradable fraction of biomass and EPS.

3.5.3.1 Decay coefficient

A decay reactor with the volume of 3.5 l was set-up using hydrogenotrophic biomass from the main reactor, nitrate as electron acceptor and the synthetic groundwater contained micronutrients. The initial biomass concentration in the reactor was 1000 mg l⁻¹. In order to eliminate the diffusion of oxygen, nitrogen gas was bubbled into the reactors in a closed system. Temperature and pH were maintained constant at 12C° and 7.5, respectively. Temperature and pH were maintained constant at 12C° and 7.5,

respectively. Nitrate samples were taken every day and analyzed for nitrate a nitrite, and soluble COD. The nitrogen uptake rate in the reactor was calculated and was plotted versus starvation period to yield the decay coefficient.

3.5.3.2 Biodegradable fraction of biomass (fd)

The same reactor was described for measuring decay coefficient, was used for measuring the biodegradable fraction of active biomass. COD samples were taken every day and analyzed for total COD. The total COD decline in the reactor was plotted versus starvation period to yield the biodegradable fraction of biomass. The biodegradable fraction of biomass was calculated from the slope of the graph plotting COD decline versus starvation time divided by decay coefficient.

3.5.3.3 Extracellular polymeric substances (EPS)

EPS was measured based on the steaming extraction method (Zhang et al., 1999). Total COD measurements were used in place of protein and carbohydrate measurements. It should be noted that this method is only accurate when there is no organic carbon in the flocs. In the present study, the feed did not contain any organic carbon and therefore no trapped organics in the flocs were expected.

100 ml of steady state biomass was rinsed with deionised water and total COD was measured under completely mixed condition. The sample was steamed in an autoclave at 80°C and 1 bar for 10 minutes. Then the sample was centrifuged at 8000 g for 10 minutes. The supernatant was filtered and the soluble COD was measured. The soluble COD divided by total COD represented fraction of EPS in the biomass.

3.5.4 Results and Discussion

3.5.4.1 Justification of the assumptions used for method development

One of the assumptions in the simplification of equation (3.11) was that nitrate consumption due to endogenous respiration is dominant only when there is no other source of energy in the reactor. The validity of the assumption can be explored as shown in Figure 3. 7 and Figure 3. 8 At the beginning of the starvation condition the soluble COD in the reactor increased rapidly from 7 to 45 mg l⁻¹. The soluble COD is suggested to come from the hydrolysis of EPS, as the biomass used in the starved reactor was fed only inorganics. It appears that the bacteria use the hydrolyzed EPS for maintenance and survival as the rapid reduction in electron acceptor concentration was observed in the first 3 days of starvation. When the EPS is depleted, the rate of nitrate consumption is almost linear and caused solely by endogenous respiration of the active biomass. The linear portion of Figure 3. 8, which is showing nitrate consumption over time, will be used for calculating decay coefficient. Another interesting observation from Figure 3. 8 is the mixotrophic behaviour of hydrogen-dependent denitrifiers. Hydrolyzed EPS is a source of organic carbon and can be utilized only under heterotrophic condition. The rapid nitrate reduction in first three days is in correlation with the second term of equation (3.11). EPS and soluble microbial products released through metabolic processes in the bacteria are biodegradable and kinetics of their degradation is well understood under aerobic condition (Lu et al., 2002; Henze et al., 1987; Rittmann and McCarty, 2001). It appears that they can be a source of organic carbon for the heterotrophic fraction of biomass under anoxic conditions as well.

The increase in soluble COD during starvation period suggests that non-biodegradable fraction of active biomass is not only particulate as it was proposed by Lapsidou and Rittmann, soluble COD is also produced during decay.

Figure 3. 7 Soluble COD concentrations under starvation conditions

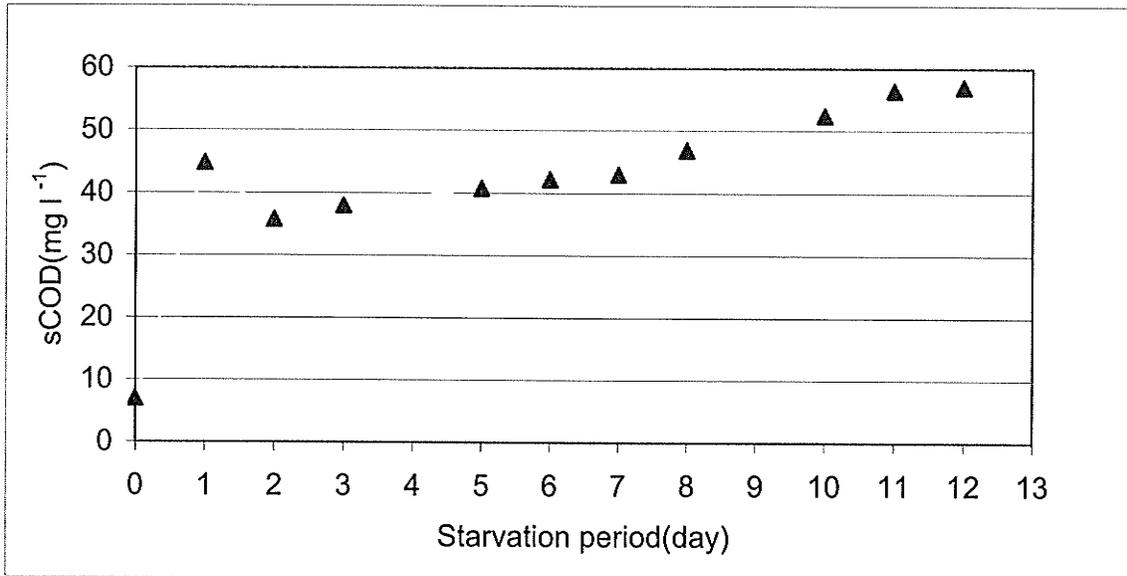
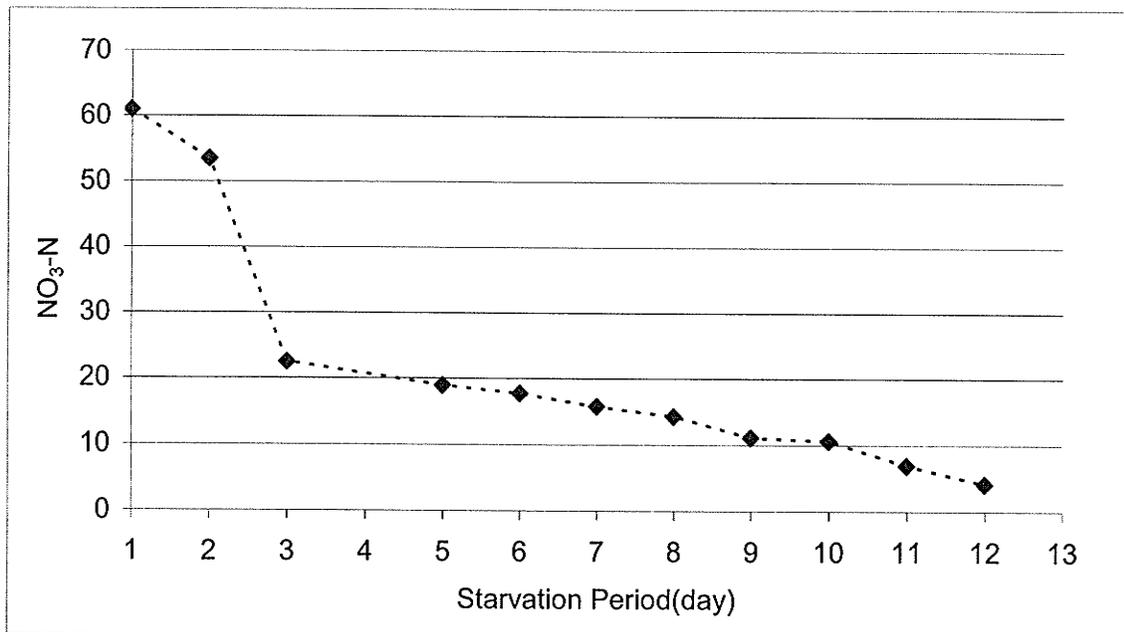


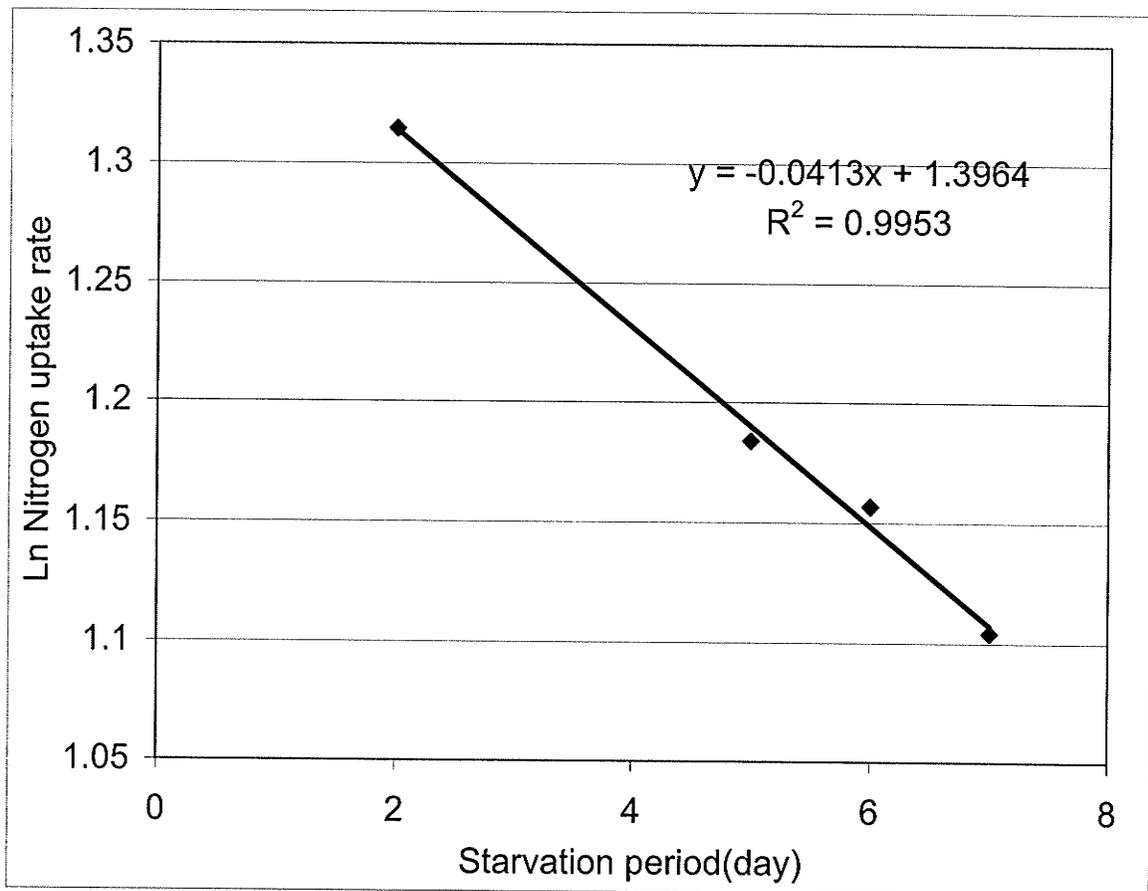
Figure 3. 8 Concentration of electron acceptor under starvation conditions



3.5.4.2 Decay and active fraction of biomass

Plotting nitrogen uptake rate versus time resulted in a decay coefficient of 0.041 day^{-1} . Once the decay coefficient is determined, the first term of equation (3.16) $b_A \cdot f_d \cdot X_{A0}$ can be used for estimating active fraction of the biomass. X_{A0} in equation 3.16 represents active biomass concentration, by which active biomass is defined as active bacteria excluding the extracellular polymeric substances (EPS). In order to calculate the concentration of active biomass the biodegradable fraction of active biomass (f_d) is required.

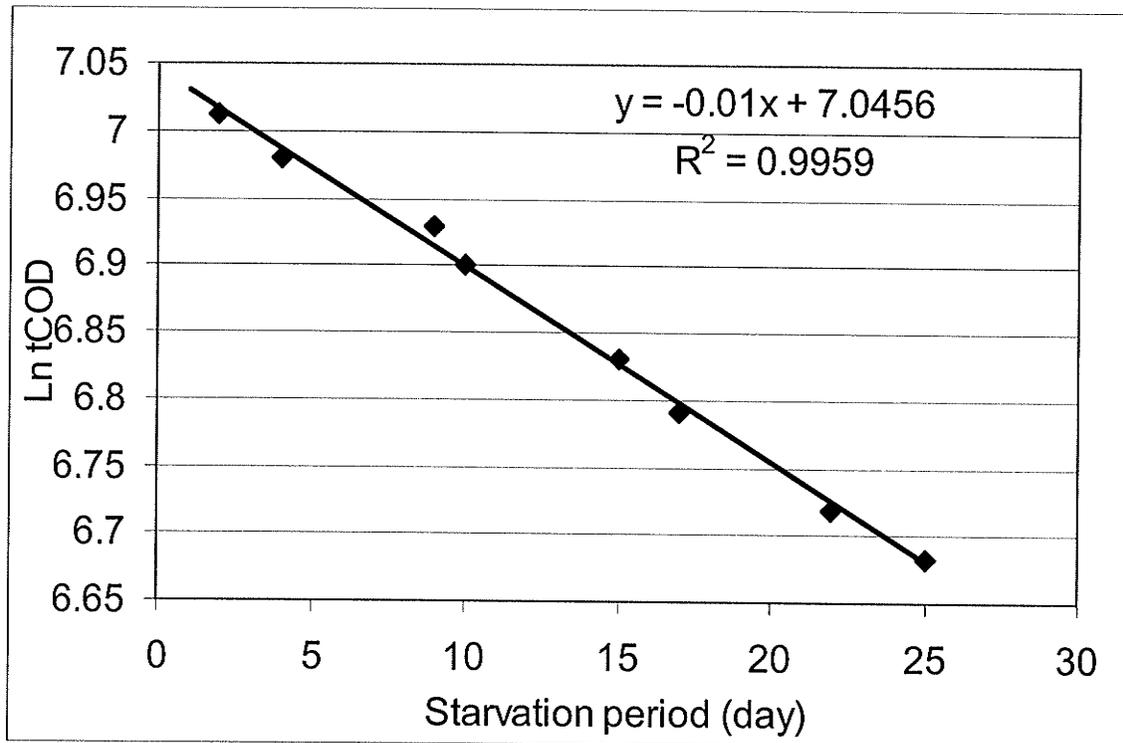
Figure 3.9 Nitrogen uptake rates versus starvation time



3.5.4.3 Biodegradable fraction of active biomass (f_d)

Biodegradable fraction of active biomass was calculated using the slope of the graph plotting total COD versus starvation time divided by decay coefficient. The value of 24% was obtained for biodegradable fraction of active biomass.

Figure 3. 10 Total COD versus starvation time



Knowing the f_d , the first term of equation 3.16 ($b_A f_d X_{A0}$) can be used for estimating active fraction of the biomass by using 24% for f_d . The value of 410 mg l^{-1} was obtained for the active biomass; this can be compared to the initial biomass concentration in the decay reactor, which was 1000 mg l^{-1} , suggesting 41% of biomass was active.

The biomass used in decay reactor was obtained from the main reactor at steady state. It included active biomass, extracellular polymeric substances and cell debris. The concentration of cell debris can be calculated through the following equation using active biomass concentration, biodegradable fraction of active biomass (f_d) and decay coefficient obtained previously.

$$X_i = b_A \cdot (1 - f_d) \cdot X_A \cdot \theta_x \quad (3.18)$$

The value of 256 mg l^{-1} is obtained for the concentration of cell debris using this equation. Combining the data obtained for cell debris and active biomass concentration, the concentration of extracellular polymeric substance (EPS) can be estimated. The value of 334 mg l^{-1} is obtained for EPS, which shows that a great portion of biomass is occupied by inert polymers.

The calculated EPS is higher in compare to EPS concentration obtained by steam extraction method. The average concentration of 210 mg l^{-1} was obtained through steam extraction method; – the value is 37 % lower than the calculated value. The higher EPS content resulted from calculation is expected as the extraction methods are limited. There are several extraction methods, including EDTA extraction, cation exchange resin, formaldehyde extraction, steam extraction and ultracentrifuge extraction. However, different quantity of EPS seems to be extracted by different method (Zhang et al., 1998; Brown and Lester, 1980; Hong and Liu, 2002). Furthermore, none of the methods used for extraction of EPS can assure that all of EPS is extracted.

3.5.4.4 True Yield

Equation 1.30 shows the relation of observed yield (Y_{Obs}), true yield(Y), decay coefficient (b_A) and solids retention time (θ_x).

$$Y_{Obs} = \frac{Y}{1 + \theta_x \cdot b_A} + \frac{(1 - fd)(b_A)Y\theta_x}{1 + \theta_x \cdot b_A} \quad (1.30)$$

The second term in the equation 9 represents the cell debris accumulated in the biomass. The true yield in equation 1.30 is the yield for active biomass production when EPS is included as a part of active biomass. Using the observed yield and fd , true yield was calculated as 0.65 g active cell+EPS/ g NO₃-N. In the case that EPS is excluded from biomass the yield of 0.27 g active biomass / g NO₃-N is obtained.

In other research studies on hydrogen-dependent denitrification, values ranging from 0.22 to 1.2 have been reported as it is shown in Table 3. 3.

Table 3. 3 True yield of hydrogenotrophic denitrifiers

True Yield mg VSS mg NO ₃ -N ⁻¹	Reference
0.78	Pierkiel, (2002)
1.2	Benedict, (1996)
0.22	Rittmann &McCarty,(2001)
0.65	Current study

Surprisingly, the true yield for hydrogenotrophic denitrifiers is high in comparison to heterotrophic denitrifiers, despite the general perception of lower biomass yields of autotrophs versus heterotrophs. The values reported for hydrogen dependent denitrifiers are higher than the 0.4-0.9 mg VSS mg N⁻¹ reported for heterotrophic denitrifiers (Metcalf and Eddy, 1991).

It should be noted that the yield of hydrogen dependent systems might also vary due to the effect of dissolved oxygen (Schink and Schlegel, 1978). The dissolved oxygen in the

feed can provide the condition for the growth of aerobic hydrogen-oxidizing bacteria such as *Alcaligenes eutrophus*. Therefore at different HRTs under steady state conditions, the fraction of aerobic hydrogen oxidizing bacteria in the biomass might be different due to variations in dissolved oxygen loading. The variation in true yield might come from the aerobic fraction of hydrogenotrophic biomass.

3.5.5 Conclusions

- During starvation, once the hydrogen is eliminated from the feed, EPS is hydrolyzed and used as a substrate for heterotrophic fraction of biomass, resulting in high nitrogen (nitrate) uptake rate at the start-up of the experiment.
- Once the EPS is consumed the biodegradable fraction of biomass serves as electron donor, resulting in lower and steady nitrogen uptake rate.
- During endogenous respiration not only particulate cell debris is produced but also non- biodegradable soluble COD is released to the reactor.
- Steady-state biomass obtained from the reactor operating under SRT of 20 d and loading of $0.081 \text{ g NO}_3\text{-N d}^{-1} \text{ l}^{-1}$ contained 41% active biomass, 25.6% cell debris and 33.4% EPS.
- The decay coefficient of 0.041 d^{-1} and true yield of 0.28 mg active biomass per mg $\text{NO}_3\text{-N}$ removed was obtained.

CHAPTER 4: HYDROGEN-DEPENDENT DENITRIFICATION IN ANAEROBIC SUBMERGED MEMBRANE BIOREACTOR: POTENTIAL FOR WATER REUSE

This chapter is focused on introducing a practical and efficient system for producing reusable water from municipal wastewater final effluents containing nitrate. The design is focused on designing a system, which utilizes hydrogen for stimulating biological denitrification, while producing the effluent free of suspended solids. The chapter starts with introducing the guidelines for producing reusable water, and then focuses on reactor design and optimization.

4.1 Wastewater (Water) reuse

4.1.1 Introduction

The increased water demand in the world is putting increasing pressure on water resources. The water specialists are looking at reusing final effluent for irrigation and agriculture as well as for indirect and even direct potable water supply. Water experts around the world agree that the implementation of wastewater reuse will be a major challenge in the 21st century (Marsalek et al., 2002). Currently, wastewater reuse is mainly practiced in areas that have the most stressed water resources. These areas include the Middle East, Japan, Korea, Australia, and the southwest United States. Australia, Japan and the southern United States have well-established standards governing the practice of wastewater reuse.

4.1.2 Direct potable reuse

It can be defined as either the injection of reclaimed water directly into the potable water distribution system downstream of the water treatment plant, or into the raw water supply immediately upstream of the water treatment plant. Injection could be either into

a service reservoir or directly into a water pipeline. The water used by consumers could be either undiluted, or slightly diluted, reclaimed water. There are no existing examples of direct potable water reuse in Australia. Internationally, the nearest known example is at Windhoek in Namibia where treated wastewater is blended with water from other sources prior to distribution (Asano and Levine, 1996).

4.1.2 Indirect potable water reuse

In the case of indirect water reuse the reclaimed water is cycled to the water cycle at a point well upstream of the water treatment plant. Return can be either into a major water supply reservoir, a stream feeding a reservoir, or into a water supply aquifer. The returned water is significantly diluted with other 'natural' water and there can be a real or perceived spatial or temporal separation between the point of return and the point of use (Marsalek et al., 2002). Indirect reuse is expected to be the most acceptable potable reuse approach for the community. Indirect reuse provides the necessary separation between the treatment plant and the user, depends to a lesser extent on the reliability of technology, and explicitly incorporates 'natural' processes within the reuse system to improve the water quality. In the US, there are an increasing number of examples of formal indirect potable reuse. A significant advantage of both direct or indirect potable reuse is that it allows for 100% reuse of available reclaimed water because total potable water usage will always be greater than the volume of wastewater generated. This is contrasted with non-potable reuse where the high cost of storage and distribution infrastructure limits the viability of achieving full reuse consistently.

4.1.3 Water quality indicators

The physiochemical parameters such as BOD/COD, hardness, pH, total organic carbon (TOC), taste and odour, total organic halogens (TOX), colour and total dissolved solids are used as an indicator of water quality. In case of water reuse, more detailed information about toxicological parameters of reusable water are required. The toxicological parameters can be grouped as:

Health issues regarding:

- Inorganics
- Algal Toxins
- Organics
- Pesticides
- Pharmaceuticals
- Disinfection By-products
- Hormones
- Radionuclides
- Endocrine Disruptors

In Drinking Water Guidelines many chemicals have been identified and their limit has been established. However, in the case of water reuse more restricted guidelines are expected for specific chemicals, especially when treated wastewater is planned for indirect water reuse.

4.1.4 Health concerning chemicals for water reuse

4.1.4.1 Inorganics

In the case of many inorganics, sufficient information is available for Guidelines for potable water. However, for several of the metals there is currently insufficient information for specific potable reuse and guidelines need to be developed (US EPA, 2004).

4.1.4.2 Organics

As for the organics, the issue is more complex and controversial. The question whether the presence of different organics with concentration as low as nanogram per liter has adverse effect on human health. It is important and difficult to determine what possible human health effects will develop when persons are exposed to a chemical or mixture of chemicals over a life time (US EPA, 2004).

4.1.4.3 Pesticides

Many of the chlorinated pesticides are still present in the environment due to their slow degradation, and as such may require further research in terms of potable reuse, especially when wastewater discharges are planned for indirect potable reuse (US EPA, 2004).

4.1.4.4 Disinfection by-products

A number of by-products of water disinfection have been highlighted as having the potential for formation under certain circumstances. In case of potable water reuse, the

issue is more complicated due to presence of synthetic organics in wastewater and formations of by-products during disinfection.

4.1.4.5 Radionuclides

Drinking Water Guidelines provide a reasonable introduction on the guidelines, however they do not address the potential for enhancement of radionuclide concentration in reuse applications (US EPA, 2004).

4.1.4.6 Pharmaceuticals

The 10 most commonly prescribed and non-subsidised drugs have been identified. Two important issues controlling the fate of pharmaceuticals are their metabolism and stability (US EPA, 2004).

4.1.4.7 Hormones

Hormones are considered the main biogenic compounds likely to occur in wastewater for potable reuse. Their activity as androgens or oestrogens highlights the need for further research on their potential endocrine effects when consumed orally and the their improved removal using various water treatment techniques.

4.1.5 Existing Guidelines for Direct Potable Reuse

There are no guidelines for direct potable reuse of reclaimed water. Guidelines exist for indirect portable reuse. As it was mentioned before, indirect potable reuse is defined as the return of reclaimed water to a point well upstream of the water treatment plant. Return could either be into a major water supply reservoir, a stream feeding a reservoir,

or into a water supply aquifer. The returned water is likely to be significantly diluted with other 'natural' water.

4.1.6 Existing Guidelines for Indirect Potable Reuse

4.1.6.1 Canada

In Canada, there are no guidelines for indirect potable reuse. British Columbia and Alberta are the only provinces that have developed regulatory guidance for industrial wastewater reuse. In British Columbia, uses include rangeland irrigation, silviculture applications, stream augmentation and toilet flushing (Marsalek, 2002). The City of Vernon successfully uses 100 % of its wastewater for irrigation of agricultural, silvicultural, and recreational lands, in a program that has been operational for over 20 years serving a population of 32,000 (Waller, 1998).

The province of Alberta has supported the use of treated wastewater for irrigation purposes, which currently demand over 70 % of Alberta's water resources (Marsalek, 2002). Current allowable uses for wastewater reuse are "golf courses; municipal parkland and boulevards; forested woodlots under special approval consideration; and agriculture lands used for pasture, forage, coarse grains, turf, and oil seeds. Any other crops to be considered must be first supported by scientific based studies that ensure no risk to human health or the environment" (Marsalek, 2002).

In Nova Scotia, a demonstration project has been initiated that reuses wastewater effluent from a wastewater treatment lagoon for a wetland refuge (Waller, 1998). The created wetlands have the added benefit of providing some measure of tertiary treatment, reducing coliform, suspended solids, BOD and phosphorus by up to 99 %.

4.1.6.2 US EPA Water Reuse Guidelines

A number of states in the USA, such as California, Arizona and Florida have developed guidelines and regulations addressing potable reuse of reclaimed water, however, there is considerable variation among the different state regulations. There are no federal regulations governing water reclamation and reuse in the US, hence the regulatory burden rests with the individual states (US EPA, 2004). The USEPA guidelines for water reuse published in 1992 provide guidance to states that have not developed their own criteria or guidelines. The US Environmental Protection Agency, in conjunction with the US Agency for International Development, published Guidelines for Water Reuse in 1992. The primary purpose of the document is to provide guidelines, with supporting information, for utilities and regulatory agencies in the US, particularly in states where standards do not exist or are being revised or expanded (US EPA, 2004). The guidelines address all important aspects of water reuse and include recommended treatment processes, reclaimed water quality limits, monitoring frequencies, setback distances, and other controls for various water reuse applications. The guidelines address water reclamation and reuse for non-portable applications as well as indirect potable reuse by groundwater recharge and augmentation of surface water sources of supply. The treatment processes and reclaimed water quality limits recommended in the guidelines for various reclaimed water applications are given in Table 4.1.

Table 4. 1 US EPA guidelines for water reuse (US EPA, 2004).

Type of use	Treatment	Reclaimed water quality
Urban users, food crops Raw, recreational impoundments	<ul style="list-style-type: none"> ▪ Secondary ▪ Filtration ▪ Disinfection 	<ul style="list-style-type: none"> ▪ pH= 6-9 ▪ 2NTU ▪ No detectable fecal coliform ▪ 1 mg l⁻¹ Cl₂ residual
Restricted access area Irrigation, processed food crops, nonfood crops, cooling, environment reuse	<ul style="list-style-type: none"> ▪ Secondary ▪ Disinfection 	<ul style="list-style-type: none"> ▪ pH= 6-9 ▪ 30 mg l⁻¹ BOD ▪ 30 mg l⁻¹ SS ▪ 200 focal coli/100mL ▪ 1 mg l⁻¹ Cl₂ residual
Groundwater recharge of portable aquifers by spreading	<ul style="list-style-type: none"> ▪ Site specific and use dependent ▪ secondary 	<ul style="list-style-type: none"> ▪ Site specific and use dependent
Groundwater recharge of portable aquifers by injection	<ul style="list-style-type: none"> ▪ Site specific and use dependent ▪ secondary 	<ul style="list-style-type: none"> ▪ Site specific and use dependent
Groundwater recharge of portable aquifers by spreading	<ul style="list-style-type: none"> ▪ Site specific and use dependent ▪ Secondary and disinfection 	<ul style="list-style-type: none"> ▪ Site specific ▪ Meet drinking water standards after percolation through vadose zone
Groundwater recharge of portable aquifers by injection, augmentation of surface supplies	<ul style="list-style-type: none"> ▪ Secondary ▪ Filtration ▪ Disinfection ▪ Advanced wastewater treatment 	<ul style="list-style-type: none"> ▪ pH= 6 -8.5 ▪ 2NTU ▪ No detectable fecal coliform ▪ 1 mg l⁻¹ Cl₂ residual ▪ meet drinking water standards

4.2 Producing reusable water from municipal final effluent contaminated with nitrate

Many final effluents contain high level of nitrate due to low C/N ratio in raw wastewater. (Bernard and Abraham, 2005). According to US EPA, the produced water for indirect reuse should meet drinking water guidelines. This stringent effluent quality can be achieved using advanced treatment processes after biological nutrient removal. In the case that nitrate is present in the effluent, denitrification also needs to be incorporated.

4.2.1 Denitrification Options

The denitrification process can be divided into two categories of tertiary treatment and single- sludge treatment (Rittmann and McCarty, 2001). In single sludge treatment, denitrification includes using the BOD in the wastewater influent to drive denitrification. In single sludge denitrification, the nitrate is produced in the aerobic zone by nitrifiers and directed to the anoxic zone, which can be located before, after or within the aerobic zone (nitrification). These possibilities are called pre-anoxic (Figure 4. 1), Post-anoxic (Figure 4. 2) and simultaneous nitrification denitrification (SND). The design strategy is dependent on wastewater characteristics, especially C/N ratio.

Figure 4. 1 Pre-anoxic design for denitrification

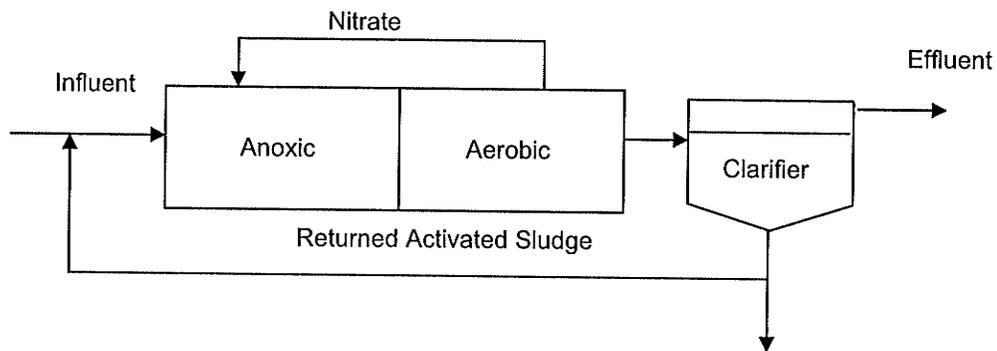
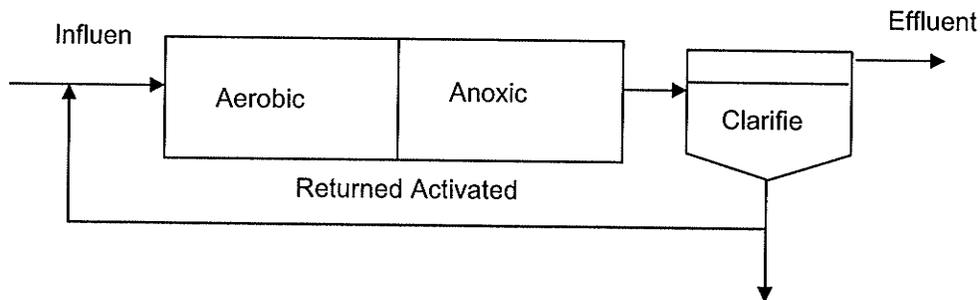


Figure 4. 2 Post-anoxic design for denitrification



To incorporate denitrification for wastewater with low C/N ratio, exogenous electron donors need to be added. The factors to be considered are the choice of appropriate electron donor and the location of addition (post anoxic or pre anoxic). Post-anoxic denitrification is chosen as an alternative due to the following reasons. It can easily be retrofitted to the existing plants. It requires less organic carbon in compared to post denitrification. In case of pre-anoxic denitrification some portion of organic carbon is degraded in the aerobic zone. Pre-anoxic denitrification can not produce an effluent free of nitrate. To choice complete nitrate removal, tertiary denitrification can be considered s an option.

4.2.2 Choice of exogenous electron donor

Depending on the choice of exogenous substrate, either heterotrophic or autotrophic denitrification can be stimulated. The electron donor should have low cost and posses a low saturation coefficient, as the lower the saturation coefficient the lower the donor carryover to the final effluent.

4.2.2.1 Heterotrophic denitrification

Almost any organic carbon can be used as exogenous energy source for heterotrophic denitrifiers. As it was mentioned before, methanol is the most commonly used exogenous carbon source only due to its economic benefits. Methanol might not be a proper donor choice for water reuse applications as it has a relatively high saturation coefficient of 9.1 mg l^{-1} (Rittmann and McCarty, 2001) resulting in methanol carryover to the final effluent.

Waste streams from food processing and beverage industries can also be used as a cheap source of electron donor to drive denitrification. These wastes have very high BOD (in the range of 10,000 mg l⁻¹) and very high C/N ratio. However, the availability of these carbon sources is a concern. Furthermore, the goal of denitrification is total nitrogen removal, and the ammonia content of these wastes might prohibit achieving this goal. Another alternative is taking advantage of autotrophic denitrifiers by using inorganic substrates as carbon source.

4.2.2.2 Autotrophic denitrification

As it was described in Chapter 1, autotrophic denitrification is a biological process that uses inorganic carbon to derive denitrification. Different electron donors can be used for autotrophic denitrification. These donors include hydrogen and elemental sulphur. Autotrophic denitrification using sulfur compounds carries several disadvantages over hydrogenotrophic denitrification, including:

- Sulfur is in solid phase and mass transfer of sulfur to the water is the Limiting step during denitrification.
- High alkalinity consumption per unit of NO₃-N
- Relatively higher biomass yield
- Sensitive to pH fluctuations
- Potentially high sulphate concentrations in the effluent that might not be appropriate for water reuse

Autotrophic denitrification using hydrogen is an attractive option for removing nitrate from the wastes with low C/N ratio. Gantzer (1995) reported the cost of methanol to be about 73% higher than hydrogen in the case that hydrogen is produced on-site. This is

not include the cost of transportation of methanol to the site. The transportation cost can be as high as the cost of the chemical itself depending on the location of the site. If hydrogen is not produced on-site, it is more expensive than methanol. The use of hydrogen carries some disadvantages that need to be addressed. These include:

- Low solubility in water
- Explosion risk when mixed with oxygen.

Up to date, a variety of reactor configurations have been used to improve the efficiency of hydrogen delivery. One such reactor configuration, which addresses effective hydrogen delivery, is called biofilm electrode reactor (Islam et al., 1998; Prosnansky & Sakakibara, 2002; Kiss et al., 2000; Sakakibara and Nakayama, 2001) . A biofilm electrode reactor is an electrochemical cell, in which water is electrolysed and hydrogen is generated. The hydrogen produced on the surface of cathode allows formation of hydrogenotrophic denitrifiers in a biofilm at the surface of the cathode. The main drawback of biofilm electrode reactors is gradual scale formation on the surface of the cathode, suppressing hydrogen production, which may cause a dramatic decrease in denitrification rates (Kiss et al., 2000).

Another alternative is using membrane gas diffusers, which allows bubble-less dissolution of hydrogen into the water. Membrane gas diffusers have been used to deliver hydrogen either to biofilm (Ergas and Reuss, 2001; Lee and Rittman, 2002), or to suspended bacteria (Rezania et al., 2005; Mo et al., 2005).

A membrane biofilm reactor is simply fabricated by immersing the membrane gas diffuser in a reactor. The membrane acts as a support medium for biofilm formation, while introducing the gas to the developed biofilm. Membrane biofilm reactors are subject to problems that might limit their application in long term operation. The

membranes are structurally fragile and will be ineffective if any physical damage occurs. Porous membrane diffusers are subject to condensation of water vapour inside the fibres lowering the hydrogen mass transfer (Ma et al., 2003). The biofilm grown on the surface of the membrane is usually thick and sheering of this biofilm requires high energy due to precipitation of inorganics inside the biofilm (Ergas and Reuss, 2001; Lee and Rittman, 2003). In addition, sloughing of biomass can result in breakthrough of organic matter in the effluent.

4.3 Objectives

The objective of this study was to produce water free of nitrate and suspended solids from nitrate contaminated water or wastewater novel bubble-less hydrogen delivery coupled to a submerged anaerobic membrane bioreactor. The new reactor configuration aimed to allow for suspended growth of biomass and effective hydrogen delivery without the use of a diffuser or electrode.

The main goals are described as follow:

- To evaluated the technical feasibility of a hydrogen delivery unit and biological treatment unit
- To remove nitrate form the contaminated water and wastewater
- To produce effluent free of suspended solids and pathogens
- To provide an organic carbon and nitrate mass balance

4.4 Design concepts

4.4.1 Hydrogen delivery unit

The process of bubbleless hydrogen delivery employed in this research is based on pressurizing some portion of the feed with hydrogen in a saturator tank and releasing the hydrogen supersaturated feed to the biological reactor. The supersaturated feed contains high dissolved hydrogen concentration. The concentration of dissolved hydrogen in the saturator is dependent on the pressure in the saturator and the mixing regime; however at equilibrium the dissolved hydrogen concentration in the saturator follows Henry's law. When the supersaturated feed is released to the reactor it goes through several transformations effecting mass transfer including, liquid-liquid mass transfer, stripping of dissolved hydrogen and micro bubble formation due to change in pressure, gas-liquid mass transfer of formed micro bubbles, and dissolved hydrogen consumption by the bacteria. The bubble-less hydrogen delivery can be achieved in the case that the hydrogen loading to the reactor is equal to the hydrogen uptake rate by the bacteria in the reactor. One of the advantages of delivering hydrogen through supersaturation is that hydrogen loading is easily adjustable by changing the flowrate of supersaturated liquid to the reactor.

4.4.2 Anaerobic submerged membrane bioreactor

Membrane bioreactors are used to retain biomass in bioreactor by membrane filtration and thereby eliminate microorganisms in the effluent and biomass washout. The application of submerged membrane bioreactors has been limited to aerobic operation. This is due to the requirement of air scouring through the membrane module for

cleaning of the membrane surface and maintaining a constant permeate flux. In the case of anaerobic membrane bioreactors, external membranes have been tested. However, to operate a submerged membrane bioreactor under anaerobic conditions, air needs to be replaced with another gas in a closed system. In this study, the nitrogen gas produced during denitrification was recycled to the membranes for scouring and reactor mixing.

4.5 Material and methods

4.5.1 Removing nitrate from water

The experimental system used for denitrification of nitrate contaminated water is illustrated in Figure 4.3. The system was composed of a hydrogen delivery unit and a biological reactor. The hydrogen delivery unit is composed of a saturator tank with the total volume of 8.5 L, a chemical feed pump, a pressure regulator valve, a mixer and a hydrogen cylinder. The reactor unit was configured as a submerged membrane bioreactor, which consisted of a cylindrical plexiglas reactor with the total volume of 10.1 L and working volume of 5.6 L, two hollow fibre membrane filters (ZW-1, by Zenon Environmental, Inc.) with nominal pore size of 0.04 μm and the total surface area of 0.094 m^2 , two permeate pumps, a gas recycle pump and a pressure regulator valve.

The saturator tank was connected to the hydrogen cylinder, which was regulated to a pressure (120 psi) less than operating pressure of the saturator (125 psi). A portion of nitrate contaminated water was pumped to the saturator tank through the feed pump. The synthetic feed was composed of 25 mg l^{-1} $\text{NO}_3\text{-N}$, 1000 mg l^{-1} NaHCO_3 , 25 mg l^{-1} KH_2PO_4 , 5 mg l^{-1} CaCl_2 , 25 mg l^{-1} $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 1 mg l^{-1} FeSO_4 . Pumping the feed to the saturator tank caused an additional pressure build-up (5 psi) in the saturator.

Increase of pressure in the saturator forced the pressure regulator valve to open and released the feed, which was supersaturated with hydrogen, to the reactor. Bubble-less hydrogen delivery was only possible when the rate of hydrogen introduced to the reactor is equal to the hydrogen uptake rate. Providing hydrogen and nitrate to the reactor stimulated the growth of hydrogenotrophic denitrifiers. Hydrogenotrophic denitrifiers metabolised hydrogen and nitrate, resulting in the conversion of nitrate to nitrogen gas, which accumulated in head space. The headspace, which was filled with nitrogen produced during denitrification, was recycled to induce mixing and membrane scouring. The excess nitrogen was released automatically to the atmosphere through a pressure regulator valve. The remaining portion of the feed water not passed through the saturator is fed to the bioreactor using a float valve to maintain constant hydraulic loading. The biomass produced in the reactors was separated from the treated water by the submerged membrane filters.

The membrane filters were operated under 300 second filtration and 30 second backwash using two separate permeate pumps. The reactor was operated at room temperature (25-28°C) with an SRT of 20 days and HRT of 175 minutes.

Nitrate (NO_3^-) and nitrite (NO_2^-) concentrations were measured by the automated cadmium reduction method (4500- NO_3^- -F) (*Standard Methods*, 1995). Total suspended solids (TSS) and volatile suspended solids (VSS) were measured according to methods 2540 D and 2540 E (*Standard Methods*, 1995), respectively. Chemical oxygen demand (COD) samples were analyzed using the Hach Digestions Vials and the Hach spectrophotometer (Hach, USA). Dissolved organic carbon (DOC) concentration was determined by Phoenix 8000 carbon analyzer (*Standard Methods*, 1995; 5310 C).

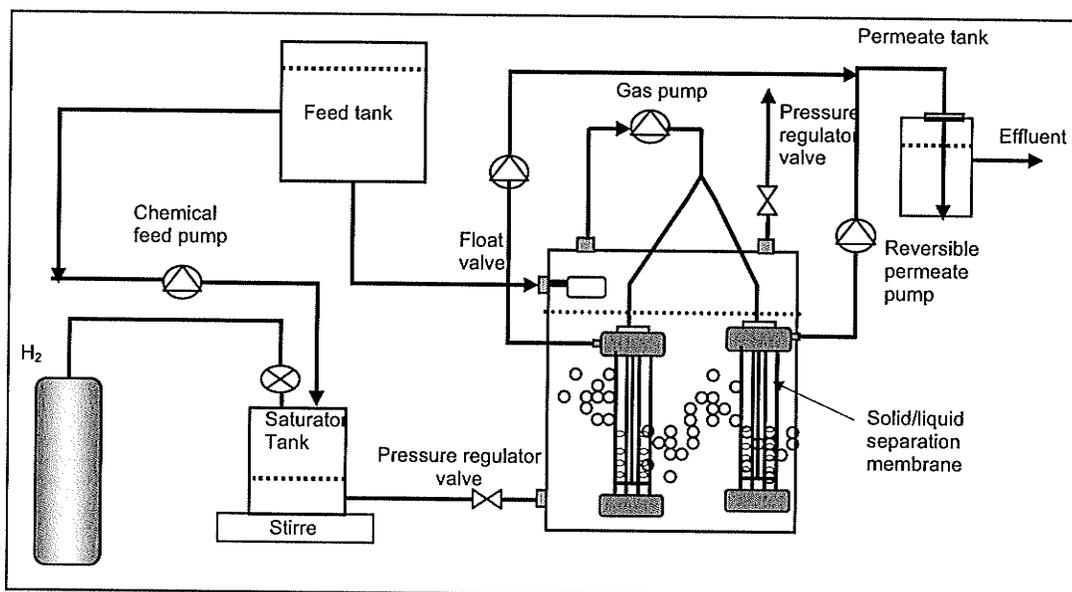
The dissolved hydrogen in the permeate tank was monitored with an online dissolved hydrogen analyzer (Orbisphere).

4.5.2 Removing nitrate from municipal final effluent

The same system described in section 4.5.1 was used for removing nitrate and suspended solids from municipal final effluent. The final effluent was obtained from Winnipeg's North End Wastewater Treatment Plant, which is pure oxygen BOD removal plant. The final effluent was spiked with nitrate to simulate the effluent from nitrified swage. A feed tank with the total volume of 25 L was used to feed both the saturator and the biological reactor. The feed tank was aerated slowly to prevent denitrification from occurring. The feed, with the average flow rate of 32 ml min^{-1} was split and directed to the MBR and the hydrogen saturator. The portion of feed directed to the saturator was supersaturated with hydrogen at pressure of 120 psi and released to the reactor.

Both the feed and effluent were sampled and analyzed using Standard Methods for nitrate, nitrite, total and soluble chemical oxygen demand, pH, alkalinity, true colour, turbidity, hardness, total dissolved solids and total coliform. The mixed liquor from the reactor was measured for volatile and total suspended solids and soluble COD. The dissolved hydrogen in the permeate tank was monitored using an online dissolved hydrogen analyzer (Orbisphere).

Figure 4. 3 Schematic of the proposed system for hydrogenotrophic denitrification of contaminated wastewater



2.5.3 Mass transfer study

An experiment was conducted to study the mass transfer of the supersaturated water released to the reactor, which was filled with pure water. Mass transfer of hydrogen into the pure water is the worst-case scenario, since no hydrogen is consumed by denitrifying bacteria, enhancing the sink for dissolved hydrogen.

In order to study the mass transfer of hydrogen into the water, the feed tank and reactor (shown in Figure 4.3) were filled with water and reactor head space was filled with nitrogen gas. Some portion of the feed (water) from the feed tank is pumped to the saturator tank with a flow rate of 37 ml min⁻¹. The saturator was already filled with hydrogen at a pressure of 120 psi. The working pressure of the regulator valve, which is located between the saturator and the reactor, was adjusted to 125 psi. The supersaturated water was introduced to the reactor through the pressure regulator valve.

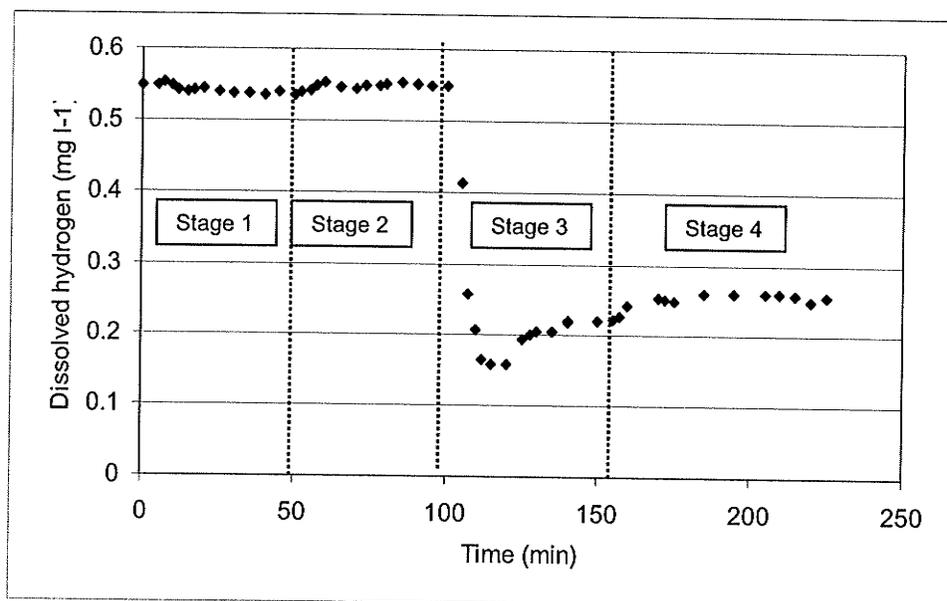
At steady-state, the flow rate of the feed pump was equal to the flow rate of the supersaturated feed released to the reactor. The supersaturated water from the saturator and the water from the feed tank were mixed in the reactor and pumped out with the flow rate of 480 ml min^{-1} . The dissolved hydrogen concentration in the reactor was monitored using an online dissolved hydrogen analyzer. The dissolved hydrogen in the saturator was measured by headspace method (Schmidt et al., 1993). The mass transfer efficiency was calculated by applying a hydrogen mass balance using dissolved hydrogen concentration in the saturator and in the reactor. Finally, the effect of mixing and headspace gas recirculation on mass transfer efficiency was studied.

4.6 Results and Discussion

4.6.1 Mass Transfer

Figure 4. 4 shows the dissolved hydrogen concentration in the reactor under steady state conditions at different stages of operation.

Figure 4. 4 Dissolved hydrogen concentration in the reactor at different stages



In the first part of the experiment (stage 1), the mixer in the saturator was turned off and there was no circulation of the head-space gases. In this case, the dissolved hydrogen in the reactor was around 0.55 mg l^{-1} . In order to calculate the mass transfer efficiency, the dissolved hydrogen concentration in the saturator is needed. The dissolved hydrogen concentration in the saturator was measured by taking samples from the pressure regulator valve, resulting in 7.18 mg l^{-1} of hydrogen. Hydrogen mass balance showed that 100% hydrogen delivery was achieved. As the second part of the experiment shows (stage 2), mixing in the saturator did not increase dissolved hydrogen concentration. This is due to the high natural flow rate maintained through the saturator (very low retention time) which render the effect of additional mixing negligible. In the third part of the experiment (stage 3), the headspace was circulated through the membrane module with the rate of 30 L min^{-1} . The dissolved hydrogen in the reactor decreased from 0.55 to 0.2 mg l^{-1} . The circulated gas stripped out some portion of the dissolved hydrogen. However, as the system is closed, the stripped hydrogen will eventually be available to the bacteria. Another way to eliminate the need for headspace recirculation and subsequent hydrogen stripping is to use external (recirculated) membranes for water filtration. The cost of high flow recirculation, however, can be prohibitive to the use of external membranes in many applications. In the last step of testing (stage 4), decreasing the head space gas recirculation flow rate from 30 L min^{-1} to 15 L min^{-1} increased the dissolved hydrogen concentration from 0.2 to 0.25 mg l^{-1} .

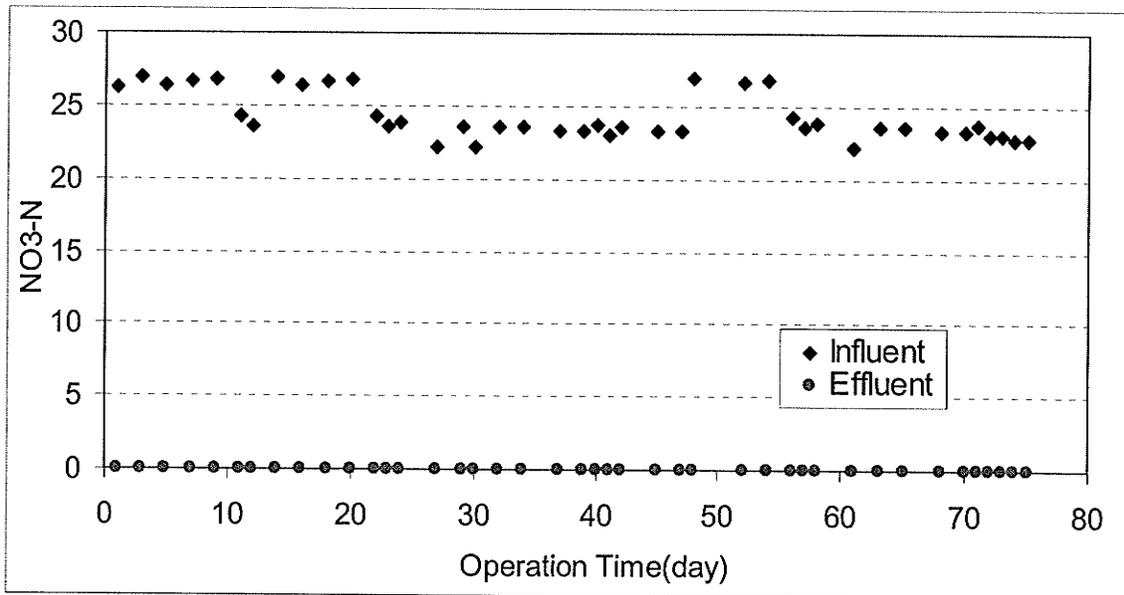
4.6.2 Reactor performance in removing nitrate from water

4.6.2.1 Nitrate removal

At the start up, some portion of the feed (16 ml min^{-1}) was directed to the saturator and the rest (17 ml min^{-1}) was introduced into the reactor. The clogging of the miniature pressure regulator valve introducing the supersaturated feed to the reactor was a problem. The particular valve used in this study was designed for liquids, free of suspended solids and could not handle suspended solids and organic matter resulting from bacteria growth in the saturator. However, clogging is not anticipated to be a concern in full-scale applications, as better working valves have been applied successfully in full-scale dissolved air flotation systems working under the similar concept.

To minimize the bacterial growth in the saturator, nitrate feed free was separately used to deliver dissolved hydrogen to the reactor.

Figure 4. 5. Reactor performance regarding nitrate removal from water under steady-state

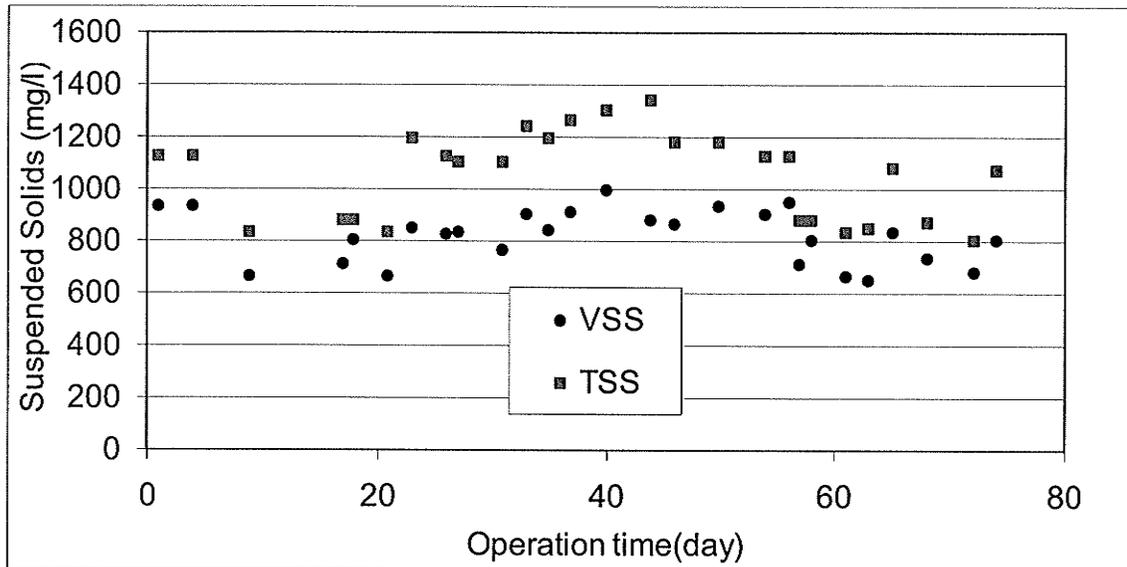


As shown in Figure 4. 5, the membrane bioreactor system was effective in complete nitrate removal from the synthetic feed, as nitrate concentration was reduced from 25 mg l⁻¹ NO₃-N to below detectable level at the loading of 0.11 kg N m⁻³ d⁻¹. No nitrite accumulation was observed throughout the experiment. The dissolved hydrogen concentration in the effluent changed between 0.05 to 0.1 mg l⁻¹. Complete denitrification was achieved at dissolved hydrogen concentrations as low as 0.001 mg l⁻¹. The dissolved hydrogen concentration in the effluent was changing due to the fact that the supersaturated water flow rate was decreasing over time. The hydrogen delivery system was operated in a way that the hydrogen pressure was 120 psi and extra pressure for opening of pressure regulator valve was provided by a chemical feeding pump. Over time the partial pressure of hydrogen in the headspace was decreasing due to hydrogen dissolution causing the level of water to rise. Controlling the water level in the saturator is the key for continuous dissolution of gas into the water in the saturator. The level of water in the saturator was controlled manually, resulted in less fluctuation in the dissolved hydrogen concentration.

4.6.2.2 Biomass concentration

Figure 4.6 shows the concentration of volatile and suspended solids at steady state condition, with average values of 803± 108 mg l⁻¹ for VSS and 1033 ± 166 mg l⁻¹ for TSS.

Figure 4. 6 Suspended solids concentration at steady-state condition



The inorganic fraction of TSS can be attributed to the precipitation of inorganics as it was explained in section 2.5.3. The observed yield of 0.36 mg VSS/mg N was calculated, where the solids retention time of 20 days.

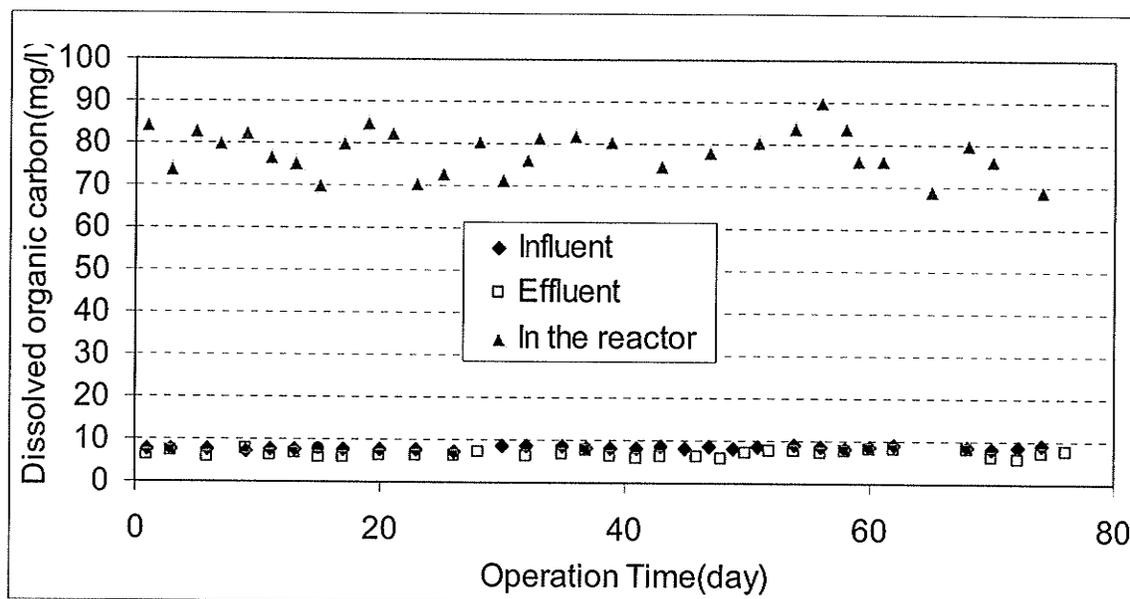
4.6.2.3 Organic carbon mass balance

Under steady state conditions the organic carbon mass balance can be written as follow:

$$0 = \text{DOC (In)} - \text{DOC (Out)} + \text{DOC (generation)} - \text{DOC (degradation)} \quad (4.1)$$

Figure 4. 7 shows the soluble organic carbon concentration at steady state condition. The influent contained some organic carbon, as tap water was used in the preparation of synthetic feed. The organic carbon exist in tap water is composed most likely of natural organic matter or disinfection by-products produced during chlorination.

Figure 4. 7 Dissolved organic carbon concentration at steady- state condition



During autotrophic denitrification, generation of organic carbon occurs due to the release of soluble microbial products. Soluble microbial products (SMP) can be classified into utilization-associated products (UAP) and biomass associated products (BAP). UAP are associated with substrate metabolism and produced at a rate proportional to substrate utilization, which in this study is the denitrification rate. BAP are associated with biomass decay and are produced at a rate proportional to biomass concentration (Barker and Stuckey, 1999). In order to assess the fate of organic carbon, the biodegradability kinetics of organic carbon is required. The degradation of the organic carbon requires heterotrophic activity. Although the conditions in the reactor are favourable to autotrophic growth, degradation of SMP in the reactor is possible. It has been found that all of hydrogen-dependent denitrifiers are mixotrophic as they are able to use inorganic carbon under autotrophic and organic carbon under heterotrophic

conditions (Szekeres et al., 2002). Soluble microbial products (SMP) are slowly biodegradable and kinetics of their degradation is well understood under aerobic conditions (Lu et al., 2002; Henze et al., 1987; Rittmann and McCarty, 2001). SMP were shown to have high saturation coefficients and low utilization rates, and therefore require long hydraulic retention time to degrade. When nitrate is the electron acceptor, even lower degradation rates are expected. In this study it was assumed that there was no degradation of SMP at a hydraulic retention time of three hours. To confirm this assumption, the wasted biomass from the reactor was transferred to a batch reactor and spiked with nitrate. No hydrogen was provided and nitrogen gas was bubbled into the reactor to provide anoxic conditions. As SMP were the only electron donor, nitrate consumption rates would represent the biodegradability of SMP. Negligible amounts of nitrate were consumed during the 3 hours batch test, which validates the original assumption that SMP is largely non-biodegradable. As shown in Figure 4. 7, the concentration of organic carbon in the influent and effluent were very similar. The organic carbon in the effluent can result from SMP produced during denitrification or from organic carbon carryover from the feed.

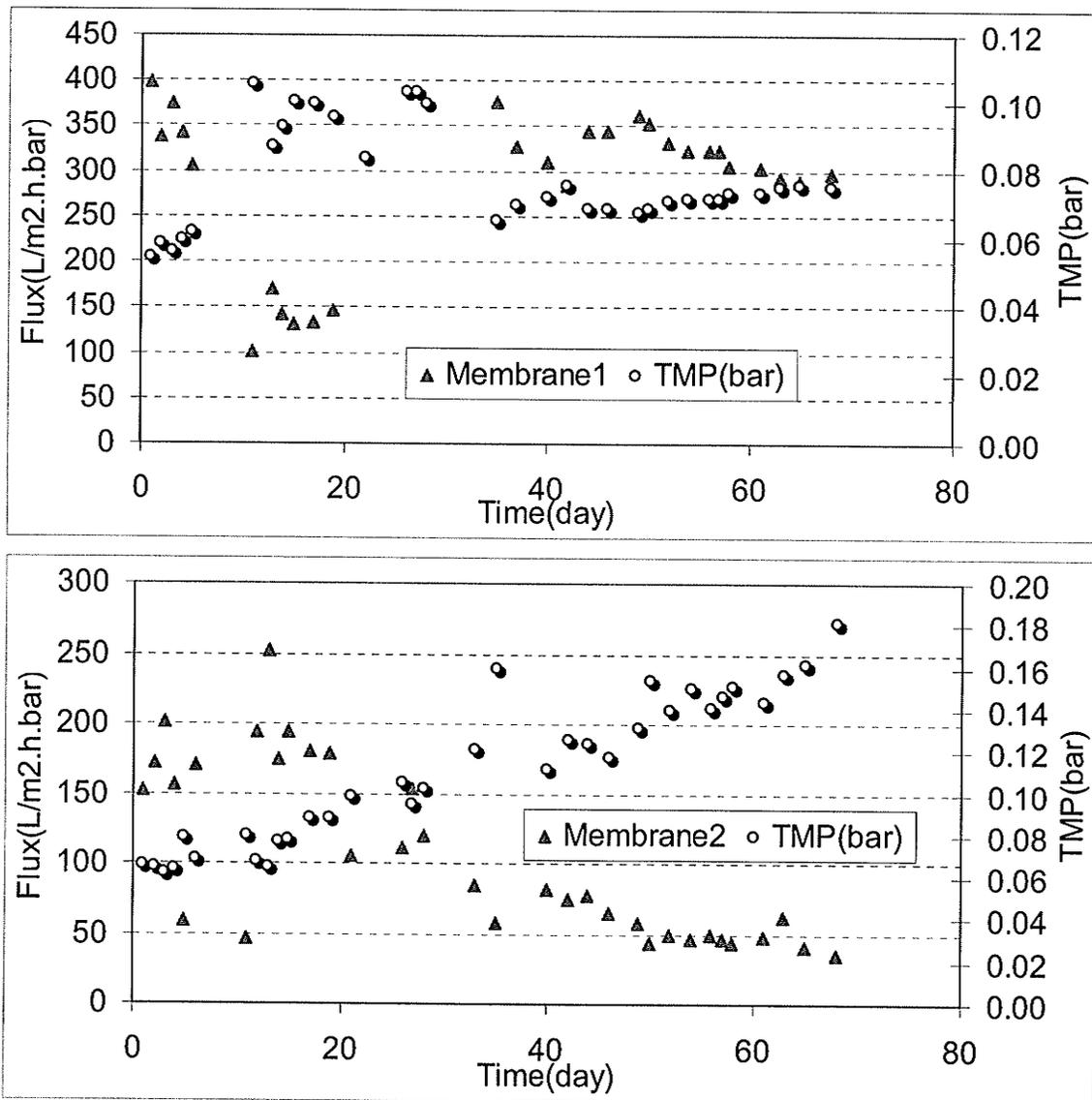
A simple test was conducted to access if the organic carbon in the feed was possibly rejected by the membrane. The test showed that almost all of the soluble organic carbon in the feed passed through the membrane. Therefore it is expected that most of the organic carbon in the effluent is a result of organic carbon carryover not SMP breakthrough. In a previous study using the same the same membrane filters, 81% of SMP was retained in the reactor (Rezania et al, 2005). This suggests that effluent organic matter is closely related to feed water DOC content and when nitrate

contaminated groundwater containing low levels of organic carbon is tested, low effluent DOC can be expected. Applying the mass balance (Eq. 4.1) the rate of SMP production was calculated as 0.18 mg DOC per mg $\text{NO}_3\text{-N}$ removed. This value might slightly change at different solids retention time (SRT), as SMP production is closely related to biomass concentration.

4.6.2.4 Membranes performance at steady state condition

Two separate membranes connected to different pumps were submerged in the reactor. Figures 4.8(a) and(b) show the flux and transmembrane pressure (TMP) of these membrane at steady state condition. Both membranes were operated under the condition of 5 minutes filtration and 30 seconds backwash. As the system was a closed system, the trans membrane pressure not only was affected by fouling and cake formation but also by flow condition and headspace pressure of the reactor. As it is shown in the figure the behaviour of the membranes was not the same. The behaviour of the membranes can be explained as follows: The initial increase in the flux of membrane 1 was due to pre cleaning. It was experimentally found that chemically cleaned membranes show more resistance at the start up and gradually achieve higher flux. This might be due to change in polarity of the membrane and biofilm formation on the membrane surface.

Figure 4. 8 Membranes performance at steady- sates condition



The constant transmembrane pressure in membrane 1 can be explained by the concept of critical flux. Critical flux is defined as a flux below which significant membrane fouling does not occur. The critical flux is related to different factors such as hydrodynamics and scouring rate (Howell et al., 2004). Apparently membrane 1 was operated below critical flux with minimum degree of fouling. Although membrane 2 was operated under

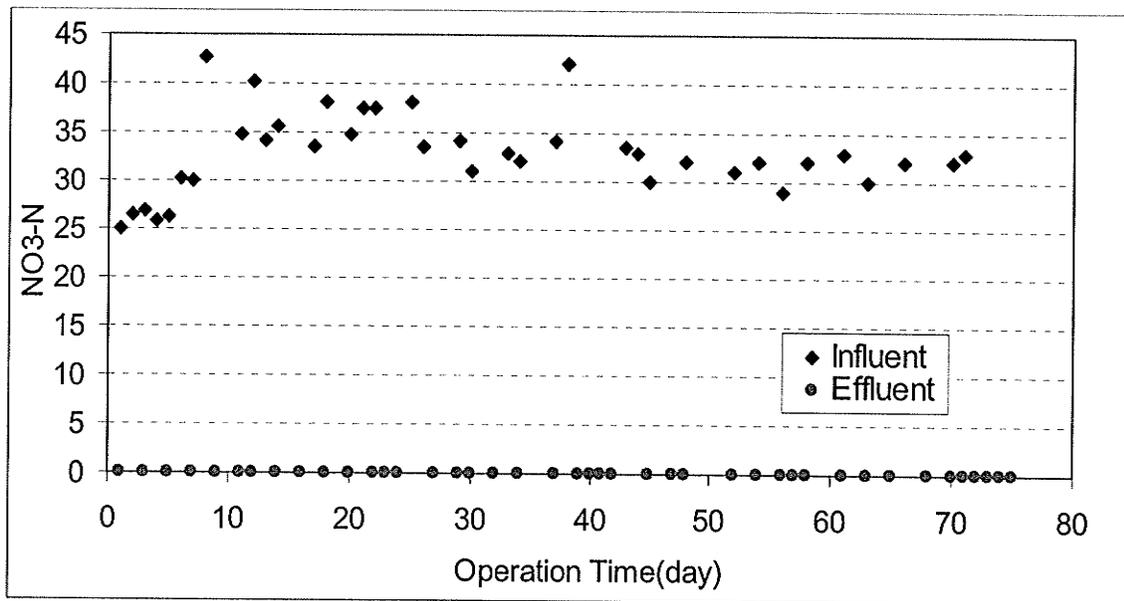
lower flux compared to membrane 1; it was subject to increased fouling. This fouling was found to be the results of lower scouring applied to second membrane. The nitrogen (headspace) scouring rate of membrane 1 was 18 L min^{-1} compared to 12 L min^{-1} of membrane two. This shows the huge impact of membrane scouring on membrane operation and fouling.

4.6.3 Removing nitrate from municipal final effluent

4.6.3.1 Nitrate removal

The system was successful in removing nitrate in the municipal final effluent from $33 \text{ mg NO}_3\text{-N l}^{-1}$ to below detectable level without any nitrite accumulation (Figure 4. 9). The portion of feed (17 ml min^{-1}) directed to the saturator was free of nitrate (was not spiked with nitrate) in order to prevent the growth of denitrifiers in the saturator and clogging of the pressure regulator valve.

Figure 4. 9 Reactor performance regarding nitrate removal under steady-state conditions



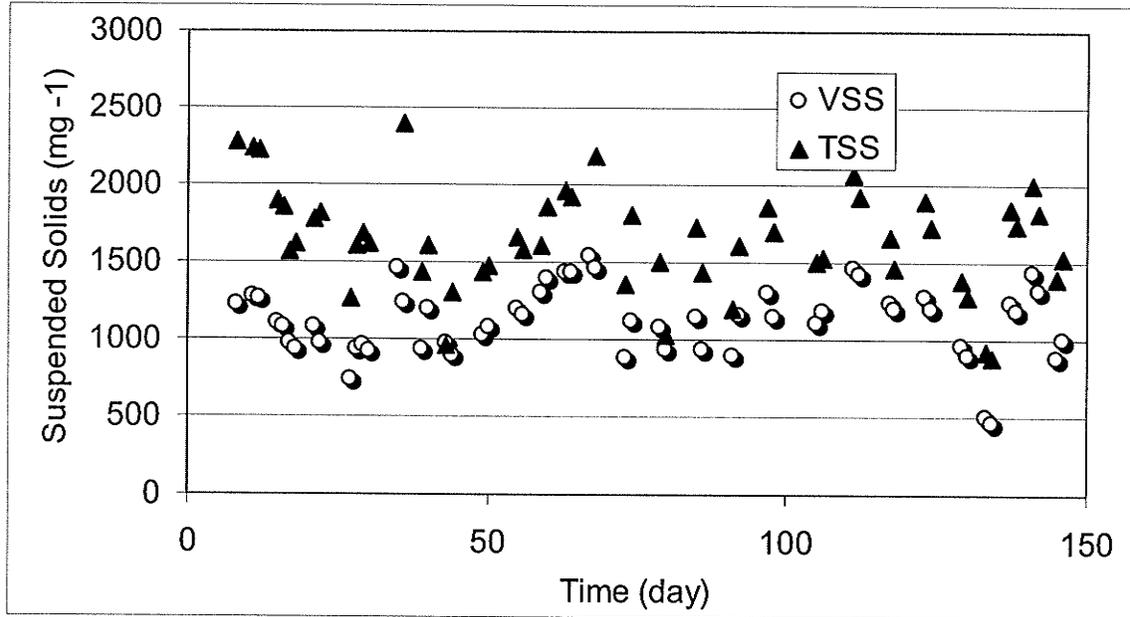
As it was mentioned before, clogging is not a concern in full scale application if an appropriate valve is chosen. The good performance of these valves has been proven in systems such as dissolved air flotation working under the same concept.

The dissolved hydrogen concentration in the effluent was constant at $0.1 \pm 0.03 \text{ mg l}^{-1}$. Complete denitrification was achieved at dissolved hydrogen concentrations as low as 0.001 mg l^{-1} . Controlling the feed level in the saturator was the key to the continuous delivery of hydrogen. In this study, the feed level was controlled manually by removing the increased level every three days. This can be automated by simply using a level sensor and an extra valve.

4.6.3.2 Biomass concentration

At steady-state conditions the average concentration of suspended solids was $1127 \pm 235 \text{ mg l}^{-1}$ for VSS and $1645 \pm 327 \text{ mg l}^{-1}$ for TSS. The inert fraction of total suspended solids was attributed to two sources of particulate inerts in the feed and precipitated inorganics, such as the combination of Ca^{2+} and Mg^{+2} with carbonate or hydroxide (OH^-) due to increase in pH during biological denitrification. The reduction of total dissolved solids is another indicator of precipitation in the reactor (results will be presented in the following sections).

Figure 4. 10 Suspended solids concentration at steady-state condition



4.6.3.3 Organic carbon mass balance

Under steady state conditions the organic carbon mass balance can be written as:

$$0 = \text{COD (In)} - \text{COD (Out)} + \text{COD (generation)} - \text{COD (degradation)} \quad (2.2)$$

The feed contained $39 \pm 9 \text{ mg l}^{-1}$ soluble organic carbon. Since the feed water has undergone secondary treatment, the remaining organic carbon can be attributed to the non-biodegradable fraction of raw wastewater and the slowly biodegradable soluble COD produced during substrate utilization. In order to assess the fate of organic carbon, the biodegradability kinetics of organic carbon is required.

The degradation of organic carbon requires heterotrophic activity. Although the condition in the reactor is favourable to autotrophic growth, the degradation of SMP in the reactor is possible. It has been found that all of hydrogen-dependent denitrifiers are

mixotrophic as they are able to use inorganic carbon under autotrophic and organic carbon under heterotrophic conditions (Szekeres et al., 2002). Soluble microbial products (SMP) are slowly biodegradable and kinetics of their degradation is well understood under aerobic condition (Lu et al., 2002; Henze et al., 1987; Rittmann and McCarty, 2001). SMP were shown to have high saturation coefficients and low utilization rates, and therefore require long hydraulic retention times to degrade. When nitrate is the electron acceptor, even lower degradation rates can be expected. In this study it was assumed that there is not degradation of SMP within three hours hydraulic retention time. To confirm this assumption, the wasted biomass from the reactor was transferred to a batch reactor and spiked with nitrate. No hydrogen was provided and nitrogen gas was bubbled into the reactor to provide anoxic condition. As SMP were the only electron donor, nitrate consumption rate represents the biodegradability of SMP. Negligible amount of nitrate was consumed during the 3 hr batch test, which validates the assumption of non-biodegradable SMP. As shown in Figure 4. 11, the organic carbon content of the effluent ($17 \pm 4 \text{ mg l}^{-1}$ as COD) was consistently lower than that of the influent feed. The mass balance for COD is presented in Table 4. 2. The organic carbon removal was mostly achieved by membrane rejection. The organic carbon which passed the membrane, could either originate from the incoming feed or from SMP produced during denitrification. A greater portion is expected to come from the feed, since in a previous study using the same membrane, 81% of SMP was rejected by the membrane (Rezania et al, 2005).

Figure 4. 11 Soluble COD concentration at steady state condition.

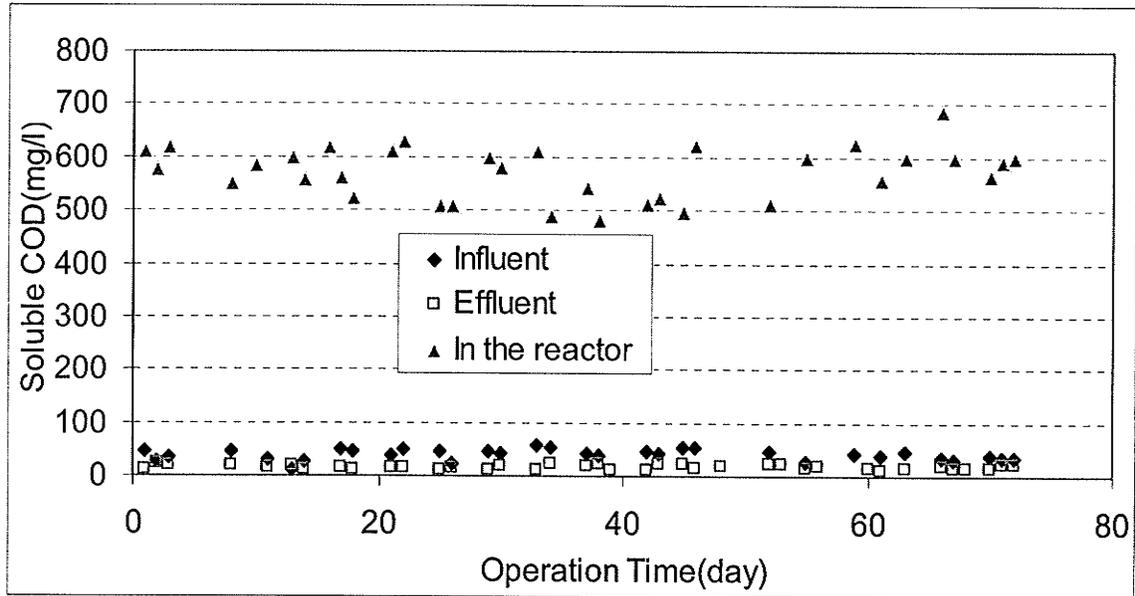


Table 4. 2 Organic carbon mass balance.

Parameter	Unit	Value
Total COD removed	%	72
Soluble COD removed	%	56
SPM produced	mg COD mg N ⁻¹	0.41

4.6.3.4 Water quality indicators

Table 4. 3 shows physical and chemical characteristics of the produced water. The produced water could meet all of the drinking water guidelines except for colour and practice level for total organic carbon. Colour and dissolved organic carbon can be reduced to the maximum contaminated limit using post-treatment with granular activated carbon.

Table 4. 3 Physical and chemical properties of produced water

Parameters	Unit	Influent	Effluent	US EPA Guidelines
Soluble COD	mg l ⁻¹	39±9	17±4	-
Total COD	mg l ⁻¹	62±14	17±4	-
Dissolved organic Carbon	mg l ⁻¹	16.5±3	9±0.3	5*
Dissolved Hydrogen	mg l ⁻¹	0	0.1±0.03	-
Nitrate	mg N l ⁻¹	33±4	0	10
Alkalinity	mg CaCO ₃ l ⁻¹	237±37	308±48	-
pH	-	7.8±0.4	9.3±0.14	6.5-8.5
Turbidity	Ntu	20±5	0.16±0.09	2
True Colour	Hu	30	25±5	15
Hardness	mg l ⁻¹	324±65	170±10	
Total Dissolved Solids	mg l ⁻¹	580±100	76±20	500
Total Coliform	CFU/100ml	2x10 ⁶	ND	ND
Iron	mg l ⁻¹			0.6
Copper	mg l ⁻¹		0.1	0.1
Zinc	mg l ⁻¹		0.035	0.09

ND None detectable

* Level of practice

4.6.4 Conclusions

The concept of introducing gas supersaturated feed to a gas consuming reactor can be used for efficient gas delivery. However, the liquid level in the saturator needs to be kept constant by using a controller, which assures continuous gas flow from the gas tank to the saturator tank. The hydrogen delivery system was efficient, achieving almost 100%

hydrogen transfer rate. The transfer of hydrogen to the membrane bioreactor effectively stimulated the growth of hydrogen-dependent denitrifiers, and complete nitrogen removal was achieved. The nitrogen gas produced during denitrification was sufficient for membrane scouring and reactor mixing. Both membrane filters were successful in separating the biomass produced during denitrification from the treated water. The produced water met drinking water guidelines in terms of total coliforms, nitrate, hardness and turbidity. Colour and dissolved organic carbon did not meet USEPA Guidelines for potable water and would thus require post treatment if direct water reuse was intended. The designed system showed good potential for wastewater reuse applications.

CHAPTER 5: ENGINEERING SIGNIFICANCE

This chapter focuses on evaluating hydrogenotrophic denitrification as an alternative for upgrading North End Wastewater Treatment Plant to BNR plant. The particular reactor configuration considered for upgrade is a membrane bioreactor coupled with a hydrogen delivery unit, which is described in Section 4.5.2.

5.1 Current wastewater treatment in the City of Winnipeg, and the need for denitrification

The North End Water Pollution Control Centre (NEWPCC) in Winnipeg, Manitoba, currently treats about 70% of Winnipeg's wastewater. It services most of the old City of Winnipeg, part of St. Boniface, all of East, West, North and Old Kildonan, Transcona and part of St. James. The rest of the city is serviced by the West End Water Pollution Control Centre (WEWPCC) in Charleswood and the South End Water Pollution Control Centre (SEWPCC) in St. Vital. The NEWPCC treats the sludge produced from the SEWPCC and the WEWPCC as well as NEWPCC with a centralized sludge treatment system. The design parameters and the actual flow for the current NEWPCC as well as the target effluent quality are summarized in Table 5. 1 and Table 5. 2, respectively.

Table 5. 1 NEWPCC design and actual parameters

Parameter	Description
Design population	395,000
Population served	374,000
Design ADWF	302 ML/d
Actual ADWF	160 ML/d

Notes: ADWF = Average Dry Weather (winter) Flow
*Source: The City of Winnipeg, NEWPCC fact sheet

Table 5. 2. Current effluent quality and anticipated effluent requirements at the NEWPCC

Parameter	Current effluent quality	Target effluent quality
BOD ₅ , mg l ⁻¹	26	10
TN, mg l ⁻¹	36	10
TP, mg l ⁻¹	4.2	1
TSS, mg l ⁻¹	18	15

The current operating permit for the City's wastewater treatment facilities specifies treated effluent BOD₅ and TSS limits of 30 mg l⁻¹ each. Although the effluent from the current plant meets the City's limit, the plant is expected to meet more stringent future effluent limits including total nitrogen (TN) and total phosphorus (TP) as presented in Table 5. 2 Accordingly, the plant needs to be upgraded from the current BOD and TSS removal system to a full biological nutrients removal (BNR) system.

5.2. Objectives

Two different design alternatives for upgrading the current BOD removal plant to full BNR plant are proposed. The main goals are:

- To design full BNR system alternatives for the NEWPCC with an aid of the BioWin 2.1 program
- To evaluate the best option for retrofitting the NEWPCC based on the cost and produced water quality

5.3 Analysis of the current system

The NEWPCC provides screening, grit removal, primary sedimentation and activated sludge secondary treatment prior to discharging the treated effluent to the Red River. Side-stream processes include anaerobic digestion of combined thickened primary and waste activated sludges and power generation from the digester gas.

The influent wastewater first passes through bar screens with 12 mm openings, and the flow then enters four aerated grit chambers. After passing through the grit chambers, the wastewater goes onto the three circular primary clarifiers. Following the primary treatment, the influent wastewater flows into six high purity oxygen (HPO) reactor tanks arranged into three trains for secondary treatment. After the treatment at the HPO reactors, the waste stream flows to a series of rectangular and circular secondary clarifiers.

Primary sludge from the primary clarifiers and waste activated sludge (WAS) from the secondary clarifiers are collected and then pumped to the mesophilic anaerobic digesters. Sludge gas produced from the anaerobic digesters is stored in the gas storage sphere and used as fuel in boilers to heat the treatment plant as well as the sludge in the digesters. Digested sludge is pumped from the sludge holding tanks to centrifuges in the dewatering building. Currently, only three out of the six available centrifuges are being used. Before the digested sludge enters the centrifuges, a polymer is added to aid in the separation of liquids and solids. The centrate, which has a high concentration of ammonia (approximately 800 – 900 mg l⁻¹) is returned to the main interceptor to enter the plant for treatment. The dewatered biosolids (sludge cake) is pumped through the biosolids cake line to the biosolids cake storage bins. The biosolids cake is temporarily stored in these covered bins until it is loaded onto trucks inside the dewatering building. It is then taken to agricultural land where it is applied as fertilizer all year round.

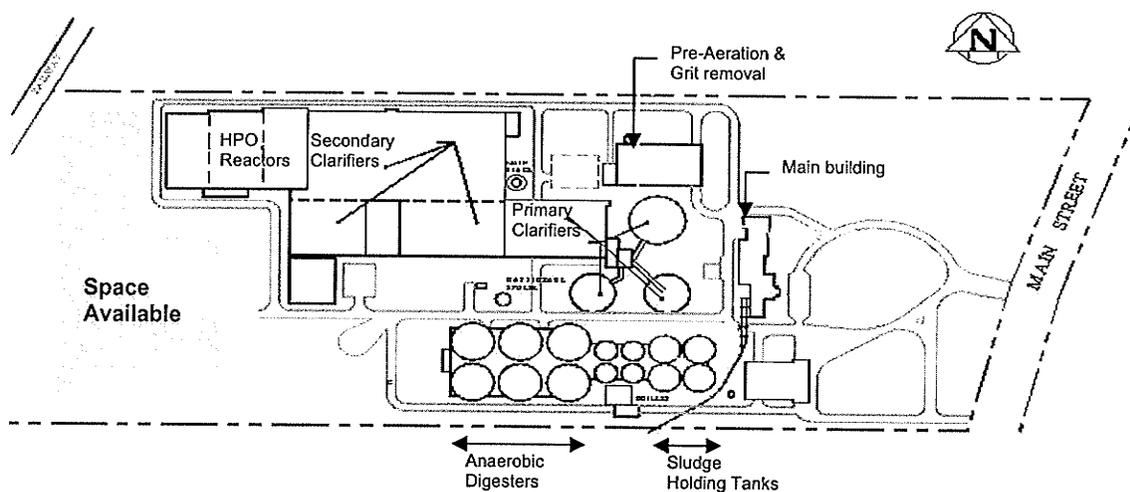
The configuration of the overall current plant is summarized in Table 5.3.

Table 5. 3 Configuration of the unit processes at NEWPCC

Unit	Capacity
(4) Bar screens	
(4) Grit chambers	1925 m ³
(3) Circular primary clarifiers	24300 m ³
(6) Oxygen reactors	31200 m ³
(26) Secondary clarifiers - (10) circular clarifiers - (16) rectangular clarifiers	41275 m ³
(6) Anaerobic digesters	44800 m ³
(4) Gas storage tank	15400 m ³
(6) Centrifuges for sludge dewatering	19-22L/s/unit
Biosolids cake storage bins	495 tonnes of sludge

Most of the facilities at the NEWPCC are located west of the main building, and the south west of the current plant will be considered to be available for the new construction as illustrated in Figure 5.1.

Figure 5.1 NorthEnd Wastewater Treatment Plant site plan



5.4 Upgrade design approaches

Based on the problem analysis of the current system, two different design approaches will be applied to upgrade the current BOD plant to a full BNR plant. Basically, the goal is to retrofit the current plant to a BNR plant to produce reusable water.

The innovative approach taken to implement BNR at the NEWPCC is to use a membrane bioreactor (MBR). MBR is a process that combines activated sludge process and membrane separation. MBR process was selected because:

- It eliminates secondary clarifiers.
- Depending on operational parameters the need for sludge thickening might be eliminated.
- The membranes produce high effluent quality by membrane without disinfection
- It is easy to fit into existing process.
- It has a small footprint.

The membrane bioreactors need to be chosen based on wastewater characteristics. Winnipeg's wastewater lacks enough organic carbon to achieve complete denitrification. Therefore an exogenous electron donor needs to be added to achieve complete denitrification. Two different membrane bioreactor designs proposed for the upgrade. The designs differ based on the type of electron donor used (methanol or hydrogen), and place of application (pre-denitrification and post-denitrification).

5.5 Upgrade approach 1: Pre-denitrification in a BNR-MBR using methanol as exogenous electron donor

The process flow diagram of the proposed design is shown in Figure 5. 2. The system is a modified A2/O process, which follows simultaneous nitrification-denitrification (SND)

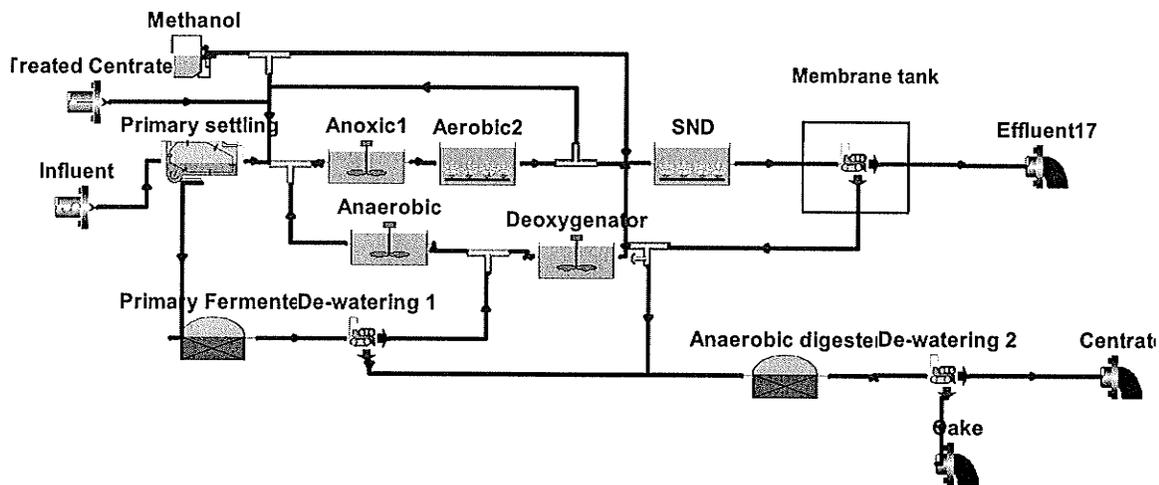
membrane cell retention. The suggested membrane modules are ZeeWeed® ZW-500 membranes with nominal pore size of 0.04µm. 360 membrane cassettes are required for this system according to the estimations by ZENON. These cassettes will be installed in 20 trains (with room for 440 cassettes as a safety factor), and the dimensions of a single cassette are 1.74m (L) x 2.12m (W) x 2.56m (D). Each train will house 18 cassettes and the dimensions of each train are 3.05m (L) x 44.81m (W) x 3.65m (D).

The upgraded treatment plant provides screening, grit removal, primary sedimentation and activated sludge secondary treatment prior to membrane filtration and discharging the effluent to the Red River. The activated sludge used for nutrient removal incorporates both denitrification and phosphorus removal.

The nitrification takes place in the aerobic reactor and partially in the SND reactor. The nitrate produced during nitrification is removed in three different reactors, Anoxic1, deoxygenator and SND reactor. The organic carbon required for denitrification is provided by exogenous methanol and biodegradable COD produced from fermentation of primary sludge. The phosphorus release, takes place in both reactors in RAS line. The first reactor (deoxygenator) removes both oxygen and nitrate from the RAS and allows the maximum phosphorus release in the following anaerobic reactor. The Anaerobic reactor in the RAS provides the optimum condition for phosphorus release by providing the volatile fatty acids (VFA) produced in the fermenter to the phosphorus accumulating bacteria. The aerobic phosphorus uptake, takes place in Aerobic 2 and SND. The SND reactor enhances both nitrogen removal and phosphorus removal as phosphorus uptake can still occur at dissolved oxygen concentrations as low as 0.4 mg l⁻¹.

Side-stream processes include fermentation of primary sludge, anaerobic digestion of combined thickened primary and waste activated sludge and separate centrate treatment. Separate centrate treatment reduces the ammonia loading from the centrate to the main stream by 8%. The primary fermentation of primary sludge was implied to produce volatile fatty acids, which enhances denitrification and phosphorus removal.

Figure 5. 2 Schematic of MBR pre-denitrification plant using methanol as exogenous electron donor



The design and operational parameters of each unit is summarized in Table 5.4. In addition, the steady-state concentrations of nutrients in each unit are provided in Appendix 1 as well. For instance, the change in nitrate concentration in each reactor can be followed through Appendix 1.

Table 5. 4 Operating parameters for BNR-MBR process using methanol for denitrification

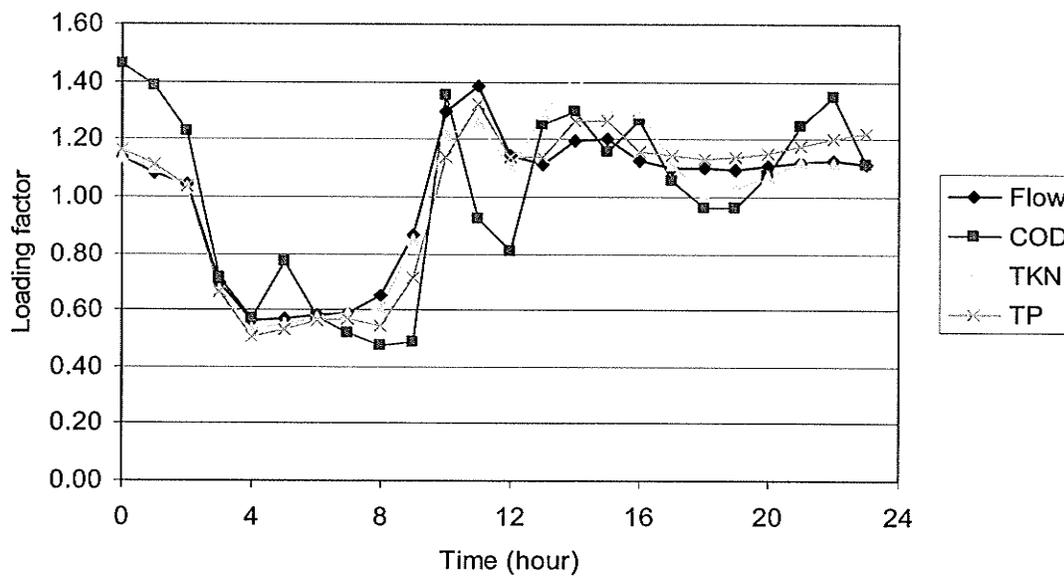
Unit	Parameter	Value (unit)
Primary clarifier	Volume	24.8 ML/d
	SOR	52.5 m ³ /m ² d
	HRT	1.82 hr
Anoxic 1	Volume	14 ML
	HRT	0.3 hr
	Temp	12 °C
Aerobic 2	Volume	40 ML
	HRT	0.9 hr
	Temp	12 °C
SND	Volume	24 ML
	HRT	1.1 hr
	Temp	12 °C
Methanol	Flow	47520 kg d ⁻¹
Deoxygenator	Volume	2 ML
	HRT	0.2 Hr
	Temp	12 °C
Anaerobic	Volume	3 ML
	HRT	0.3 hr
	Temp	12 °C
Membrane unit	Flow	465.34 ML/d
	Volume	10 ML
	Temp	12 °C
Fermenter	Volume	0.8 ML
	HRT	0.6 day
	Temp	15 °C
Anaerobic digester	Volume	45 ML
	SRT	20.5 day
	Temp	35 °C
Centrate bioreactor	Volume	4.5 ML
	HRT	1.29 day
	SRT	40 day
	Temp	30 °C
	DO	3 mg l ⁻¹

5.5.1 Dynamic simulation

5.5.1.1 Developing wastewater flow pattern and loads

A flow and load pattern was developed for the BioWin 2.1 modeling that will be used for the simulation of the BNR alternatives. The flow and load patterns were developed for the whole year using the hourly peak factors (Figure 5. 3) and projected seasonal flows and loads (Table 5.5). The hourly variation in loads was applied to the projected primary effluent flows and loads to obtain the predicted flows and contaminant concentrations for the year 2041.

Figure 5. 3 Loading factors for flow, COD, TKN and TP for average one day



Projected diurnal primary effluent flows and concentrations for the whole year are shown in

Figure 5. 4 to Figure 5. 7. All the figures were plotted at hourly intervals to obtain diurnal flows and concentrations during one year. The flow and loads are the anticipated values for the year 2041

Table 5. 5 Projected flows and loads to secondary treatment at the NEWPCC for the year 2041

Periods	Flow condition	Flow	TSS	BOD	COD	TKN	TP
		(ML/d)	(Kg d ⁻¹)				
Winter	Average	211	22,809	34,414	68,828	8,862	1,245
	Maximum month	237	27,682	41,475	82,950	10,144	1,398
	Maximum week	250	32,335	47,053	94,106	11,373	1,650
	Maximum day	260	42,036	61,169	122,338	14,785	2,145
Spring	Average	390	34,905	33,969	67,938	9,672	1,443
	Maximum month	571	59,670	46,023	92,045	11,249	1,827
	Maximum week	705	89,107	58,968	117,936	13,322	2,228
	Maximum day	710	115,839	76,658	153,317	17,319	2,896
Summer	Average	291	27674	30380	60761	8352	1251
	Maximum month	381	40424	37338	74676	9639	1524
	Maximum week	449	52800	53856	107712	13376	2042
	Maximum day	686	68640	70013	140026	17389	2654
Fall	Average	250	21100	30975	61950	8425	1125
	Maximum month	312	29640	36941	73882	9890	1373
	Maximum week	364	34248	43003	86005	10300	1828
	Maximum day	526	44522	55903	111807	13390	2377

Figure 5. 4 NEWPCC 2041 diurnal primary effluent flow for one year

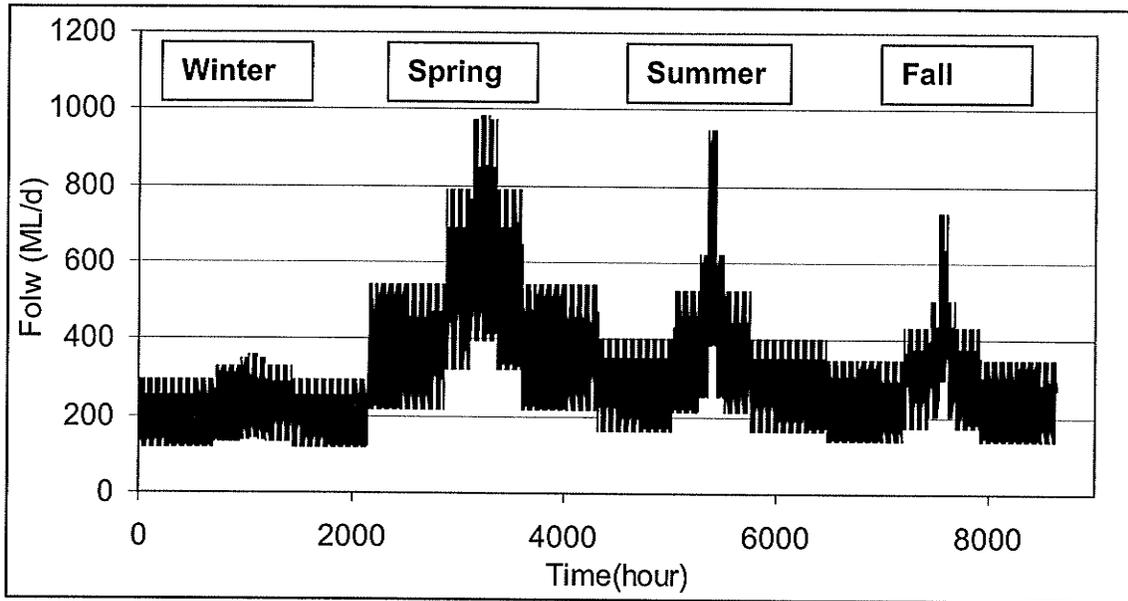


Figure 5. 5 NEWPCC 2041 primary effluent COD projections for one year

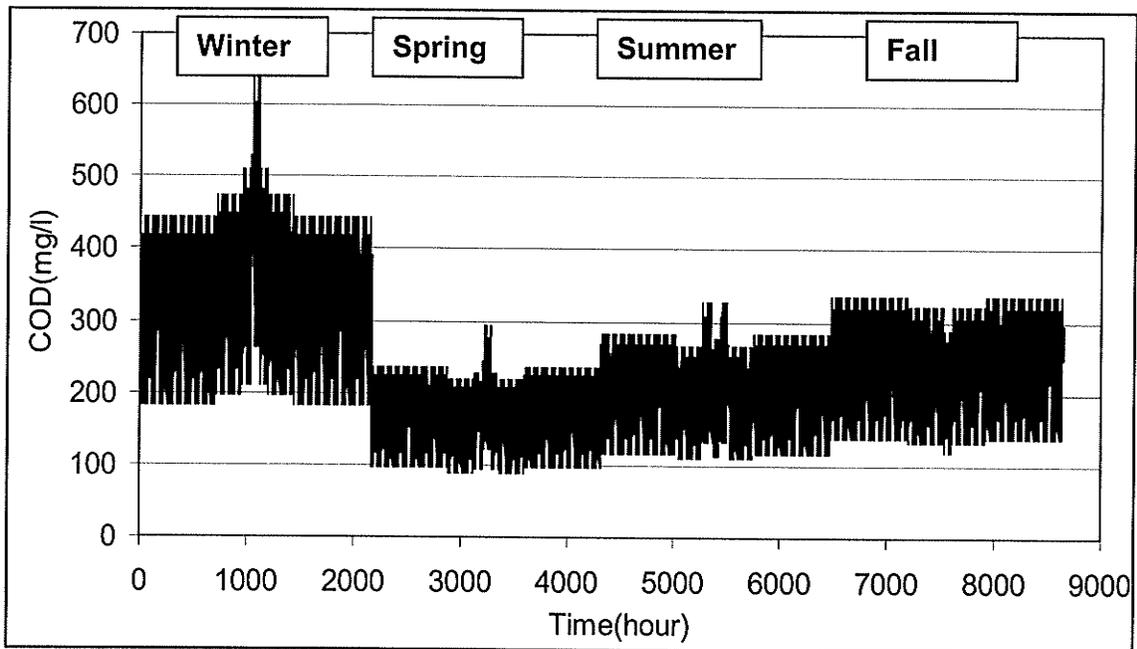


Figure 5. 6 NEWPCC 2041 primary effluent TKN projections for one year

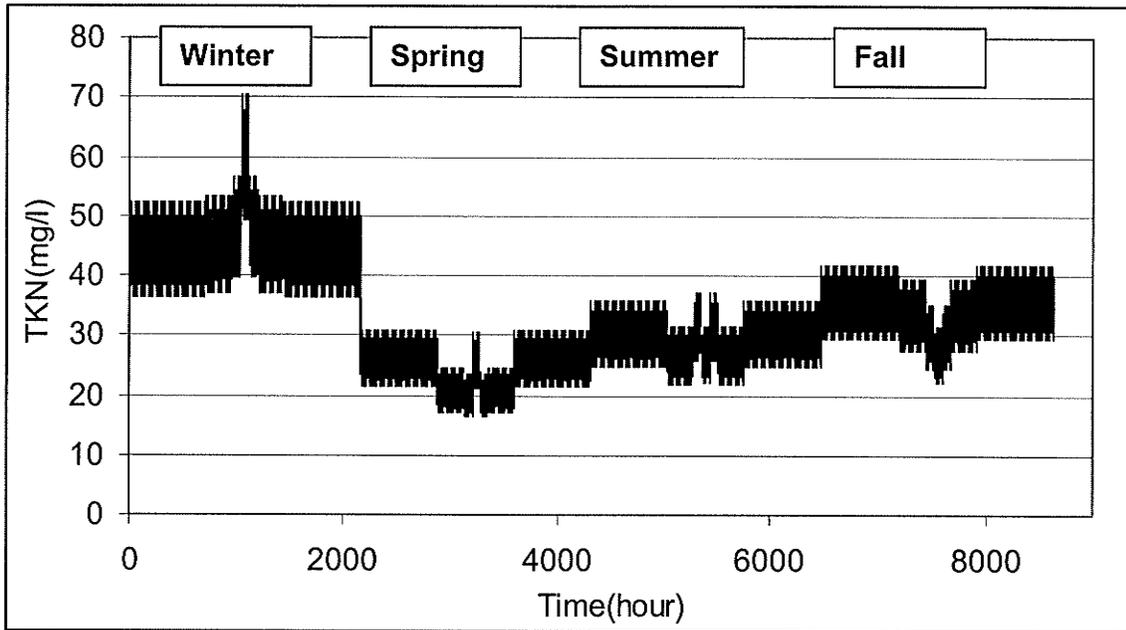
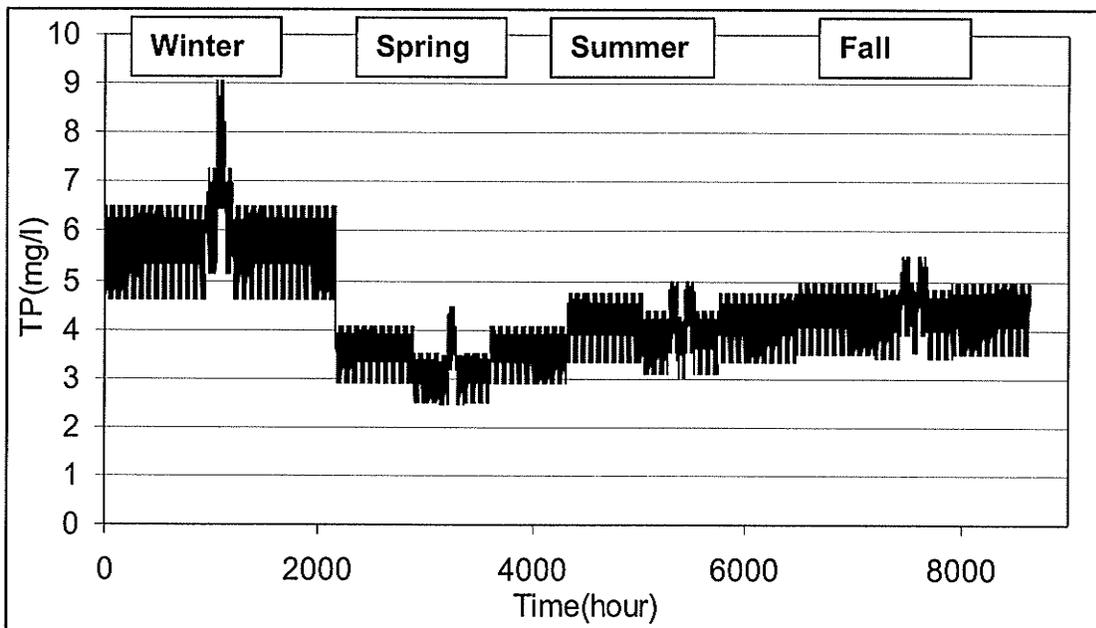


Figure 5. 7 NEWPCC 2041 primary effluent TP projections for one year



5.1.2 Dynamic simulation results

The projected flows and loadings were used to simulate the performance of the BNR-MBR plant using methanol for denitrification for the whole year. The performance of the plant is presented in the following Figures 5.8 to 5.10.

Figure 5. 8 The system performance in COD removal

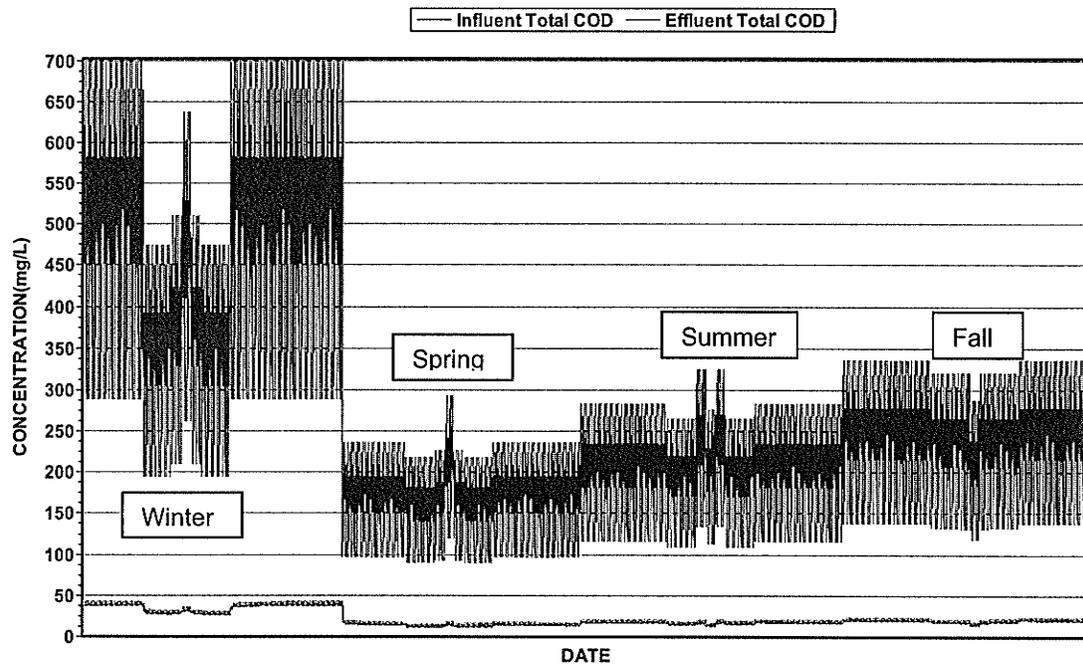


Figure 5. 9 The system performance in nitrogen removal

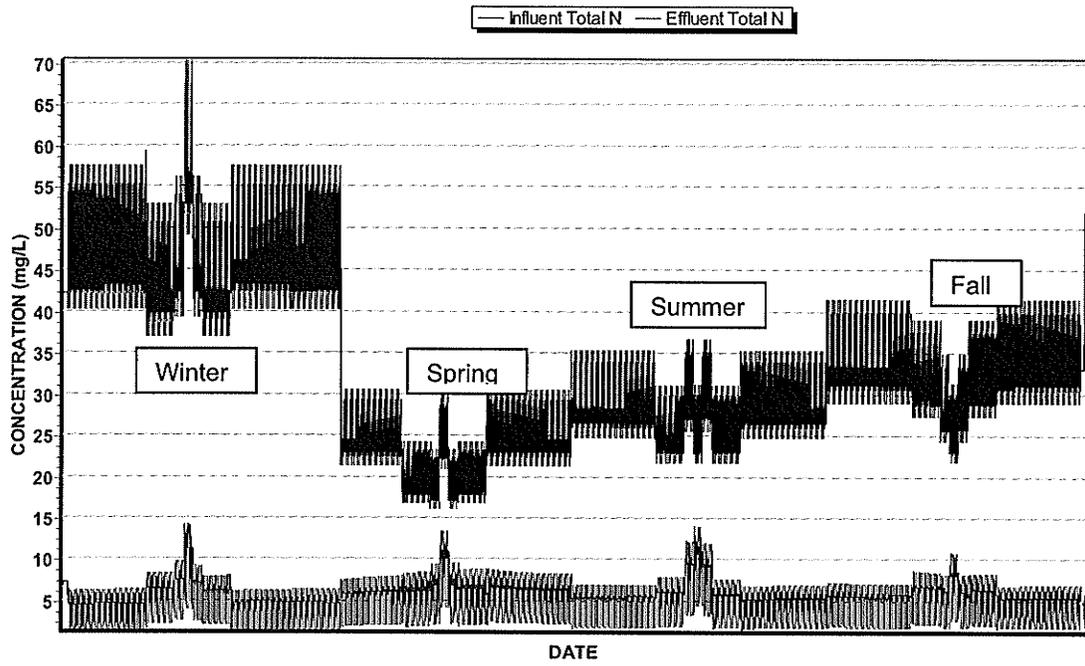
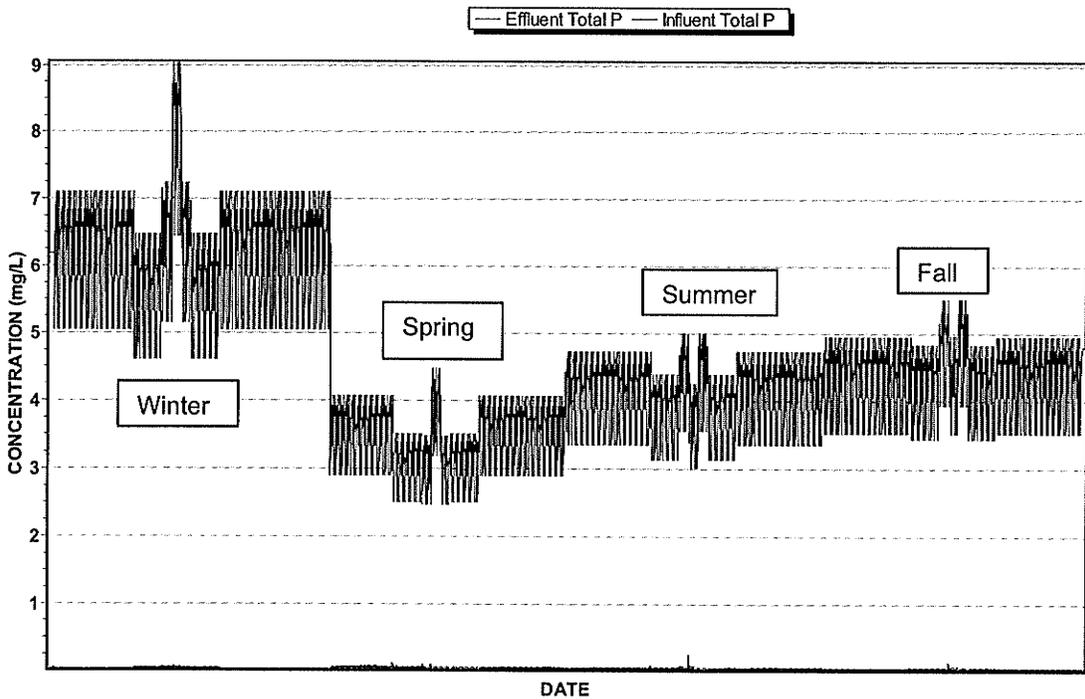


Figure 5. 10 The system performance in phosphorus removal



As shown in the Figures the designed system was able to achieve the discharge limits for the whole year. The BOD in the effluent was less than 3 mg l^{-1} for the whole year. The designed system was successful in phosphorus and nitrogen removal.

5.5.2 Steady state simulation

The steady state simulation results are presented in Table 5. 6. The achieved effluent quality meets the effluent discharge guidelines.

Table 5. 6 The anticipated effluent quality at steady-state conditions

Parameters	Conc. (mg l^{-1})	
	Influent	Effluent
Volatile suspended solids	83.89	0
Total suspended solids	98.89	0
Filtered COD	91.28	17.9
Total COD	225.49	17.9
Soluble PO ₄ -P	2.17	0.05
Total P	4.34	0.05
Filtered TKN	25.03	1.19
Particulate TKN	4.72	0
Total Kjeldahl Nitrogen	29.75	1.19
Filtered Carbonaceous BOD	56.51	0.31
Total Carbonaceous BOD	111.96	0.31
Total N	29.75	5.27
Total inorganic N	22.31	4.4
Alkalinity	200	195.59
pH	7.3	8.36
Ammonia N	22.31	0.33
Nitrate N	0	4.07

The dynamic concentration of the parameters in each unit is presented in Appendix 1.

5.6 Upgrade approach 2: Post-denitrification in a membrane bioreactor using hydrogen as exogenous electron donor

The process flow diagram of the second alternative before implementing denitrification is shown in Figure 5. 11. As a denitrification option the anaerobic submerged membrane bioreactor is used as a polishing step for both denitrification and filtration of

the final effluent. The process flow diagram of the denitrification system is also shown in Figure 4.3. In the polishing step, the final effluent is divided into two portions. A portion is directed to the saturator tank, where it is supersaturated with hydrogen and the other portion is introduced to the membrane bioreactor. The supersaturated portion of final effluent with hydrogen and the remaining portion are finally mixed in the bioreactor to stimulate autotrophic denitrification.

Figure 5. 11 Process flow diagram of upgrade design using hydrogen for post-denitrification of final effluent

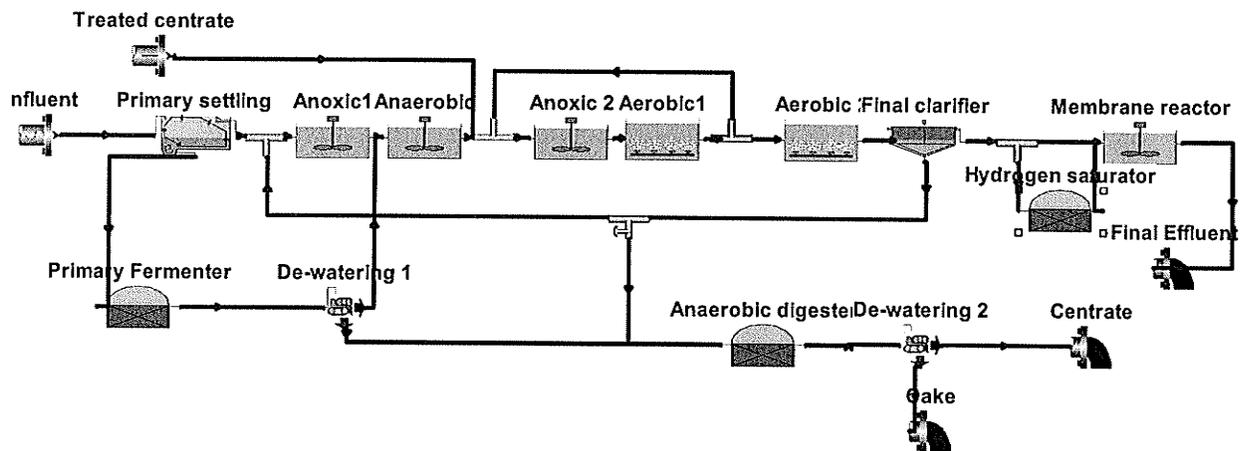


Table 3. 4 Operating parameters for plant before the pos-denitrification

Unit	Parameter	Value (unit)
Primary clarifier	Volume	24.8 ML/d
	SOR	52.5 m ³ /m ² d
	HRT	1.82 hr
Anoxic 1	Volume	14 ML
	HRT	0.4hr
	Temp	12 °C
Anaerobic	Volume	19 ML
	HRT	0.9 hr
	Temp	12 °C
Anoxic 2	Volume	14 ML
	HRT	0.3 Hr
	Temp	12 °C
Aerobic 1	Volume	40 ML
	HRT	0.9 hr
	Temp	12 °C
Aerobic 2	Volume	24 ML
	HRT	1.1 hr
	Temp	12 °C
Fermenter	Volume	0.8 ML
	HRT	0.6 day
	Temp	15 °C
Anaerobic digester	Volume	45 ML
	SRT	20.5 day
	Temp	35 °C
Centrate bioreactor	Volume	4.5 ML
	HRT	1.29 day
	SRT	40 day
	Temp	30 °C
Final clarifier	Volume	60 ML
	SOR	21.8 m ³ /m ² .day
	SLR	94.2 kg/m ² .day

5.6.1 Steady state simulation

Due to limitation of BioWin 2.1 in incorporating hydrogenotrophic denitrification, the quality of denitrified final effluent was anticipated based on laboratory experiments (see chapter 4). First the proposed design before denitrification (Figure 5. 11) was simulated using BioWin 2.1 software and the steady state quality of final effluent was obtained. The simulated effluent quality was used as the feed to the proposed denitrification system. And the quality of final denitrified effluent was anticipated based on the data obtained through laboratory experiment.

Table 5.7 Estimated effluent quality before and after denitrification using hydrogen as external electron donor

Parameters	Conc. (mg l ⁻¹)		
	Influent	Effluent (before denitrification)	Effluent after polishing
Volatile suspended solids	83.89	6.71	0
Total suspended solids	98.89	8.7	0
Filtered COD	91.28	13.48	4.31
Total COD	225.49	23.29	4.31
Soluble PO ₄ -P	2.17	1.9	<1.9
Total P	4.34	2.61	<2.61
Filtered TKN	25.03	1.4	<1.4
Particulate TKN	4.72	0.67	<0.67
Total Kjeldahl Nitrogen	29.75	2.06	<2.06
Filtered Carbonaceous BOD	56.51	0.35	-
Total Carbonaceous BOD	111.96	3.1	-
Total N	29.75	13.73	<2.06
Total inorganic N	22.31	12.19	-
Alkalinity	200	196.59	-
pH	7.3	8.3	-
Ammonia N	22.31	0.52	-
Nitrate N	0	11.67	0

Nitrate, total and soluble COD were the quality indicator parameters, which were estimated using the data obtained from laboratory experiment.

5.7 Evaluating the alternatives

The two design alternatives were compared in terms of effluent quality and cost. The cost analysis included capital cost and operation and maintenance cost. The details of cost analysis are presented in Appendix 2.

The cost and effluent quality comparison are summarized in Table 5.8 and Table 5.9. In terms of produced effluent quality the MBR using hydrogen was superior to the option involving methanol. The capital cost of the MBR with hydrogen was higher than the

MBR with methanol. However the operating cost of MBR with hydrogen was lower. The difference mostly comes from the different cost of methanol and hydrogen. The cost of chemical addition with methanol and hydrogen are calculated to be 0.5-2 C\$ /kg N for hydrogen and 0.97-5 C\$/kg N for methanol. This is in the case that hydrogen is produced on site and bulk methanol is purchased. The operating cost of hydrogen is directly proportional to the cost of electricity. The operating cost in an industrial plant is 4.5-5 kw.h/m³ of hydrogen. Therefore the cost of denitrification varies at different locations. Low cost of electricity in Manitoba allows lower cost of denitrification in compared to the other provinces. The stoichiometric cost of denitrification with methanol based of the methanol cost obtained form Tampa Wastewater Plant is 0.97 C\$/ kgN. However, the actual methanol consumption in the plant can be higher depending on the place of application up to 5 C\$/ kgN. For instance if the methanol is added to the first anoxic zone in the plant a large portion of methanol is consumed in the aerobic zone due to rate of internal recycle. The minimum annual cost of methanol and hydrogen addition was calculated to be 1.7 and 3.4 million C\$/Year respectively. In order to compare the cost of two alternatives the present worth value of two alternatives was calculated using 8% as interest rate for the design period of 40 years.

Table 5. 8 Steady state effluent quality comparison of each alternative

Parameter	MBR with methanol	An. MBR with hydrogen
TCOD mg l ⁻¹	17.9	4.3
TN, mg l ⁻¹	5.2	2.06
TP, mg l ⁻¹	0.05	1
Nitrate, mg l ⁻¹	4	0

Table 5.9 Cost comparison of each alternative

Cost	Methanol	Hydrogen	Unit
Operating(excluding chemical cost)	5.8	6	million C\$/Year
Capital	108	159	million C\$
Chemical addition	0.97-5	0.5 -2	C\$/Kg N
Minimum chemical cost	3.4	1.7	million C\$/Year
Maximum chemical cost	17. 7	7	million C\$/Year
Present worth based on minimum chemical cost	148.5	179.2	million C\$
Present worth based on maximum chemical cost	319	241.4	million C\$

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

Hydrogen-driven denitrification seems to be a promising technology for removing nitrate from water and wastewater containing low degree of organic carbon. It was found that hydrogenotrophic denitrifiers can be easily grown using waste activated sludge as seed, hydrogen as a substrate, and inorganic carbon as carbon source. The kinetics and microbial ecology of steady-state hydrogenotrophic denitrifiers were studied. It was confirmed that hydrogenotrophic denitrifiers have the ability to use both organic and inorganic carbon, as during starvation the consumption of soluble organic carbon was observed. It was proven that conversion of nitrate to nitrogen gas is carried out under autotrophic conditions by the same group of bacteria. This is in contrast to previous studies, proposing that there are two different groups of bacteria involved in hydrogenotrophic denitrification, autotrophic nitrate reducers and heterotrophic nitrite reducers.

The kinetics of nitrate and nitrite reduction was studied at two different temperature of 12 °C and 25 °C. A zero order kinetic model was proposed for nitrate and nitrite reduction and found to be highly correlated with experimental result. The zero order kinetic model also confirm very low hydrogen and nitrate half saturation coefficients. The rate of both nitrate and nitrite reduction increased with pH at both temperatures. The optimum pH for nitrate and nitrite reduction was found to be 9.5 at 25°C and 8.5 and 12 °C. Although, denitrification rates were slower than the values reported for heterotrophic denitrification with methanol but they were comparable.

The kinetics study also led to the development of a novel approach for studying microbial cultures. The developed method was mainly focused on addressing the

limitation of volatile suspended solids measurement as a representative for active bacteria. Volatile suspended solids represent the summation of viable bacteria, cells debris and extra cellular polymeric substances. The developed method was applied to the process of hydrogenotrophic denitrification. The model was based on nitrate uptake rate under starvation conditions. During starvation, once the hydrogen is eliminated from the feed, EPS is hydrolyzed and used as a substrate by heterotrophic fraction of biomass, resulting in high nitrogen (nitrate) uptake rate at the start-up of the experiment. Once the EPS is consumed, the biodegradable fraction of biomass serves as the electron donor, resulting in lower and steady nitrogen uptake rates. During endogenous respiration, not only particulate cell debris is produced but also non- biodegradable soluble COD is released to the reactor. It was found that steady-state biomass obtained from the reactor operating under SRT of 20 d and loading of $0.081 \text{ g NO}_3\text{-N d}^{-1} \text{ l}^{-1}$ contained 41% active biomass, 25.6% cell debris and 33.4% EPS. The decay coefficient of 0.041 d^{-1} and true yield of 0.28 mg active biomass per mg $\text{NO}_3\text{-N}$ removed was obtained.

From engineering prospective, the design should address effective hydrogen delivery, biomass retention and cost. An anaerobic submerged membrane bioreactor was designed as the biological treatment unit and was operated in modes of batch and continuous feed. Two different methods were used for hydrogen delivery. These included bubbles-less hydrogen dissolution using porous membrane gas diffusers and hydrogen supersaturated feed. Both nitrate contaminated groundwater and wastewater effluents were used for testing.

For nitrate removal from groundwater, a hydrogenotrophic denitrification system, which consisted of a sequencing batch membrane bioreactor, was evaluated for simultaneous removal of nitrate and soluble microbial products (SMP). The hydrogen gas was delivered to the membrane bioreactor using porous membrane diffusers. 100% nitrate removal efficiency was achieved at nitrate loadings of $0.328 \text{ kg N m}^{-3} \text{ d}^{-1}$. Denitrification rates varied at different hydrogen pressure to the diffuser, ranging from 17 to 20 $\text{mg NO}_3\text{-N gVSS}^{-1} \text{ h}^{-1}$ at hydrogen pressures between 0.28-0.55 atm. Compared to reactor configurations in other research studies, higher denitrification rates were obtained with this system at the low operating temperature of 10-12 °C. During the aerobic period, 81% of SMP produced within the anoxic phase, was rejected by the membrane, 9% was biodegraded, and 5% was passed through the membrane. Precipitation of solids was observed, and at the SRT of 20 days, the average TSS and VSS concentrations were 2322.5 mg l^{-1} and 1162.6 mg l^{-1} , respectively. The precipitation of inorganics affected both the membrane gas diffuser and the membrane filter. Inorganic fouling was found to be due to the precipitation of calcium and phosphate ions. The chemical composition of the primary foulant was determined to be $\beta\text{Ca}_3(\text{PO}_4)_2$. Inorganic fouling in membrane filters can be easily controlled by acid cleaning of the membrane at the time of chemical backwash. The failure of the microporous membrane diffuser was attributed to the condensation of water in the fibres, which allowed precipitation of inorganics inside the fibre. It can be concluded that micro-porous membrane gas diffusers are not suitable for hydrogen delivery into the bioreactor. Instead, composite or dense membranes can be used for gas delivery. Hydrogen driven denitrification systems are vulnerable to precipitation of calcium or magnesium with phosphate or carbonate at high pH. This

might be controlled by using the mixture of carbon dioxide and hydrogen instead of pure hydrogen in the gas feed.

To address the problems associated with using membrane gas diffusers, a novel hydrogen delivery was designed and coupled with an anaerobic submerged MBR to produce reusable water from nitrate contaminated final effluent. Hydrogen delivery was based on supersaturating some portion of the feed with hydrogen in a high pressure vessel (saturator) and delivering the hydrogen supersaturated feed to the reactor. It was found that the concept of introducing hydrogen through super-saturation can be used for efficient gas delivery. The liquid level in the saturator needs to be maintained constant by using a level controller, which would assure continuous gas flow from the gas tank to the saturator tank. The hydrogen delivery system was efficient in achieving almost 100% hydrogen transfer rate. The transfer of hydrogen to the membrane bioreactor effectively stimulated the growth of hydrogen-dependent denitrifiers, and complete nitrogen removal was achieved at a loading rate of $0.14 \text{ kgN m}^{-3}\text{d}^{-1}$. The nitrogen gas produced during denitrification was sufficient to be recycled for membrane scouring and reactor mixing. Both membrane filters were successful in separating the biomass produced during denitrification from the treated water. In addition the membranes were effective in rejecting 52% soluble COD. The produced water met drinking water guidelines in terms of total coliforms, nitrate, hardness and turbidity. Color and dissolved organic carbon did not meet USEPA Guidelines for potable water and would thus require post treatment if water direct reuse was intended. The designed system showed good potential for wastewater reuse applications. However, the potential cost of implementing the designed system into full scale needed to be addressed. As a

potential application, the developed system (MBR coupled with hydrogen delivery unit) was chosen as one of the alternatives to retrofit Winnipeg's North End Wastewater Treatment Plant to a full BNR plant. The other alternative was chosen to be pre-anoxic MBR using methanol for denitrification. The alternatives were compared based on produced effluent quality and capital and operating cost. The BioWin simulation results in combination with the results from lab testing suggests better produced effluent quality in terms of total COD and total nitrogen when hydrogen was used as external electron donor for denitrification. The capital cost of hydrogenotrophic denitrification was higher than heterotrophic denitrification with methanol. The operational cost of hydrogenotrophic denitrification was almost half of denitrification with methanol in the case that hydrogen is produced on site in Manitoba. In long-term operation, hydrogenotrophic denitrification can be more favourable. Furthermore, the cost of sludge treatment is expected to be lower for hydrogenotrophic denitrification due to lower yield of hydrogenotrophic denitrifiers. Finally it can be concluded that hydrogenotrophic denitrification is an attractive option for water and wastewater denitrification wherever the cost of electricity (on-site hydrogen production) is low.

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Appendix .1 Steady-state concentrations of wastewater constituents in different units of MBR plant when methanol was used as exogenous electron donor.

Table 1 The steady-sate concentration of nutrients in different units
Influent

Parameters	Conc. (mg l ⁻¹)	Mass rate (kg d ⁻¹)
Volatile suspended solids	83.89	27443.3
Total suspended solids	98.89	32350.27
Particulate COD	134.21	43902.74
Filtered COD	91.28	29861.68
Total COD	225.49	73764.42
Soluble PO ₄ -P	2.17	709.34
Total P	4.34	1418.68
Filtered TKN	25.03	8188.71
Particulate TKN	4.72	1544.45
Total Kjeldahl Nitrogen	29.75	9733.16
Filtered Carbonaceous BOD	56.51	18486.94
Total Carbonaceous BOD	111.96	36625.43
Total N	29.75	9733.16
Total inorganic N	22.31	7299.87
Alkalinity	200	65426.21
pH	7.3	
Volatile fatty acids	6.76	2212.93
Total precipitated solids	0	0
Total inorganic suspended solids	15	4906.96
Ammonia N	22.31	7299.87
Nitrate N	0	0

Treated centrate		
Parameters	Conc. (mg l ⁻¹)	Mass rate (kg d ⁻¹)
Volatile suspended solids	0.37	0.73
Total suspended solids	0.37	0.73
Particulate COD	0.6	1.17
Filtered COD	0.4	0.8
Total COD	1	1.97
Soluble PO ₄ -P	151.23	297.92
Total P	302.46	595.85
Filtered TKN	1.46	2.87
Particulate TKN	0.21	0.42
Total Kjeldahl Nitrogen	1.67	3.29
Filtered Carbonaceous BOD	0.25	0.49
Total Carbonaceous BOD	0.5	0.98
Total N	386.13	760.68

Total inorganic N	385.71	759.85
Alkalinity	6	11.82
pH	7.3	
Volatile fatty acids	0.03	0.06
Total precipitated solids	0	0
Total inorganic suspended solids	0	0
Ammonia N	1.25	2.47
Nitrate N	384.46	757.39

Methanol		
Parameters	Conc. (mg l⁻¹)	Mass rate (kg d⁻¹)
Volatile suspended solids	0	0
Total suspended solids	0	0
Particulate COD	0	0
Filtered COD	118800	47520
Total COD	118800	47520
Soluble PO4-P	0	0
Total P	0	0
Filtered TKN	0	0
Particulate TKN	0	0
Total Kjeldahl Nitrogen	0	0
Filtered Carbonaceous BOD	83911.26	33564.5
Total Carbonaceous BOD	83911.26	33564.5
Total N	0	0
Total inorganic N	0	0
Alkalinity	0	0
pH	4.27	
Volatile fatty acids	0	0
Total precipitated solids	0	0
Total inorganic suspended solids	0	0
Ammonia N	0	0
Nitrate N	0	0

Primary settling		
Parameters	Conc. (mg l⁻¹)	Mass rate (kg d⁻¹)
Volatile suspended solids	46.72	5737.59
Total suspended solids	52.02	6387.91
Particulate COD	74.74	9178.77
Filtered COD	144.02	17685.84
Total COD	218.76	26864.62
Soluble PO4-P	2.87	352.82
Total P	3.89	477.37
Filtered TKN	34.83	4276.72
Particulate TKN	2.39	294.01
Total Kjeldahl Nitrogen	37.22	4570.73

Filtered Carbonaceous BOD	89.16	10949.05
Total Carbonaceous BOD	120.04	14741.28
Total N	37.22	4570.73
Total inorganic N	31.21	3832.17
Alkalinity	200	24560.39
pH	7.3	
Volatile fatty acids	10.67	1310.63
Total precipitated solids	0	0
Total inorganic suspended solids	5.3	650.32
Ammonia N	31.21	3832.17
Nitrate N	0	0

Primary sludge		
Parameters	Conc. (mg l⁻¹)	Mass rate (kg d⁻¹)
Volatile suspended solids	13113.75	16628.24
Total suspended solids	14710.51	18652.93
Particulate COD	20978.88	26601.22
Filtered COD	134.1	170.04
Total COD	21112.98	26771.25
Soluble PO4-P	2.92	3.7
Total P	316.69	401.56
Filtered TKN	35.34	44.81
Particulate TKN	719.86	912.79
Total Kjeldahl Nitrogen	755.2	957.6
Filtered Carbonaceous BOD	83.02	105.27
Total Carbonaceous BOD	8750.48	11095.61
Total N	755.2	957.6
Total inorganic N	31.55	40.01
Alkalinity	200	253.6
pH	7.3	
Volatile fatty acids	9.94	12.6
Total precipitated solids	0	0
Total inorganic suspended solids	1596.76	2024.69
Ammonia N	31.55	40.01
Nitrate N	0	0

Primary Fermenter		
Parameters	Conc. (mg l⁻¹)	Mass rate (kg d⁻¹)
Volatile suspended solids	3555.94	4508.94
Total suspended solids	5264.15	6674.94
Particulate COD	5593.76	7092.89
Filtered COD	14960.33	18969.69
Total COD	20554.09	26062.58
Soluble PO4-P	201.46	255.45
Total P	316.82	401.73

Filtered TKN	390.77	495.5
Particulate TKN	368.6	467.39
Total Kjeldahl Nitrogen	759.37	962.89
Filtered Carbonaceous BOD	10546.09	13372.45
Total Carbonaceous BOD	11073.86	14041.65
Total N	759.37	962.89
Total inorganic N	387.45	491.29
Alkalinity	204.66	259.51
pH	6.41	
Volatile fatty acids	14912.16	18908.62
Total precipitated solids	0	0
Total inorganic suspended solids	1708.2	2166
Ammonia N	387.45	491.29
Nitrate N	0	0

De-watering 1 in		
Parameters	Conc. (mg l⁻¹)	Mass rate (kg d⁻¹)
Volatile suspended solids	0	0
Total suspended solids	0	0
Particulate COD	0	0
Filtered COD	14908.29	10092.91
Total COD	14908.29	10092.91
Soluble PO ₄ -P	200.42	135.68
Total P	200.42	135.68
Filtered TKN	389.81	263.9
Particulate TKN	0	0
Total Kjeldahl Nitrogen	389.81	263.9
Filtered Carbonaceous BOD	10509.53	7114.95
Total Carbonaceous BOD	10509.53	7114.95
Total N	389.81	263.9
Total inorganic N	386.49	261.66
Alkalinity	204.36	138.35
pH	6.43	
Volatile fatty acids	14861.41	10061.17
Total precipitated solids	0	0
Total inorganic suspended solids	0	0
Ammonia N	386.49	261.66
Nitrate N	0	0

De-watering 1 (sludge cake)		
Parameters	Conc. (mg l⁻¹)	Mass rate (kg d⁻¹)
Volatile suspended solids	7624.46	4506.06
Total suspended solids	11293.49	6674.46
Particulate COD	11993.85	7088.37

Filtered COD	14950.97	8836.02
Total COD	26944.82	15924.39
Soluble PO4-P	201.32	118.98
Total P	448.75	265.21
Filtered TKN	390.66	230.88
Particulate TKN	790.49	467.18
Total Kjeldahl Nitrogen	1181.15	698.06
Filtered Carbonaceous BOD	10539.53	6228.86
Total Carbonaceous BOD	11671.01	6897.57
Total N	1181.15	698.06
Total inorganic N	387.32	228.91
Alkalinity	204.3	120.74
pH	6.43	
Volatile fatty acids	14903.08	8807.72
Total precipitated solids	0	0
Total inorganic suspended solids	3669.04	2168.4
Ammonia N	387.32	228.91
Nitrate N	0	0

Anaerobic digester		
Parameters	Conc. (mg l⁻¹)	Mass rate (kg d⁻¹)
Volatile suspended solids	6952.64	14763.94
Total suspended solids	8988.43	19086.94
Particulate COD	10363.14	22006.12
Filtered COD	292.2	620.48
Total COD	10655.33	22626.6
Soluble PO4-P	519.28	1102.69
Total P	830.32	1763.18
Filtered TKN	292.93	622.03
Particulate TKN	690.08	1465.38
Total Kjeldahl Nitrogen	983.01	2087.41
Filtered Carbonaceous BOD	53.67	113.97
Total Carbonaceous BOD	1370.12	2909.45
Total N	983.01	2087.41
Total inorganic N	278.65	591.71
Alkalinity	213.21	452.74
pH	7.72	
Volatile fatty acids	75.94	161.27
Total precipitated solids	0	0
Total inorganic suspended solids	2035.79	4323
Ammonia N	278.65	591.71
Nitrate N	0	0

De-watering 2		
Parameters	Conc. (mg l⁻¹)	Mass rate (kg d⁻¹)

Volatile suspended solids	0	0
Total suspended solids	0	0
Particulate COD	0	0
Filtered COD	291.8	555.15
Total COD	291.8	555.15
Soluble PO4-P	519.68	988.69
Total P	519.68	988.69
Filtered TKN	293.01	557.45
Particulate TKN	0	0
Total Kjeldahl Nitrogen	293.01	557.45
Filtered Carbonaceous BOD	53.34	101.47
Total Carbonaceous BOD	53.34	101.47
Total N	293.01	557.45
Total inorganic N	278.72	530.27
Alkalinity	213.02	405.27
pH	7.89	
Volatile fatty acids	75.47	143.58
Total precipitated solids	0	0
Total inorganic suspended solids	0	0
Ammonia N	278.72	530.27
Nitrate N	0	0

De-watering 2 (sludge cake)		
Parameters	Conc. (mg l⁻¹)	Mass rate (kg d⁻¹)
Volatile suspended solids	66869.89	14778.25
Total suspended solids	86454.5	19106.44
Particulate COD	99668.97	22026.84
Filtered COD	291.81	64.49
Total COD	99960.77	22091.33
Soluble PO4-P	519.67	114.85
Total P	3511.97	776.15
Filtered TKN	293.01	64.75
Particulate TKN	6636.77	1466.73
Total Kjeldahl Nitrogen	6929.77	1531.48
Filtered Carbonaceous BOD	53.35	11.79
Total Carbonaceous BOD	12730.71	2813.49
Total N	6929.77	1531.48
Total inorganic N	278.72	61.6
Alkalinity	213.02	47.08
pH	7.89	
Volatile fatty acids	75.48	16.68
Total precipitated solids	0	0
Total inorganic suspended solids	19584.6	4328.2
Ammonia N	278.72	61.6
Nitrate N	0	0

Centrate		
Parameters	Conc. (mg l⁻¹)	Mass rate (kg d⁻¹)
Volatile suspended solids	0	0
Total suspended solids	0	0
Particulate COD	0	0
Filtered COD	291.8	555.15
Total COD	291.8	555.15
Soluble PO ₄ -P	519.68	988.69
Total P	519.68	988.69
Filtered TKN	293.01	557.45
Particulate TKN	0	0
Total Kjeldahl Nitrogen	293.01	557.45
Filtered Carbonaceous BOD	53.34	101.47
Total Carbonaceous BOD	53.34	101.47
Total N	293.01	557.45
Total inorganic N	278.72	530.27
Alkalinity	213.02	405.27
pH	7.89	
Volatile fatty acids	75.47	143.58
Total precipitated solids	0	0
Total inorganic suspended solids	0	0
Ammonia N	278.72	530.27
Nitrate N	0	0

Anoxic1		
Parameters	Conc. (mg l⁻¹)	Mass rate (kg d⁻¹)
Volatile suspended solids	4821.46	4022620.9
Total suspended solids	5765.15	4809963.3
Particulate COD	6971.87	5816742.5
Filtered COD	62.54	52178.94
Total COD	7034.41	5868921.4
Soluble PO ₄ -P	23.7	19773.76
Total P	479.92	400408.54
Filtered TKN	6.53	5446.84
Particulate TKN	473.2	394794.59
Total Kjeldahl Nitrogen	479.72	400241.43
Filtered Carbonaceous BOD	3.22	2684.9
Total Carbonaceous BOD	2100.03	1752093.5
Total N	479.74	400256.19
Total inorganic N	5.55	4627.31
Alkalinity	195.86	163408.42
pH	8.02	
Volatile fatty acids	0.92	763.74
Total precipitated solids	0	0
Total inorganic suspended	943.7	787342.31

solids		
Ammonia N	5.53	4612.55
Nitrate N	0.02	14.76

Aerobic2		
Parameters	Conc. (mg l⁻¹)	Mass rate (kg d⁻¹)
Volatile suspended solids	4813.67	4016125.3
Total suspended solids	5795.6	4835362.8
Particulate COD	6956.74	5804124.9
Filtered COD	29.98	25011.29
Total COD	6986.72	5829136.2
Soluble PO4-P	1.75	1458.91
Total P	480.5	400889.95
Filtered TKN	1.71	1430.73
Particulate TKN	474.62	395980.46
Total Kjeldahl Nitrogen	476.33	397411.19
Filtered Carbonaceous BOD	0.36	297.31
Total Carbonaceous BOD	2085.37	1739857.4
Total N	479.13	399744.17
Total inorganic N	3.43	2862.34
Alkalinity	195.38	163004.94
pH	8.31	
Volatile fatty acids	0	3.4
Total precipitated solids	0	0
Total inorganic suspended solids	981.93	819237.52
Ammonia N	0.63	529.35
Nitrate N	2.8	2332.99

SND		
Parameters	Conc. (mg l⁻¹)	Mass rate (kg d⁻¹)
Volatile suspended solids	4792.78	1602306.3
Total suspended solids	5778.02	1931687.7
Particulate COD	6923.67	2314697.6
Filtered COD	28.26	9449.05
Total COD	6951.94	2324146.7
Soluble PO4-P	0.03	9.96
Total P	480.29	160569.54
Filtered TKN	1.26	421.04
Particulate TKN	474.24	158546.32
Total Kjeldahl Nitrogen	475.5	158967.36
Filtered Carbonaceous BOD	0.31	104.25
Total Carbonaceous BOD	2065.3	690465.31
Total N	476.43	159279.29
Total inorganic N	1.05	349.61
Alkalinity	195.59	65389.81
pH	8.36	

Volatile fatty acids	0	0.32
Total precipitated solids	0	0
Total inorganic suspended solids	985.24	329381.42
Ammonia N	0.11	37.68
Nitrate N	0.93	311.93

Deoxygenator		
Parameters	Conc. (mg l⁻¹)	Mass rate (kg d⁻¹)
Volatile suspended solids	7639.46	1593342.3
Total suspended solids	9182.9	1915254
Particulate COD	11038.16	2302201.3
Filtered COD	58.4	12179.44
Total COD	11096.56	2314380.8
Soluble PO4-P	14.24	2970.95
Total P	764.01	159346.75
Filtered TKN	1.19	247.76
Particulate TKN	754.5	157365.18
Total Kjeldahl Nitrogen	755.69	157612.93
Filtered Carbonaceous BOD	6.51	1358.33
Total Carbonaceous BOD	3304.07	689121.4
Total N	755.7	157614.21
Total inorganic N	0.17	36.39
Alkalinity	195.6	40795.21
pH	8.33	
Volatile fatty acids	1.1	229.48
Total precipitated solids	0	0
Total inorganic suspended solids	1543.44	321911.66
Ammonia N	0.17	35.12
Nitrate N	0.01	1.28

Anaerobic		
Parameters	Conc. (mg l⁻¹)	Mass rate (kg d⁻¹)
Volatile suspended solids	7621.48	1594753.1
Total suspended solids	9119.32	1908168.4
Particulate COD	11015.86	2305008.8
Filtered COD	78.79	16485.43
Total COD	11094.65	2321494.2
Soluble PO4-P	37.86	7922.63
Total P	761.03	159242
Filtered TKN	2.98	623.37
Particulate TKN	750.44	157026.29
Total Kjeldahl Nitrogen	753.42	157649.66
Filtered Carbonaceous BOD	1.68	351.3
Total Carbonaceous BOD	3298.91	690279.18
Total N	753.42	157649.67

Total inorganic N	2	418.96
Alkalinity	195.76	40960.72
pH	8.3	
Volatile fatty acids	1.62	339.5
Total precipitated solids	0	0
Total inorganic suspended solids	1497.84	313415.21
Ammonia N	2	418.95
Nitrate N	0	0.01

APPENDIX 2. Cost estimation

Table A-1. Cost estimation for the first alternative (MBR using methanol for denitrification)C\$

Category	Item description	Expected value
Capital cost	Membrane tank+new aerobic zone building	4,571,028
	Membrane Unit	66,500,000
	Miscellaneous site pipeworks	3,917,000
	Existing tankage modifications	1,208,000
	Sub Total	76,196,028
	Contingencies (20%)	15,964,776
	Sub Total	91,435,233
	Engineering (15%)	13,715,285
	City & administration (3%)	2,743,057
	Total	107,893,575
O&M Cost	Labour	569,000
	Power	1,944,444
	Cleaning chemicals	360,000
	Suggested annual membrane accrual	2,280,000
	Utilities	420,000
	Consumables	73,000
	E&M materials	88,000
	Miscellaneous	20,000
	Annual methanol addition	4,625,280
	Total per year	10,379,724
Centrate capital Cost	Existing tank modification	304,000
	Contingencies (20%)	60,800
	Sub Total	364,800
	Engineering (15%)	54,720
	City & administration (3%)	10,944
Total	430,464	
Centrate O&M Cost	Labour	117,000
	Power	140,000
	Utilities	38,000
	Consumables	219,000
	E&M materials	50,000
	Miscellaneous	6,000
	Total per year	570,000

Table A-2 Cost estimation for the second alternative (Anaerobic MBR using hydrogen for denitrification)C\$

Category	Item description	Expected value
Capital cost	Membrane tank+new aerobic zone building	6,758,559
	Membrane Unit	66,500,000
	Miscellaneous site pipeworks	3,917,000
	Existing tankage modifications	1,208,000
	Sub Total	76,196,028
	Contingencies (20%)	15,964,776
	Sub Total	91,435,233
	Engineering (15%)	13,715,285
	City & administration (3%)	2,743,057
	Total	159,527,589
O&M Cost	Labour	569,000
	Power	1,944,444
	Cleaning chemicals	360,000
	Suggested annual membrane accrual	2,280,000
	Utilities	420,000
	Consumables	73,000
	E&M materials	88,000
	Miscellaneous	20,000
	Annual Hydrogen cost	2,384,164
	Total per year	8,138,608
Centrate capital Cost	Existing tank modification	304,000
	Contingencies (20%)	60,800
	Sub Total	364,800
	Engineering (15%)	54,720
	City & administration (3%)	10,944
	Total	430,464
Centrate O&M Cost	Labour	117,000
	Power	140,000
	Utilities	38,000
	Consumables	219,000
	E&M materials	50,000
	Miscellaneous	6,000
	Total (year 2000)	570,000
	Total of 40 years	7,307,400

Table A-3 Required volumes for the first alternative (MBR using methanol for denitrification)

Unit	Required Volume(m3)
Anoxic	14000
Aerobic	40000
SND	24000
Membrane	8000
Deoxygenator	2000
Anaerobic	3000
Primary fermenter	800
Centrate Reactor	4500
Centrate Clarifier	4000
Total	100300

Table A-4 Required volumes for the second alternative (Anaerobic MBR using hydrogen for denitrification)

Unit	Required Volume(m3)
Anoxic	24000
Aerobic	64000
Anaerobic MBR	8000
Anaerobic	19000
Primary fermenter	800
Centrate Reactor	4500
Centrate Clarifier	4000
Hydrogen Saturator	24000
Total	148300