Feasibility of Industrial Wastewater as External Carbon Source to Enhance Total Nitrogen Removal from Digested Sludge Liquor

by

Jiazhong Chen

A Thesis Submitted to the Faculty of Graduate Studies of

The University of Manitoba

in Partial Fulfilment of the Requirements of the Degree of

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Department of Civil Engineering

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ABSTRACT

The carbon to nitrogen ratio of below 1:1 in digested sludge liquor, i.e. centrate, need to be elevated to at least 4:1 by the addition of external carbon sources to enhance total nitrogen removal. The applicability as exogenous carbon sources of four industrial wastewaters, i.e. potato processing, canola processing and oil refining, biodiesel production byproduct (mainly glycerol) and deicing liquid from Winnipeg International Airport (mainly glycol), was evaluated because of their high carbon but negligible ammonia content and easy accessibility in Manitoba, Ca. Three bench-scale sequencing batch reactors (SBRs) were operated at 10 d SRT (solids residence time), 2.5 d HRT (hydraulic residence time) and room temperatures (21±2 °C). One reactor was aerated all the time while alternating aerobic/anoxic condition was maintained in the other two reactors during the investigation. Nitrate uptake rate (NUR) and ammonia uptake rate (AUR) tests were performed to determine specific denitrification rate (SDNR) and specific nitrification rate (SNR) after a steady state was achieved. Both glycerol and potato wastewater showed promising potential with SDNRs of 13 mgNO_3-N/gVSS*h and 12 mgNO_3-N/gVSS*h, respectively. The SDNRs were better than 10 mgNO_3-N/gVSS*h with methanol but lower than 17 mgNO_3-N/gVSS*h with ethanol. The additional benefit was that the bacterial response times needed were only 4 days and 2 days for glycerol and potato wastewater, respectively. The times were lower than 6 d response for ethanol and much lower than 26 days for methanol. Glycol was also a good substrate to replace purchased carbon sources as it required a 3-day response time and could enhance the SDNR to 8 mgNO_3-N/gVSS*h. However, the canola oil wastewater was eliminated

because of the lowest SDNR of 5 mgNO_3-N/gVSS*h it produced, a long response time of 24 days required, and the inhibitory effect on nitrification it exerted. Compared to the reference SNR of 36 mgNH_3-N/gVSS*h, a hindered SNR of 10 mgNH_3-N/gVSS*h was detected when canola oil wastewater was used. Potato wastewater, a byproduct of biodiesel production (glycerol) and deicing wastewater from WIA (glycol) were recommended in this study as good substitutes to the purchased chemicals as an external carbon source in denitrification.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS AND SYMBOLS

ADWF	Average Dry Weather Flow
ANAMMOX	ANaerobic AMMonium OXidation
AOB	Ammonia Oxidizing Bacteria
AUR	Ammonia Uptake Rate
BABE	BioAugmentation Batch Enhanced
BOD_5	5-day Biochemical Oxygen Demand
BNR	Biological Nutrient Removal
centrate	sludge reject liquor
C	Carbon
C/N	Carbon to Nitrogen ratio
CO_2	Carbon dioxide
COD	Chemical Oxygen Demand
bCOD	biodegradable Chemical Oxygen Demand
bsCOD	biodegradable soluble Chemical Oxygen Demand
bpCOD	biodegradable particulate Chemical Oxygen Demand
sCOD	soluble Chemical Oxygen Demand
DAF	Dissolved Air Floatation
DNP	DeNitrification Potential
DNR	DeNitrification Rate
DO	Dissolved Oxygen
F/M	Food to Microorganism ratio
H^+	Proton
H_2O	Water
H_2SO_4	Sulfuric acid
HNO_2	Nitrous acid
HPOAS	High Purity Oxygen Activated Sludge
HRT	Hydraulic Retention Time
InNitri	Inexpensive Nitrification
K_N	Specific Nitrification Rate
K_{DN}	Specific Denitrification Rate

MBR	Membrane BioReactor
MLSS	Mixed Liquor Suspended Solids
MLVSS	Mixed Liquor Volatile Suspended Solids
N	Nitrogen
N_2	Nitrogen gas
N_2O	Nitrous Oxide
$NaHCO_3$	Sodium bicarbonate
NH_3-N	Ammonia Nitrogen
NH_4^+	Ammonium ion
NEWPCC	North End Water Pollution Control Centre
NO	Nitric Oxide
NOB	Nitrite Oxidizing Bacteria
NO_2-N	Nitrite Nitrogen
NO_3-N	Nitrate Nitrogen
NUR	Nitrate Uptake Rate
O	Oxygen
O_2	Oxygen gas
O_3	Ozone
OH^-	Hydroxyl ion
ORP	Oxidation Reduction Potential
P	Phosphorous
R1	Reactor No. 1
R2	Reactor No. 2
R3	Reactor No. 3
RAS	Returned Activated Sludge
RBC	Rotary Biological Contactor
RPM	Rotation Per Minute
SBR	Sequencing Batch Reactors
SDNR	Specific DeNitrification Rate
SEWPCC	South End Water Pollution Control Centre
SHARON	Single reactor system for High rate Ammonium Removal Over Nitrite
SND	Simultaneous Nitrification and Denitrification
SNdN	Simultaneous Nitrification and Denitrification

SNR	Specific Nitrification Rate
SRT	Solids Retention Time
TCOD	Total Chemical Oxygen Demand
TKN	Total Kjeldahl Nitrogen
TN	Total Nitrogen
TSS	Total Suspended Solids
USEPA	United States Environmental Protection Agency
VSS	Volatile Suspended Solids
WAS	Wasted Activated Sludge
WEF	Water Environment Federation
WERF	Water Environment Research Foundation
WEWPCC	West End Water Pollution Control Centre
WIA	Winnipeg International Airport
WPCC	Water Pollution Control Centers
WW	WasteWater
WWTP	WasteWater Treatment Plant

1.0 INTRODUCTION

1.1 Background

There are three wastewater treatment plants (WWTPs) currently operating in the City of Winnipeg, Manitoba, Canada: North End Water Pollution Control Centre (NEWPCC), South End Water Pollution Control Centre (SEWPCC) and West End Water Pollution Control Centre (WEWPCC). These three WWTPs are all fully carbon or chemical oxygen demand (COD) removal facilities, i.e. they do not remove nutrients contained in wastewater: nitrogen (N) and phosphorous (P). These two nutrients, however, can cause various problems when they enter a natural body of water, including but not limited to depletion of Oxygen (O_2) in water, algae blooms, eutrophication, and endocrine disrupting potential caused by nitrates. These problems have been receiving increased awareness from both public and governments, and the upgrading of the three WWTPs to full nutrient removal plants is required by Manitoba Conservation.

The total nitrogen (TN) load entering a WWTP originates from two major sources. One is the raw sewage from the collection system, and the other is centrate, generated within the system itself, from dewatering of the anaerobically digested primary sludge and wasted activated sludge (WAS), and called digested sludge liquor. The liquor is typically high in ammonia (600-1000 mg NH_3-N /L), total suspended solids (300-2000 mg TSS/L), and temperature (normally 28-40°C), but low in biodegradable carbon in form of 5-day biochemical oxygen demand (BOD_5) (normally 200 mg BOD_5/L) (Constantine et al.,

2005a; Caffaz et al. 2005). Centrate typically contributes 10-30% of the total nitrogen load in the entire influent to WWTPs despite the normally small flow compared to the raw sewage wastewater flow, typically only occupying 0.5%-2% of the total influent flow (van Loosdrecht et al., 2005; Thornton et al., 2005; Constantine et al., 2005a).

There is an existing trend to centralize sludge processing facilities at one large WWTP for a region. Such regionalization is the case in Winnipeg. The anaerobic digestion and storage tanks were built at NEWPCC, the largest WWTP in the city, to treat the biosolids generated by all three WWTPs. This trend towards separate sludge processing facilities to treat the biological sludge from a large region is also present in other communities such as New York, San Diego, United Kingdom (Barnes, 2000; Jeavons et al., 1998), and South Africa (Pitman, 1999). The centralization minimizes the construction of sludge processing facilities, facilitates the sludge handling and control, but it adds much more nitrogen load to the supernatant from the anaerobic digestion of sludge than when the facility treated the primary sludge and WAS generated only by its mother plant. This supernatant is ultimately recycled to the influent of the treatment plant and its high nitrogen load could hamper the overall performance of the bioreactors. In Winnipeg, this centrate flow contributes as much as 30 percent of the total nitrogen load into NEWPCC but engages less than one percent of the total influent flow (Head and Oleszkiewicz, 2004). This reality made it necessary to remove nitrogen from the sludge liquor.

To meet the goal set by the Manitoba Conservation of removing nutrients from wastewater before discharging, it is better to control the nutrients loads at the source.

This idea also means the nitrogen should be removed from the centrate in a separate side stream treatment facility rather than in the main stream bioreactors. There are many innovative methods developed to treat centrate, for example, ANAMMOX (**An**aerobic **Amm**onium **Ox**idation) process (Kurukawa et al, 2006; van Dongen et al, 2001, Caffaz et al., 2005), SHARON (**S**ingle reactor system for **H**igh rate **A**mmonium **R**emoval **O**ver **N**itrite) process (Volcke et al, 2006; Hellinga et al, 1998), BABE (**B**io**A**ugmentation **B**atch **E**nhanced) process (Salem et al, 2003 & 2001), and InNitri (**In**expensive **Nitri**fication) process (Kos, 1998; Constantine et al, 2005a; Oleszkiewicz and Barnard, 2006). All of the processes mentioned above take advantage of the centrate characteristics of high temperature and ammonia concentration.

The most common method, however, is the conventional biological method of the combination of nitrification and denitrification processes (Hallin et al., 1996b). Nitrification is an autotrophic process, but denitrification is heterotrophic and requires easily biodegradable carbon to proceed to completion. Typically the ratio of carbon to nitrogen (C/N ratio) should be greater than 5:1 to enhance the denitrification process in a plant. However plants often face the problem of a lack of an adequate supply of indigenous carbon in their wastewater or are designed such that the indigenous carbon is removed before the denitrification stage. The carbon deficiency is more serious when the plants are additionally loaded with sludge processing liquors, i.e. centrate. The normally meager carbon resources of typical Canadian municipal sewage can be overwhelmed by the centrate, especially when the centrate is derived from a sludge processing facility handling sludge from several plants. This is the case at NEWPCC in Winnipeg. Large

plants, therefore, are forced to purchase chemicals, commonly methanol and ethanol, to avoid the depression of the full removal of nitrogen. Methanol is more common because of the cost advantage over ethanol, but its price is increasing gradually. As a result, millions of dollars are spent annually to purchase carbon in the form of methanol in large plants. In addition, the substance is also highly flammable and of a toxic nature. It would be necessary to find the replacements to the purchased organic compounds.

Manitoba is an agricultural province, therefore the industries generate typically biodegradable wastewaters with high carbon but negligible nitrogen content. Another growing industry in Manitoba is biodiesel production. The byproduct from this process is mainly glycerol, which is biodegradable and a good source of carbon for denitrification. Glycol is used for deicing airplanes at the Winnipeg International Airport (WIA), consequently the wastewater from deicing contains glycol, a biodegradable substance. Generally speaking, there is a potential for these industrial wastewaters to be used as a free external carbon source to boost the denitrification process.

Alternate carbon sources in the form of industrial waste could be identified and tested to check their applicability for total nitrogen removal from anaerobically digested sludge centrate (dewatering liquor). Four wastewaters from potato processing, canola processing and oil refining, biodiesel production (glycerol), and deicing wastewater (glycol) at the Winnipeg International Airport were compared to methanol and ethanol. The concept has tremendous sustainable development implications. The industrial waste

is not treated at a significant cost to the industry but is picked up by the municipality as a valuable raw material for their wastewater treatment process.

1.2 Goals and objectives of the study

This study was carried out in bench-scale sequencing batch reactors (SBRs). The primary goal was the development of alternate sources of biodegradable carbon for TN removal from anaerobic digested sludge liquor in municipal plants by using industry wastewater that normally has to be treated at high costs.

The objectives of this study were to:

(1) Identify which carbon source from the industrial wastes described above was best suited for the denitrification of centrate;

(2) Determine the optimum dosage that should be used;

(3) Indicate how much the rate of denitrification will be increased;

(4) Compare the rates to those of methanol and ethanol as carbon sources;

(5) Define time lag before each carbon source added starts being utilized by the denitrifiers and the time for full acclimation.

2.0 LITERATURE REVIEW

2.1 Review on centrate

Centrate is the subject that was dealt with in this study. The following sections discuss the characteristics of centrate. In addition, the methods available for centrate treatment are briefly reviewed.

2.1.1 Typical characteristics of centrate

Centrate refers to the supernatant from the centrifuges that dewater anaerobically digested biosolids which consist of primary sludge and waste activated sludge (WAS). Because of its high ammonia contents, centrate is a major source of nitrogen entering a WWTP besides the raw municipal wastewater. Table 2-1 summarizes the characteristics of centrate reported by some researchers.

Table 2-1 Reported typical characteristics of centrate

Parameters	References				
	Constantine et al. (2005a)	Carrio et al. (2003)	Ali et al. (1998)	Caffaz et al. (2005)	Arnold et al. (2000)
NH_3-N (mg/L)	910	200-740	600-1200	403-997	600-700
TSS (mg/L)	1730	360-930	1000-3000	-	<400
VSS (mg/L)	1010	-	-	-	-
COD (mg/L)	-	315-650	-	119-530	-
BOD_5 (mg/L)	200	168-297	-	9.5-87.6	-
Alkalinity ($mgCaCO_3/L$)	-	1800-2800	1500-4000	2530-4311	-
Temperature (°C)	30-40	29.4-33	30-32	-	28-32
N in centrate as % of raw wastewater	18.2%	-	-	10-30%	-

Note: VSS = Volatile Suspended Solids

This table clearly demonstrates that centrate contains up to 1200 mg/L of NH_3-N. The centrate stream is usually untreated and returned to the head of a WWTP, therefore it may contribute up to 30% total nitrogen entering a WWTP. This is a heavy burden for a biological nutrient removal (BNR) plant. When the nitrogen load from centrate coincides with the high ammonia load of the influent, the effluent ammonia concentration can easily exceed the discharge limit. Thus it is necessary to remove this nitrogen load from centrate as a side stream.

2.1.2 Centrate treatment

High temperature and high concentration of ammonia make centrate an easy substrate for high rate nitrification at short solids retention time (SRT). Biological treatment of centrate is more economical than physical or chemical treatment. Based on these observations, several new total nitrogen (TN) removal technologies have been applied to centrate treatment (van Loosdrecht et al., 2005; Constantine et al. 2005a). The N-rich digested sludge liquor could also be used to grow nitrifiers in the side stream for bioaugmentation in the main stream (Head and Oleszkiewicz, 2004), thus making it possible for the main stream process to operate below the optimum aerobic SRT.

SHARON (**S**ingle reactor system for **H**igh rate **A**mmonium **R**emoval **O**ver **N**itrite) is a process which takes advantage of the higher temperature and ammonia concentration of centrate (Volcke et al, 2006; Hellinga et al, 1998; Constantine et al., 2005a; van Loosdrecht et al., 2005). The process is based on the higher maximum growth rate of ammonia oxidizers than that of nitrite (NO_2-N) oxidizers at higher temperatures. Therefore there is a nitrite accumulation in reactors. This sets the stage for another innovative process, ANAMMOX (**An**aerobic **Amm**onium **Ox**idation) process (Kurukawa et al, 2006; van Dongen et al, 2001, Caffaz et al., 2005), which requires that the partial nitrification happen first to make nitrite available in bioreactors. The ANAMMOX process is a fully autotrophic method for TN removal. This process transforms ammonia directly to nitrogen gas under anaerobic conditions with nitrite as the electron acceptor in the absence of any organic carbon sources. ANAMMOX-bacteria cannot use nitrate

(NO_3-N) as the electron acceptor, therefore for effective nitrogen removal, nitrite is required for the process to occur. As a direct solution, the SHARON and ANAMMOX processes are connected in one system, which is termed as the combined SHARON-ANAMMOX process (van Dongen et al., 2001). The configuration of this process is shown in Figure 2-1.

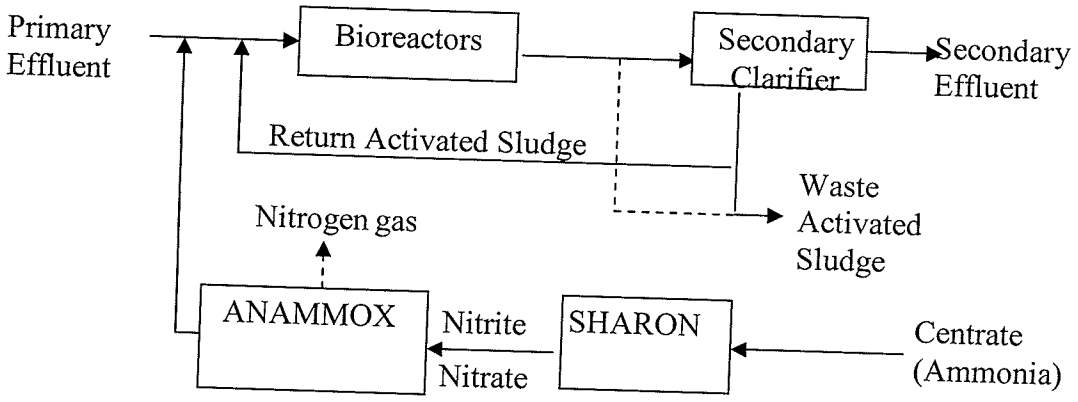


Figure 2-1 Process flow diagram for combined SHARON-ANAMMOX process (Adapted from Constantine et al., 2005a)

The centrate contains high nitrogen content, which facilitates the growth of nitrifiers. When centrate is treated with the returned activated sludge (RAS) from the main stream bioreactors, nitrifying bacteria can enrich the side stream treatment. Then the biomass containing nitrifiers can be returned to the main stream as the seed for bioaugmentation, and this seed is easy to acclimate to the main system because it is obtained from the natural population available in the same WWTP. As a result, the aerobic SRT of BNR can be reduced. Based on this theory, BABE (**Bio**Augmentation **B**atch **E**nhanced) process was introduced (Salem et al, 2003; van Loosdrecht et al., 2005; Constantine et al., 2005a). Salem et al. (2004) showed that the nitrification rate of the sludge was improved

with almost 60% by the augmentation effect of the BABE process in a full-scale application. Figure 2-2 demonstrates the configuration of the BABE process.

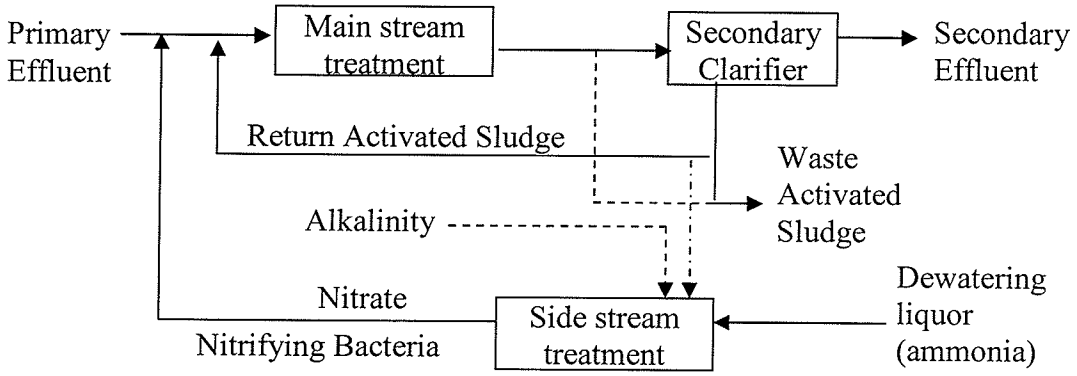


Figure 2-2 Process flow diagram for BABE process (Adapted from Constantine et al., 2005a)

InNitri (**In**expensive **Nitri**fication) process is another application of the favorable condition for nitrifiers' growth in centrate with the high ammonia concentration and temperature. This process was first introduced in Kos (1998). Similar to the BABE process, centrate is treated in a side-stream treatment, which uses the conventional activated sludge system. The enriched population of nitrifying bacteria produced in the side-stream treatment system is subsequently seeded to the main-stream treatment system. Kos (1998) demonstrated that 40% of the tankage required for nitrification in a main stream bioreactor could be reduced by recycling the nitrified centrate stream back to the main stream in a WWTP which received 33% of its N load from centrate. This is the result of the proceeding of nitrification in main stream systems at substantially reduced SRTs with the bioaugmentation of nitrifiers. The easy retrofitting of existing secondary facilities which already provide sufficient aerobic SRTs to a treatment system including anoxic zones for denitrification is another advantage of the InNitri process (Constantine

et al., 2005a). Figure 2-3, which is adapted from Constantine et al. (2005a), is the configuration of an InNitri process.

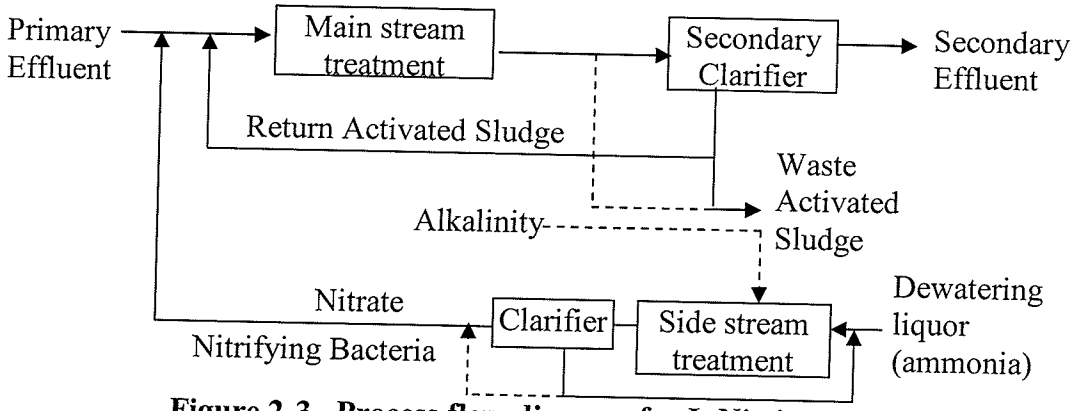


Figure 2-3 Process flow diagram for InNitri process (Adapted from Constantine et al., 2005a)

The above two figures illustrates clearly that the BABE and InNitri are two similar processes. Both of them offer bioaugmentation with the nitrifying bacteria generated in side-stream treatment systems (Oleszkiewicz and Barnard, 2006). Two major differences, however, exist between them. One is a portion of the RAS from the mainstream system is included as an additional input stream to the side-stream treatment facilities in the BABE process. The other is the conventional treatment system consisting of a bioreactor and a clarifier is typically applied in the InNitri process and the BABE process typically uses sequencing batch reactors (SBRs). The introducing of RAS into the side-stream treatment system in the BABE process can better ensure the enriched nitrifying biomass develop in both side-stream and main-stream systems and recover part of the alkalinity consumed by nitrification from the alkalinity contained in RAS. The operation of SBR in the BABE system makes it easier to incorporate a denitrification step, which provided the additional recovery of alkalinity. The introducing of RAS also has drawbacks such as the

temperature in side-stream reactors is decreased by the lower temperature in RAS, which results a bigger tank volume required in BABE compared with the InNitri process. Oleszkiewicz and Barnard (2006) stated the higher capital costs are required for the BABE process than for InNitri.

There are other treatment methods also available. Thornton et al. (2005) studied the use of mesolite, a novel clay based material, as an adsorbent for the removal of ammonia from centrate. It showed 95% of ammonia could be removed. Hydrogen-dependent denitrification in an anaerobic submerged membrane bioreactor (MBR) reported by Rezania et al. (2007) could be used in the treatment of centrate, which used the autotrophic bacteria instead of the heterotrophic bacteria.

Besides these newer technologies described above, any conventional TN removal processes can be applied in the reduction of nitrogen load from the anaerobically digested sludge liquors.

2.2 Brief information on TN removal and sequencing batch reactor (SBR)

2.2.1 Description of biological TN removal and processes for TN removal

Biological total nitrogen (TN) removal from anaerobically digested sludge liquors can be best described as the combination of two microbial activities known as nitrification and denitrification. These two processes are accomplished by the metabolic function of a vast

variety of bacteria under different environmental conditions, mainly with or without the presence of oxygen (O_2), in a series of periods within a treatment system (Hatch and Kearney, 2005).

Several operation periods can be separated out within a treatment system; each period is either aerated or un-aerated to create aerobic or anoxic conditions respectively. Therefore nitrification and denitrification are incorporated into one system. There are abundant configurations available which can achieve the reduction of TN load in wastewaters. Table 2-2 listed some of the common combined nitrification and denitrification processes (adapted from Qasim, 1998).

Table 2-2 Combined Nitrification-Denitrification Processes

Processes	Description
Alternating aeration systems	Nitrification-denitrification can be achieved either in dual-sludge system or in triple-sludge system
Attached growth processes	Aerobic and anoxic zones are maintained in attached growth reactors to achieve nitrification-denitrification. Rotary biological contactors (RBCs), nitrification/denitrification filters, fluidized bed denitrification filters are typical reactors.
Bardenpho process	Four stages: anoxic, aerobic, anoxic, and aerobic zones are included the proprietary process.
Ludzack-Ettinger process	The process consists of anoxic zone, aerobic zone, clarification and returned sludge. With internal recycle, the process is called MLE process (Modified Ludzack-Ettinger).
Oxidation ditch	Tanks upstream and downstream of the rotor were created as anoxic and aerobic conditions. Proprietary processes include Orbal, Carrousel and Bio-denitro.
Sequencing batch reactor	Aerobic and anoxic are available in a single reactor to achieve both nitrification and denitrification
Wuhrmanr process	An aerobic-anoxic reactor and a clarifier are included in the process. The head of the anoxic basin is the adding point of returned sludge and influent.

2.2.2 Sequencing batch reactor (SBR) process for TN removal

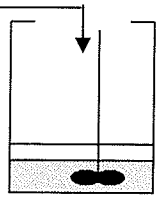
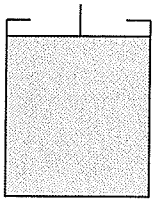
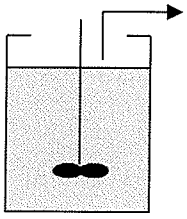
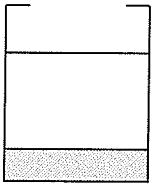
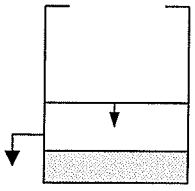
A Sequencing batch reactor (SBR) process utilizes only one fill-and-draw complete-mix reactor in which both reaction and clarification happen (Metcalf & Eddy, 2003), and several fill-and-draw cycles can be operated per day. The SBR process typically contains five phases which are carried out in sequence: fill, react, waste, settle (clarification), and draw (decant). Sometimes there is an idle period after the draw phase, which provides time for one reactor to complete its fill step before changing to another unit in a multi-tank system and for the operational flexibility at high flows. The idle phase, however, is not a necessary period, so it is usually excluded from the SBR system. Because all of the operations, including the sedimentation, are performed in one reactor, the secondary clarifier is eliminated from the configuration. In addition, there is no RAS portion in the system since there are no solids lost in the reaction step and no need to return solids to sustain the constant solids contents in the reaction zone (Metcalf & Eddy, 2003).

During the reaction period in a SBR system, thorough mixing is provided to ensure the homogeneous distribution of the mixed liquor usually by independent mixers. The separation of stirring from aeration can supply flexibility for operations and is especially useful during the fill period for the anoxic operation. The mixed liquor can be either aerated or un-aerated to create aerobic and anoxic conditions suited for nitrifiers and denitrifiers respectively. Consequently, the TN is removed from wastewaters biologically (WERF, 2003; Metcalf & Eddy, 2003).

Wasting of the activated sludge in a SBR reactor is a necessary step in order to assure that the right quantity of the solids is maintained in the system and the activity of the solids is not reduced, both of which contribute to the optimum performance of the SBR. Wasting usually occurs at the end of the reaction period, resulting in a more uniform discharge of solids than at the end of the settling phase (WERF, 2003; Metcalf & Eddy, 2003).

SBR systems are inexpensive, effective, and highly flexible in operation, therefore it is used in small- and medium-sized communities and in many research studies (Dytczak et al., 2007; Louzeiro et al., 2002; Gali et al., 2005). In addition, SBRs have been reported as an effective solution to treat centrate in order to remove the high nitrogen content (Arnold et al., 2000; Rostron et al., 2001; Wett et al., 1998). An example of bench scale SBR operation conditions is demonstrated in Table 2-3. The example is based on the condition of 10d of SRT, 4/3d of HRT (Hydraulic Retention Time), and one fill and draw cycle per day (WERF, 2003; Metcalf & Eddy, 2003).

Table 2-3 Bench scale SBR operation conditions (Adapted from WERF, 2003; Metcalf & Eddy, 2003)

Stage	Percent of cycle time (%)	Percent of total volume (%)	Reactor condition	Operation
Fill	25	From 25 to100		Wastewater is added to reactor until reach max. volume Air off Mixing on
React (excluding fill)	35	100		Reactor volume constant Air on/off Mixing on
Waste	5	From 100 to 90		Mixing and aeration on Waste 10% mixed liquor
Settle	20	90		Mixing or aeration off Allow sludge to settle
Draw	15	From 90 to 25		Mixing or aeration off Decant 65% of total volume supernatant

Note: One fill-and-draw cycle per day, SRT=10d, HRT=4/3d

2.3 Metabolic properties of bacteria

Familiarity with the metabolic properties of bacteria is the necessary foundation to better understand both nitrification and denitrification since they are bacteria-based processes.

These two microbial processes can be well designed, optimized, and operated with better comprehension.

Bacteria, like other living things, need to survive and reproduce. To maintain this activity, they need to ingest materials from the surrounding environment, form new cells, and obtain energy to sustain the action. There are three basic requirements: a carbon source for the formation of new cellular material, a source of energy, and other inorganic elements, known as nutrients, such as nitrogen, phosphorus, magnesium, calcium, potassium, and sulfur. The formation of new cells may also require other organic nutrients such as amino acids, purines, pyrimidines, and vitamins. The organic nutrients are commonly referred as growth factors (Metcalf & Eddy, 1991).

Carbon dioxide (CO_2) and carbon contained in organic materials are the two sources from which bacteria obtain carbon for the growth of cells. Therefore microorganisms are divided into two categories based on the different carbon sources. One group is called autotrophs, which obtain cell carbon from CO_2. The other is called heterotrophs, which requires organic carbon to form cell tissues. A net input of energy is needed for the incorporation of CO_2 to new biomass because this conversion is a reductive process. Therefore more energy is needed for autotrophs to survive and reproduce than for heterotrophic bacteria. As a result, lower growth rate and biomass yields are usually observed among autotrophs (Metcalf & Eddy, 2003).

Energy is also commonly gained from two sources: light and chemical oxidation-reduction reactions. Phototrophs refer to the microorganisms that use light as their energy source, while the energy produced by chemical oxidation-reduction reactions is utilized by another type of bacteria for cell synthesis, chemotrophs. Both phototrophs and chemotrophs can either be autotrophs or heterotrophs. Thus four additional sub-categories are developed according to different carbon sources and energy sources: photoautotrophs, chemoautotrophs, photoheterotrophs, and chemoheterotrophs (Metcalf & Eddy, 1991). Nitrifying bacteria (nitrifiers) belong to chemoautotrophs, which gain energy from the oxidation of reduced ammonia and obtain carbon from CO_2 (Metcalf & Eddy, 2003). Therefore the nitrifiers are slow-growth and low-yield bacteria, resulting in a long SRT required in the nitrification process.

For the chemical oxidation-reduction reactions from which chemotrophs derive energy, there are electron donors and electron acceptors present. The reactions are completed by the transfer of electrons from donors to acceptors. Both organic and inorganic matter can act as electron donors and electron acceptors. Chemoautotrophs usually use inorganic compounds as electron donors while organic compounds are commonly utilized by chemoheterotrophs. Electron acceptors mainly include oxygen, nitrate and nitrite, and organic compounds. The bacteria which use nitrate and nitrite as electron acceptors can reduce them to nitrogen gas, which is then released to the atmosphere, resulting in an act of removing nitrogen. These bacteria are known as denitrifiers. Denitrifiers are facultative aerobic bacteria, which means they can use nitrate and nitrite as electron acceptors without the presence of oxygen (anoxic condition) and transfer electrons to

oxygen with its presence (aerobic condition) (Metcalf & Eddy, 2003). Denitrifiers can obtain a wealth of energy with the oxidation of organic compounds but less energy is required in cell synthesis than nitrifiers. Therefore denitrifiers are fast growing bacteria and they are less demanding than nitrifiers. However, for nitrogen removal, there should be no oxygen presenting in bioreactors, i.e. providing anoxic periods. The classification of microorganisms is summarized in Table 2-4.

Table 2-4 Microorganisms categorization

Microorganism category	Typical reaction	Source of carbon	Donor of electron	Acceptor of electron	End products
Aerobic autotrophic	Nitrifying process	Carbon dioxide	Ammonia Nitrite	Oxygen	Nitrate Nitrite
Facultative heterotrophic	Denitrifying process	Organic substance	Organic substance	Nitrate Nitrite	Nitrogen Carbon dioxide Water
Aerobic heterotrophic	Aerobic oxidation	Organic substance	Organic substance	Oxygen	Carbon dioxide Water

2.4 Nitrification Process

2.4.1 Description of nitrification

Nitrification is referred as the reaction of the transformation of ammonia to the end product of nitrate with the presence of oxygen by aerobic autotrophs (nitrifying bacteria). This process is an essential and fundamental step in the biological total nitrogen removal because it creates the basis for denitrification to proceed (Hall et al., 1980; Qasim, 1998;

Carley et al., 1991). It is clearly shown in the previous table that nitrifiers can utilize both ammonia and nitrite as their electron donors. Therefore, nitrification itself is a two-step biological process. The first step is to oxidize ammonia to nitrite by ammonia oxidizing bacteria (AOB), and the second step is to combine nitrite with more oxygen to become nitrate by nitrite oxidizing bacteria (NOB). Typical AOBs include *Nitrosomonas* and *Nitrosospira*, which are able to complete the first step. The conversion of ammonia to nitrite can be expressed in the following Eq. (2-1) (Hall et al., 1980; Kraume et al., 2005).

$$NH_4^+ + 1.5O_2 \rightarrow 2H^+ + H_2O + NO_2^- \qquad \text{Eq. (2-1)}$$

The produced nitrite is further oxidized to nitrate by the activities of another group of bacteria – the NOB, which include *Nitrobacter* and *Nitrospira*. The reaction is (Hall et al. 1980; Kraume et al., 2005):

$$NO_2^+ + 0.5O_2 \rightarrow NO_3^- \qquad \text{Eq. (2-2)}$$

Combining the two equations, the overall reaction is obtained as Eq. (2-3) (Metcalf & Eddy, 2003).

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \qquad \text{Eq. (2-3)}$$

This chemical oxidation-reduction reaction is the energy source for the nitrifiers to form new biomass. Because they are autotrophic microorganisms, they use CO_2 as their carbon source. The complete reaction including cell synthesis is described in Eq. (2-4) (USEPA, 1975; Hatch et al., 2005):

$$NH_4 + 1.83O_2 + 1.98HCO_3^- \rightarrow 0.021C_5H_7NO_2 + 0.98NO_3^- + 1.041H_2O + 1.88H_2CO_3 \quad \text{Eq. (2-4)}$$

It can be concluded that nitrification is an oxygen and alkalinity consuming process. Nitrifiers, as a group of autotrophs, have a low yield rate and growth rate. The most important parameter is solids retention time (SRT), which should be maintained high enough in bioreactors for total nitrogen removal treatment, promoting healthy growth of nitrifiers and preventing their washouts. In addition, appropriate dissolved oxygen (DO) and alkalinity levels should be retained during the nitrification period when centrate is treated.

2.4.2 Environmental factors influencing nitrification

Nitrifying bacteria are sensitive to ambient environmental conditions. There are many factors influencing their metabolic activities, including DO, temperature, pH, alkalinity, free ammonia, and toxicity etc. (Metcalf & Eddy, 2003). These factors create barriers for the efficient treatment of centrate.

Nitrification is an aerobic process, and oxygen is used as the electron acceptor in the energy-generating oxidation-reduction reaction. Low DO concentration restrains the nitrification rate; specifically nitrite oxidation step is hampered greater than ammonia oxidation stage. Hanaki et al. (1990 b) reported that at a DO of 0.5 mg/L, the conversion of ammonia to nitrite could proceed to completion with a long retention time whereas the nitrite oxidation was totally inhibited. Similar results were also demonstrated by Ruiz et al. (2003). Besides the authors stated that ammonia accumulated, i.e. the ammonia reductase was hindered, when DO became below 0.5 mg/L and all ammonia was oxidized to nitrate with increased DO concentrations of over 1.7 mg/L. The phenomenon of slightly affected ammonia conversion and inhibited nitrite oxidation at low DO concentration results in a nitrite accumulation present in solutions (Grunditz et al., 2001; Pollice et al., 2002). It is also known that un-ionized nitrous acid (HNO_2) produced by the gathering of nitrite is an inhibitor for nitrification and is behind the prohibited nitratation rate (Yoo et al., 1999; Zheng et al., 1994).

Ruiz et al. (2003) found that the inhibitory effect of DO on oxidation of nitrite to nitrate took place when DO reached 1.4 mg/L. Thereafter the percentage of nitrite accumulation increased with the continued decreasing DO concentration, which climaxed to 65% at the DO of 0.7 mg/L, where 98% of ammonia was oxidized. When DO decreased to below 0.7 mg/L, the negative influence of DO on ammonia oxidizers occurred, resulting in the declining percentage of both oxidized ammonia and nitrite accumulation. To avoid depression of nitrification by DO, a DO of 2 mg/L is usually required to be maintained in an aerobic phase for the full ammonia removal with the end product of nitrate (Stenstrom

et al., 1980). The accumulation of nitrite is probably attributed to the difference in saturation constant in terms of DO between the nitrifiers and denitrifiers. In addition, the higher rate of ammonia oxidation than that of nitrite conversion at low DO levels could be another factor (Hanaki et al., 1990 b).

Like other biochemical reactions, the nitrification process is influenced by temperature. It was reported that the nitrification rate decreases with the declining of temperature (Louzeiro et al. 2002; Ilies et al., 2001). WEF (1998) described that the nitrification activity was very limited when temperatures were below 10°C. The influence of a sudden decrease in temperature on nitrification rate was studied by Head and Oleszkiewicz (2004), who reported that 58%, 71% and 82% of average decreases in nitrification rate were observed when the biomass, which was acclimated to 20 °C, 25 °C and 30 °C respectively, was cooled to 10 °C. Thus it can be concluded that there is approximately 50% decrease in nitrification rate corresponding to every 10 °C of temperature drop. However Oleszkiewicz and Berquist (1988) found that the temperature above 7 °C had a non-pronounced effect on ammonia oxidizing process but the negative impact became vary pronounced when temperatures decreased to the range of 2 °C -7 °C. The same authors demonstrated a general trend for that ammonia removal rate was deteriorated gradually responding to the decrease of ambient temperatures. The response had no continuity. The consequence of the reduction in nitrification rates under lower temperatures may be explained by the slower activity of bacteria enzymes.

Nitrification is pH-sensitive and will proceed at a significantly decreased rate when pH values are below 6.8 (Metcalf & Eddy, 2003). USEPA (1993) reported the nitrification rate at pH of 5.8-6.0 was only 10-20% of the rate when water pH was 7.0. Groeneweg et al. (1994) also studied the influence of pH on the nitrification rate and found that the maximum ammonia oxidation rate was achieved between 7.5-8.0, which was comparable with the values presented by Metcalf & Eddy (2003), and the bacteria activity dropped dramatically when pH values dropped to 6.0-6.7. While Ruiz et al. (2003) concluded that under an acclimated-biomass condition, nitrification process can proceed to completion, which meant no accumulation of nitrite, within the pH range of 6.45 – 8.95, but beyond this pH range, complete inhibition on ammonia consumption occurred.

Since the activity of nitrifying bacteria is limited when pH increases to over 9 or decreases to below 6.45, and the nitrification process consumes alkalinity, which reduces pH, lowest pH in the range of 7.0-7.2 was required to maintain an optimal nitrification rate in bioreactors as indicated by Metcalf & Eddy (2003). However, centrate does not contain enough alkalinity to sustain the bulk liquid pH above this lowest pH for the completion of full nitrification (Arnold et al., 2000; Ali et al., 1998). Sodium bicarbonate can be added as alkalinity to elevate pH values in mixed liquors.

Free ammonia is a toxic compound that inhibits not only ammonia oxidizing bacteria (AOB) but also nitrite oxidizing bacteria (NOB) (Yun et al., 2003; Turk et al., 1989). Free ammonia concentration is highly dependent on pH and temperature: it increases with the rising of both pH values and temperatures (Anthonisen et al., 1976). Free ammonia

seriously inhibits the activity of NOB at a concentration in the range of 0.1-1.0 mg NH_3-N/L while AOB, which is less sensitive than NOB, is hindered when the free ammonia concentration is between 10-150 mg NH_3-N/L (Kim et al., 2006). Centrate has the nature of high concentration of NH_3-N and temperature. Therefore the free ammonia level, when centrate is treated, should be properly maintained.

Biodegradable COD in mixed liquor is another factor influencing the performance of ammonia oxidizers and nitrite oxidizers. It is widely known that nitrification rate decreases with increased organic carbon. Dytczak et al. (2007), who studied the ozonization of RAS to provide extra organic carbons to main stream, stated the nitrification rate in the main reactors would decrease when the readily biodegradable carbon was introduced to aerobic zones, possibly resulting from the direct destruction of nitrifying bacteria and the competition from the faster growth of heterotrophs with more carbon available. Hanaki et al. (1990 a) also reported that the activity of heterotrophs can cause an inhibitory effect on ammonia oxidation. The same trend was demonstrated in the research of Takach (1999) as shown in Figure 2-4. In this nitrification study, raw sewage was used. It also showed that a higher ratio of carbon to nitrogen (C/N) hindered nitrification rate. The inhibition on nitrification caused by biodegradable carbon may be attributed to the low DO concentration because heterotrophys consumes a large amount of oxygen to oxidize organic substance.

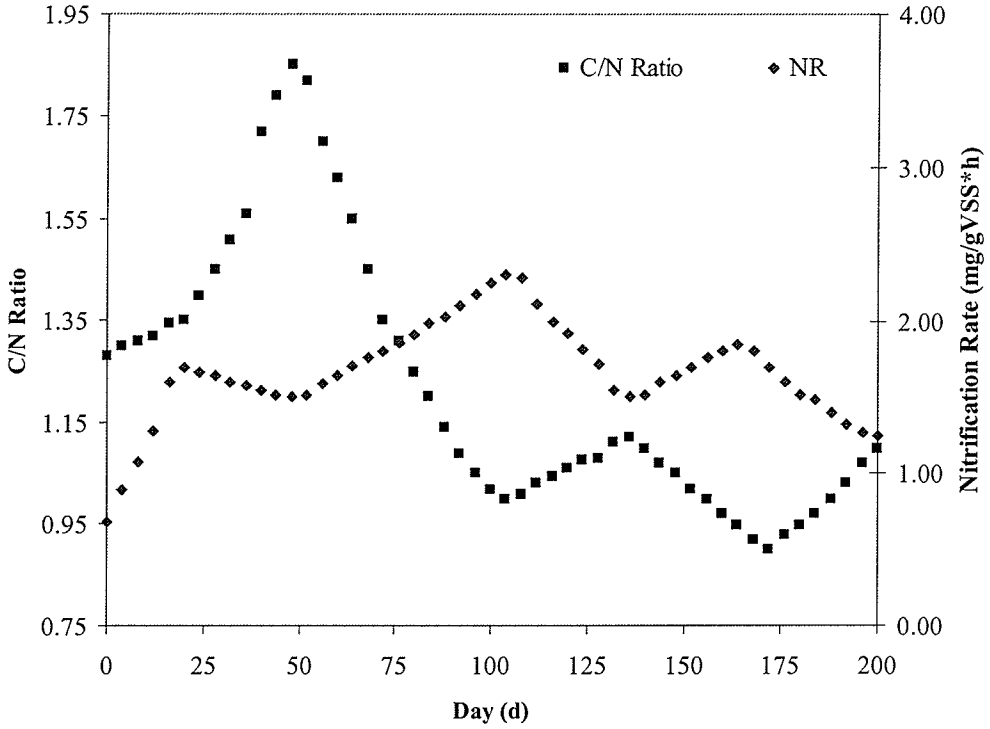


Figure 2-4 Relationship between C/N ratio and nitrification rate (Adapted from Takach, 1999)

When the negative effects of biodegradable carbon on nitrification process are combined with other limiting factors, like DO, the inhibition would become more predominant. Such deterioration is the case in the study, shown in Figure 2-5, which was undertook by Hanaki et al. (1990 b). The research demonstrated that the ultimate effluent ammonia concentration decreased to below 2 mg/L in all four cases under the condition of sufficient DO and prolonged HRT. However the reduction rate of effluent ammonia concentration became slower with the higher influent COD concentration, which meant carbon substrate restrained nitrification process. When DO concentration was limited to 0.5 mg/L, the final effluent ammonia concentration increased case by case with the enlargement of influent COD amount. The results clearly indicated the inhibitory effects of influent COD on nitrification process could be worsened by the limiting DO

concentration. Therefore the influence of carbon sources on the nitrification process should be checked after they are added to bioreactor.

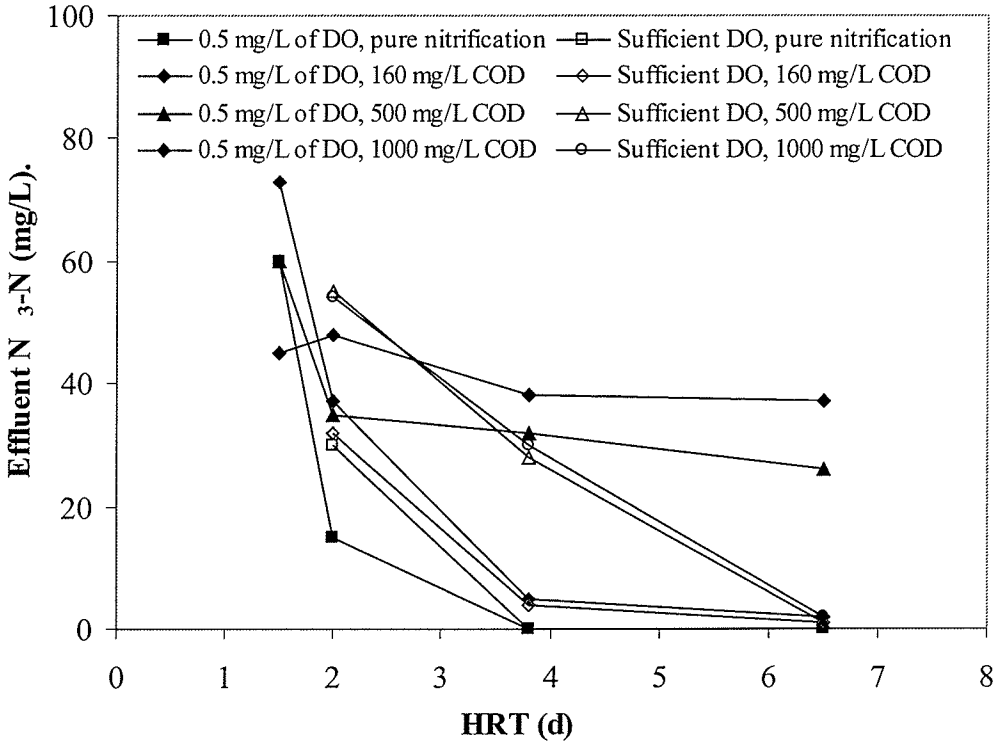


Figure 2-5 Combined effects of DO and influent COD on nitrification (Adapted from Hanaki et al. 1990 b)

2.5 Denitrification process

2.5.1 Description of denitrification

Denitrification is the second and integral stage of the two-step TN biological removal process. The dissimilative reduction of nitrate to gaseous nitrogen by denitrifying bacteria is known as the denitrification process. Many facultative heterotrophic microorganisms are capable of performing this function (Kraume et al., 2005; Qasim,

1998; Carley et al., 1991). The denitrifiers absorb organic materials from outer surroundings to use not only as carbon source, but also as the electron donors. They gain energy from transferring the electrons from organic materials to nitrate and nitrite. Nitrate and nitrite which combine with electrons become nitrogen gas and are ultimately removed from wastewater in gas phase, resulting in the removal of TN (Carley et al., 1991; Metcalf & Eddy, 2003).

The conversion of nitrate to nitrogen gas follows the pathway from nitrate to nitrite, to nitric oxide, to nitrous oxide, and to nitrogen gas as in Eq. (2-5) (Kraume et al., 2005; Metcalf & Eddy, 2003).

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad \text{Eq. (2-5)}$$

This transformation utilizes organic matters and produces energy for the new cell synthesis. The reproduction also needs organic compounds as the carbon source. The overall energy and biomass synthesis reaction can be described in the Eq. (2-6) (Qasim, 1998; Metcalf & Eddy, 2003; Hatch et al., 2005).

$$NO_3^- + Organic \quad matter \rightarrow \\ New \quad biomass + N_2 + CO_2 + H_2O + OH^- \qquad \text{Eq. (2-6)}$$

It is obvious then that organic compounds present in centrate are of essential importance to the TN removal from centrate.

2.5.2 Environmental factors influencing denitrification

Denitrifiers functions to convert nitrate to nitrogen gas without the presence of oxygen. The heterotrophic denitrifying bacteria are more robust than nitrifiers and less sensitive to the environmental conditions. The factors that impact their performance include DO, temperature, pH and available biodegradable carbon source. The impact of DO and carbon source dominates.

In terms of the effects of pH, denitrifying bacteria are not as sensitive as nitrifiers, especially when the pH in solutions is maintained in the optimum pH range for denitrification process, which has been reported to be between 7 and 8 by Thomas et al. (1994), and Wang et al. (1995). However, when pH values lessen to below 7, the influence of pH becomes predominant. Both Napier et al. (1988) and Silverstein et al. (1993) reported a pH of below 5.8 significantly prohibited the denitrification of a wastewater with high concentrations of nitrate (1000-9000 mg/L NO_3-N). The dramatic decreasing of denitrification rate with a pH lower than 7 was also demonstrated by Randall et al. (1992) and Glass et al. (1997, 1998). The decreased denitrification rate may be attributed to the inhibitory impact of unionized nitrous acid species accumulating in the acidic conditions.

When pH values in solutions fall into the alkaline range, the effects of pH become less noticeable than when pH is below neutral value. Glass et al. (1998) stated that when pH values were 7.5, 8.5, and 9, denitrification was achievable and the total time needed for

complete denitrification, which meant no residuals of both nitrite and nitrate present, was similar under the three conditions. However, when denitrification is separated into two individual processes, nitrate reduction and nitrite reduction, the pH effects on these two sub-processes are significantly different. The specific nitrate reduction rate increased from 25 to 54 mg NO_3-N/ g MLSS* h when pH value rose from 8.5 to 9 (MLSS stands for Mixed Liquor Suspended Solids). On the contrary, the specific rate of nitrite reduction remained unchanged (Glass et al. 1998). The constant nitrite reduction rate may be explained by the inhibition of nitrate on it. As a result, there was nitrite accumulation happening in reactors (as shown in Figure 2-6). Furthermore, the accumulation became more obvious as the pH rose.

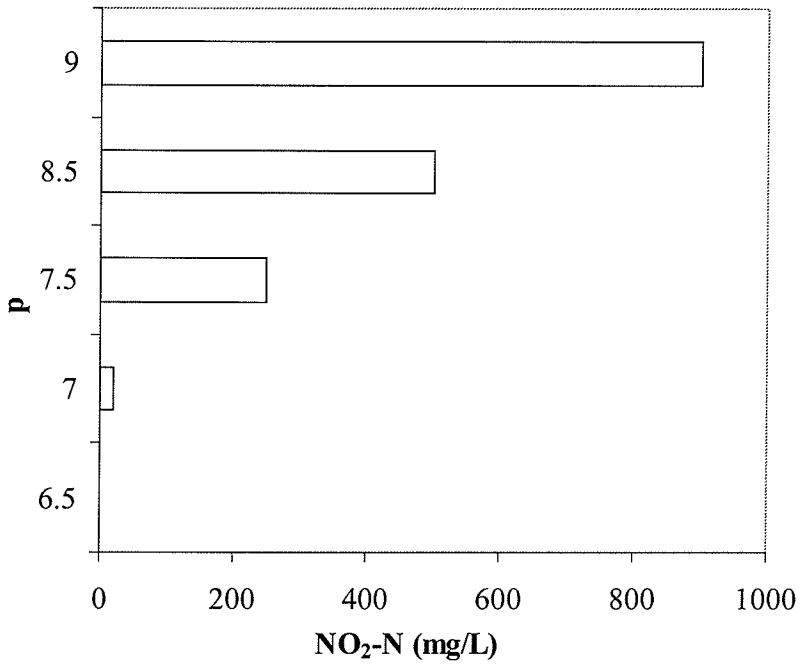


Figure 2-6 Effects of pH on maximum nitrite accumulation (Adapted from Glass et al., 1998)

Glass et al. (1998) also demonstrated that the suppression of the rate of nitrite reduction increased to 63 % from 33% when pH increased to 9 from 7.5, but the same nitrite reduction rates were obtained. Therefore with the absence of nitrate and the same pH change, the specific nitrite reduction rate increased significantly. As a result, after the entire consumption of nitrate, nitrite was converted to gaseous nitrogen at a much faster pace. Consequently, as stated earlier, the overall time for complete denitrification remained constant. It is worth to mention that denitrification process can produce alkalinity, which increases the pH in solution. In fact, approximately half of the alkalinity consumed by nitrifying bacteria could be recovered by denitrification process (Qasim, 1998).

Temperature is another factor influencing the denitrification process. Mokhayeri et al. (2006) stated that temperature had a more significant impact on denitrification than pH and proper temperature should be maintained in bioreactors. A study conducted by Ilies and Mavinic (2001) indicated that an approximate 15 % decrease in denitrification rate was experienced immediately after temperature was changed to 17 ^{o}C from 20 ^{o}C.

Oleszkiewicz and Berquist (1988) showed the general trend that specific denitrification rate (K_{DN}) decreased as temperature declined. The highest K_{DN} of 21 g N/kg VSS*d was achieved at 15 ^{o}C and under 2 ^{o}C, 3 g N/kg VSS*d, the lowest K_{DN}, was measured. It was also noted that in the temperature range of 7 ^{o}C to 15 ^{o}C, the K_{DN} was comparable in both 8h-cycle reactors and12h-cycle reactors. In contrary, the influence of temperature on specific denitrification rate became more significant in 12h-cycle reactors than in 8h-

cycle reactors when temperature dropped under 7 °C, mainly because the shorter anoxic period of 4 h per day in 12h-cycle reactors compared with the 6 h overall anoxic time per day in 8h-cycle reactors. In addition, the response of denitrification to declining temperature was not continuous. As noted, the K_{DN} decreased more quickly in the temperature rage of 7 °C to 2 °C than in the range of 7 °C to 15 °C. Oleszkiewicz and Berquist (1988) presented that the temperature correction factor was 1.06 when temperature lowered from 15 °C until 7 °C and a greater number of 1.3 was for the range of 7 °C to 2 °C. The difference in these two temperature correction factors backed the fact of the discontinuous response of K_{DN} to temperature. The similar results were also demonstrated by Carrera et al. (2003).

Denitrifiers are facultative aerobic microorganisms which means they can uptake oxygen and nitrate as electron acceptors with or without the presence of oxygen, respectively. For TN removal, the absence of oxygen should persist in bioreactors, which facilitates the denitrifiers to utilize nitrate in lieu of oxygen to proceed. If the DO concentration is high, DO can restrain the nitrate reduction enzyme, which results in the inhibition of the nitrate reduction. Therefore DO is one of the most important factors influencing denitrification process. There are many limiting thresholds reported by various researchers. Pochana and Keller (1999) and Qasim (1998) related that the denitrification performs with highest efficiency with zero DO concentration and the effectiveness of denitrification process decreased with DO concentration higher than 0.2 mg/L.

The effect of DO concentration on denitrification rate was also studied in a research conducted by Munch et al. (1996). The authors found that the response of denitrification rate to increasing of DO was not constant over the aerated react period. They demonstrated that when DO increased to 1 mg/L, the denitrification rate dropped to only around 30 % of the highest rate of denitrification, which was achieved with zero DO concentration. When the DO concentration was further increased to over 2 mg/L, the speed of the decreasing in denitrification rate became slower than when DO was below 1 mg/L, which meant the DO over 2 mg/L had a less influence on denitrification rate, mainly because the denitrification rate had already decreased to a very low level when DO reached 2 mg/L. The lowest denitrification rate was only about 0.2 mg/L/h with a high DO concentration of 9 mg/L.

In theory, the denitrification process would cease entirely at a DO concentration of 0.1 mg/L to 0.2 mg/L. However, many researchers (Ju et al., 2007; Yoo et al., 1999; Zeng et al., 2003) found that there was still denitrification present when DO concentration was greater than 0.2 mg/L. In low F/M (Food to Microorganism ratio) conditions, even when bulk liquid DO concentration was 0.5 mg/L, denitrification can happen (Oleszkiewicz, personal communication). The occurrence of denitrification at DO concentration higher than 0.2 mg/L could be explained by an oxygen diffusion limitation into the activated sludge flocs. When the bulk liquid DO concentration is low, less and less oxygen will penetrate into interior part of a floc in the course from the surface of a floc to its centre. As a result, an anoxic zone will be created at the centre of an aerobic activated sludge floc, where some heterotrophic bacteria can denitrify.

Since an aerobic zone is created on the edge of an activated sludge floc, both nitrification and denitrification processes can happen with low bulk liquid DO concentration: with nitrification at the surface of a floc and denitrification in its centre. This phenomenon is termed as simultaneous nitrification and denitrification (SND or SNdN). SND has been extensively studied over the past years by many researchers. A DO of 0.5 mg O_2 /L is generally recommended for the complete TN removal from wastewater at the same time (Pochana and Keller, 1999; Zeng et al., 2003).

Besides the above mentioned influencing factors for denitrification, carbon source is the most important parameter that significantly impacts the overall performance of the denitrifying bacteria. It is discussed in detail in the following subsection.

2.5.3 Carbon sources for denitrification

2.5.3.1 Background of carbon sources

In a TN removal system, there has to be aerobic and anoxic zones available for both biological nitrification and denitrification to occur. Denitrification can be employed either before or after nitrification. Preanoxic denitrification is the term used when the anoxic zone precedes the aerobic period. When the conversion of nitrate to gaseous nitrogen follows nitrification, this configuration is named postanoxic denitrification. In postanoxic denitrification, biodegradable carbon is removed at the aerobic zone and is not

available for the oxidation-reduction reaction of converting nitrate to nitrogen gas (Kraume et al., 2005; Metcalf & Eddy, 2003; Qasim, 1998; Carley et al.. 1991).

The denitrification process has an extreme demand on organic compounds, i.e. carbon sources. Not only the quantity of the carbon source but also the quality of the electron donors can impact the denitrification rate (Hallin et al., 1998). Basically, there are three carbon sources for the denitrifiers: organic substrate contained in raw wastewater, the residual produced by metabolic decay (endogenous carbon), and exogenous carbon added to wastewater. The external carbon sources are usually readily biodegradable chemicals and are added at the optimal dosage, thus they have the highest denitrification rate, up to 20 mg NO_3-N/gVSS*h (Kraume et al., 2005). The organic materials contained in the raw wastewater have a small portion of easily biodegradable carbon. The amount of organics compared to the nitrogen contents in raw wastewater is low, for instance, the ratio of carbon to nitrogen (C/N) is around 3:1 in raw sewage wastewater and less than 1:1 in centrate stream, so the denitrification rate using carbon sources available in raw wastewater is around 1-6 mg NO_3-N/gVSS*h (Kraume et al., 2005; Bolzonella et al., 2001), which is lower than when an external carbon source is supplied. When the third carbon source of endogenous carbon is obtained, the denitrification process becomes a fermentative process and it is a slow rate process, consequently denitrification happens at the lowest rate, 0.2-0.6 mg NO_3-N/gVSS*h (De Lucas et al., 2005; Kujawa and Klapwijk, 1999), compared to the two previous carbon sources.

For the preanoxic denitrification, the original organic matters in raw wastewater are used as carbon sources, while postanoxic denitrification utilizes the decay carbon as its sources of carbon. The preanoxic system has two major advantages over the postanoxic denitrification. It has a relatively higher denitrification rate than the configuration of denitrification zone following the aerobic tank, therefore the overall hydraulic retention time (HRT) can be lowered resulting a smaller anoxic zone (Kraume et al., 2005, Hallin et al., 1998). The other advantage is the biodegradable carbon is removed before reaching the aerobic zone, therefore the system demand on oxygen is reduced and savings can be gained on the lower aeration requirement (Kraume et al., 2005).

Since raw wastewater often has a low C/N ratio, the denitrification process can be constrained even in a preanoxic system (Trela 1998). In order to increase the denitrification rate and lower the system HRT and reactor volume, external carbon sources are required in the anoxic zones for both preanoxic and post anoxic TN removal systems (Sadick et al., 2000; Kraume et al., 2005; Caffaz et al., 2005). With the addition of external carbon sources, the denitrification rate is boosted. From a study as in Figure 2-7, it can be inferred that denitrification performs best at the highest ratio of sCOD/N (Takach, 1999). The figure showed the denitrification efficiency was increased from 50 % to 70 % as the sCOD/N ratio rose from 2:1 to 5:1.

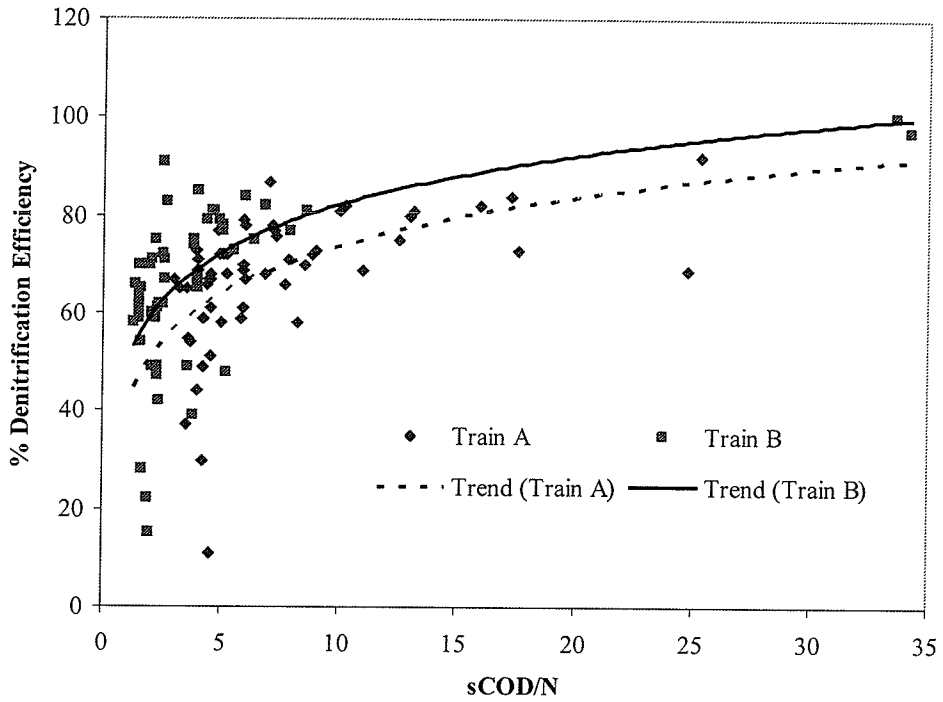


Figure 2-7 Relationship between sCOD/N and denitrification efficiency (Adapted from Takach, 1999)
sCOD = soluble COD

2.5.3.2 Carbon sources in form of single chemical compounds

Several published works (Reyes-Avila, 2004; Labelle, 2005; Punal, 2000; Monteith et al., 1979) recommend that the typical carbon to nitrogen ratio (C/N ratio) should be greater than 5:1 to facilitate the nitrogen removal process. To reach this level, possible external carbon sources which can be added to the TN removal system include purchased chemicals such as methanol, ethanol, glycol, glycerol and acetate etc., fermented primary sludge, and various industrial wastewaters, especially from the food-processing industry (Hallin et al., 1996a; Tam et al. 1992).

The addition of purchased chemicals is the most usual method utilized by many WWTPs. Methanol and ethanol are the two most frequently used chemicals with methanol more common than ethanol because of its cost advantage over ethanol. However, methanol and ethanol are highly flammable substances, and their price is climbing. Other organics used include glucose and acetate. The influence of externally added carbon sources on denitrification rate is shown in Figure 2-8.

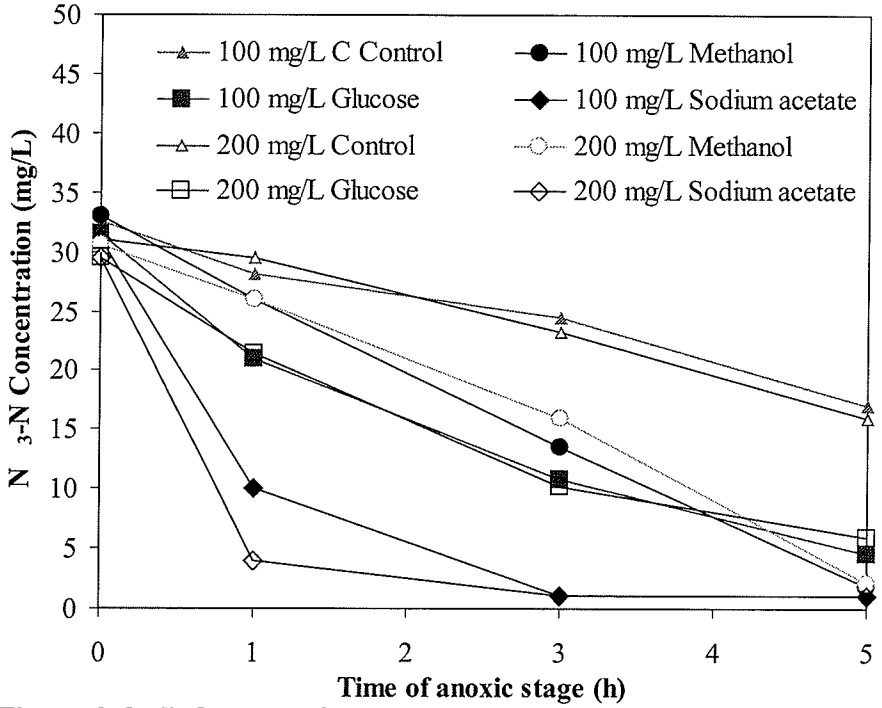


Figure 2-8 Influence of external carbon source on denitrification (Adapted from Tam et al., 1992)

The above figure demonstrated that when methanol, glucose, and sodium acetate were added to wastewater, the nitrate concentration in wastewater decreased more quickly compared to the control condition, which had no extra added carbon sources. The faster declining rate of nitrate concentration meant a greater denitrification rate. The addition of sodium acetate produced the highest denitrification rate, while the lowest enhancement

was obtained with the addition of methanol. Methanol can be utilized only by special bacteria (methylotrophs). A long acclimation time before an increase in denitrification rate is observed (Andersson et al, 1995; Nyberg et al., 1992; Hallin et al., 1998; Hallin et al., 1996a). When the denitrification rates produced by the same compound but with different dosages were compared, it was found that higher denitrification rate was produced with higher dosage of added chemicals. As the higher dosage increased the C/N ratio, it can be concluded that denitrification proceeded at a higher rate with a higher C/N ratio. This conclusion backed the findings of Takach (1999).

Hallin et al. (1996a) indicated that the denitrification process can also be intensified by ethanol, the specific denitrification rate increasing to 5 times the rate with no ethanol addition. In addition, an immediate rising of denitrification rate was observed after the addition of ethanol. Therefore, compared to methanol, ethanol instantly increased the denitrification rate (Andersson et al., 1995; Hallin et al., 1996a) and can be used in intermittent addition (Hasselblad et al., 1998).

In addition to the above mentioned external carbon substrates, other organic chemicals, such as glycerol and glycol, are also used. The impact of the intentionally added organic matters on denitrification rates has been widely researched. The results are summarized in Table 2-5. The large variations are noted in the table even with the same organic compound. The different specific denitrification rates (SDNRs) can be attributed to the different constituents' combination in individual raw wastewaters and the various amounts of denitrifying bacteria which are present in different activated sludges.

Table 2-5 Specific denitrification rates with different chemical carbon sources

Literatures	SDNR (mg NO_3-N/g VSS*h)					
	Raw Municipal Wastewater	Methanol	Ethanol	Glycerol	Glycol	Acetic
Trela, (1998)			6-9		6.5-8.4	5-9
Carley, (1991)		3-10				0.5-2
Hallin, (1996a)			7-15			
Qasim, (1998)	1-5					
Kraume (2005)	1-6					
Peng (2007)	0.3-1.53	3.2	9.6			12
Andersson (1998)	0.5-2	1.8-5.4	7.5-18			
Hallin (1998)		3-5	6-15			6-8
Akunna (1993)				7.4-10.1		23.8-27.8
Henze (1989)	2.5-6.0					
Henze (1991)	1-5					
Kujawa (1999)	0.6-1					1-3
Hallin (1996a)	1-3	9				5-8
Grab. (1985)				5-25		
Pecenka (2005)	1.1-3.9					4.5-11
Spevackova (2005)		6.6	26			

2.5.3.3 Carbon sources in form of industrial wastewaters

Denitrification rates can be boosted by the addition of organic compounds, however the process is very expensive. Millions of dollars are spent annually on purchasing carbon in the form of chemicals in large plants like Tampa FL, Blue Plains DC. Constantine et al. (2005b) reported that the Blue Plains Advanced Wastewater Treatment Plant spent between $7-8 million annually on purchasing methanol as an external carbon source. In addition, the single chemical compounds have a safety liability because of their flammability. Therefore, alternate carbon sources in the form of industrial wastes have been identified and tested to check their applicability for total nitrogen removal from wastewater. Most of the wastes were from the food-processing industry. Park et al. (2005) studied the applicability of the mecellulose wasted liquor as an external carbon source to replace methanol in a pilot plant. Some other studies are summarized in Table 2-6.

Table 2-6 Specific denitrification rate enhancement with industrial wastewaters

Literatures	Industrial wastewater type	SDNR (mg NO_3-N/g VSS*h)
Carley (1991)	Yeast waste	0.5-1
Peng (2007)	Ethanol wastewater	2-9
Sage (2006)	Dairy effluents	1.7-6.9
Cappai (2004)	Ice cream waste	5.76-7.23
	Beet-sugar waste	4.15-6.38

The conversion of nitrate to nitrogen gas was intensified when industrial wastewaters were added as external carbon substrates compared to the rates with no external carbon added. As in Table 2-5 and 2-6, the SDNR can be increased to as high as 9 mg NO_3-N/g VSS*h from the average SDNR of 3 NO_3-N/g VSS*h in raw municipal wastewaters. The wastewater from food processing industry is a good candidate for carbon source mainly because it contains a large amount of organic compounds, like carbohydrate and protein, which are easily hydrolyzed and absorbed by bacteria to be utilized in metabolic activities.

2.6 Summary

Centrate is very high in nitrogen load but has a low C/N ratio. It is better to treat it as a side stream before it contributes its nitrogen contents to the main stream bioreactors. Conventional biological TN removal processes are mostly used for the treatment of rejected sludge liquor. For the purpose of studying the optimization of the TN removal from centrate, SBR is a good choice for researchers because of its ease of operation and control. Denitrification is highly dependent on carbon sources, so low C/N ratio in centrate needs to be elevated to enhance the denitrification by the addition of external carbon substrates. Purchased organic chemicals are quite expensive and pose explosion or toxicity issues. Industrial wastewaters, especially from the food processing industry, are good alternatives to the purchased chemicals.

3.0 MATERIALS AND METHODS

3.1 Sequencing Batch Reactors (SBRs)

SBR was selected as the bioreactor for the purpose of testing the applicability of industrial wastewaters as external carbon sources to remove the nitrogen load from centrate. Three bench-scale SBRs were set up at the Environmental Engineering Laboratories at the University of Manitoba. The configuration of the bioreactors is presented in the following Figure 3-1.

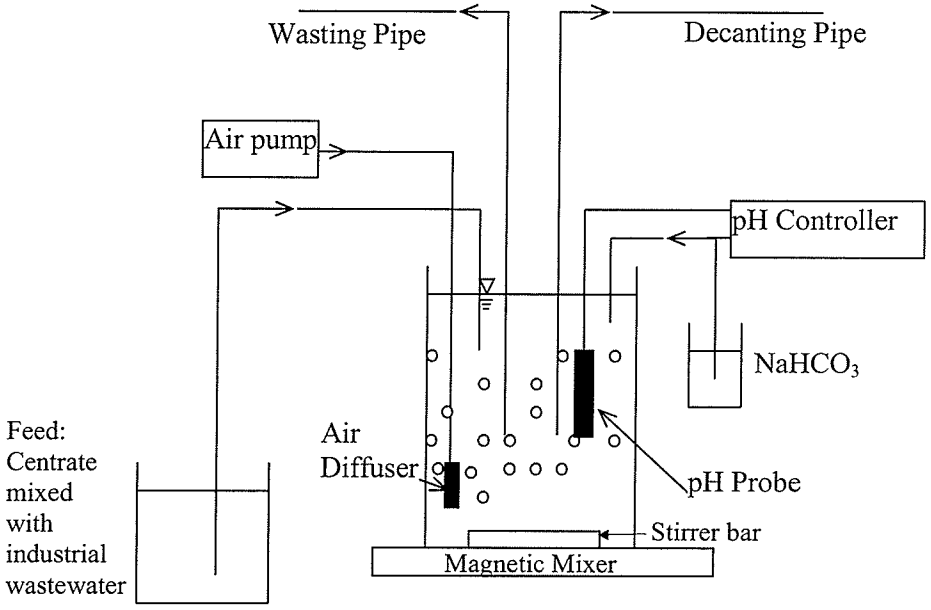


Figure 3-1 SBR configuration for centrate treatment

Each SBR was equipped with a feeding pump, a decanting pump, a wasting pump, a sodium bicarbonate pump, pH meter with automatic controller, a magnetic mixer, and an air pump (picture of SBR bioreactors shown in Appendix G). The feeding pumps were

from the Cole-Parmer Instrument Company (Catalog No. 7553-20; 6-600 RPM), while the remaining pumps were also from the Cole-Parmer Instrument company but were a different model (System model No. 7553-80; 1-100 RPM). The magnetic mixers (Fisher Scientific Isotemp®) were in operation at all times except for the settling and decanting period to ensure homogeneous mixing in reactors. These mixers together with the feeding, wasting, and decanting pumps were automatically controlled by timers following the preset sequences. Air pumps (Rolf C. Hagen Inc.; AquaClear® 20; 2,250 cc/min) were used to maintain proper dissolved oxygen (DO) levels in the bioreactors to sustain the metabolic activities of the bacteria, especially the nitrifiers, which are affected more significantly than other microorganisms are. The air pumps were also controlled automatically by timers and they were not operated following the same sequence in the three reactors.

One of the reactors (R1) was aerated all the time, therefore only the nitrification process was happening in this reactor. The other two reactors (R2 and R3) were operated under the condition of alternating aerobic and anoxic, which was obtained by turning the air pumps on and off at intervals. Consequently the full nitrogen removal was achieved in the later two reactors as the result of the presence of both biological activities of nitrifiers and denitrifying bacteria.

All reactors were run using one fill-and-draw cycle per day. Table 3-1 shows the different sequences that the air pumps followed in the three reactors. For R2 and R3, each cycle had 5 h anoxic period and 17 h 45 min aerobic period.

Table 3-1 SBRs operational sequences

Aerobic Reactor (R 1)				
Start	Steps	Lasting	Air	Mixing
12:00	Aerobic	-	•	•
10:30	Wasting	10m	•	•
10.40	Settling	1h5m	-	-
11:45	Decanting	15m	-	-

Alternating Anoxic/Aerobic Reactors (R2&R3)				
Start	Steps	Lasting	Air	Mixing
12:00	Anoxic	1h	-	•
13:00	Aerobic	1h	•	•
14:00	Anoxic	1h	-	•
15:00	Aerobic	1h	•	•
16:00	Anoxic	1h	-	•
17:00	Aerobic	1h	•	•
18:00	Anoxic	1h	-	•
19:00	Aerobic	1h	•	•
20:00	Anoxic	1h	-	•
21:00	Aerobic	-	•	•
10:30	Wasting	10m	•	•
10:40	Settling	1h5m	-	-
11:45	Decanting	15m	-	-

These three SBRs were initially seeded with the biomass from the NEWPCC in the City of Winnipeg. This WWTP, as mentioned earlier, is a high purity oxygen activated sludge (HPOAS) BOD removal only plant operated under the condition of the solids retention time (SRT) of 2.5 days and the average dry weather flow (ADWF) of 195 ML/d. A long acclimation time of approximate 30 days was needed in the beginning for the growth of ammonia and nitrite oxidizing bacteria.

The centrate used in this study was obtained from the NEWPCC in Winnipeg, the same source as the initial seed biomass. The NEWPCC sludge handling facility consists of mesophilic anaerobic digesters, sludge holding tanks, and centrifuges. The digesters receive the mixture of primary sludge and waste activated sludge from all three water pollution control centers (WPCCs) in the city. After digestion, the digested sludge is dewatered in the centrifuges and the rejected water becomes centrate. It was collected

from there and delivered to the environmental engineering labs at the University of Manitoba by Physical Plant staff. The delivery was made three times per two weeks and each time consisted of 3 containers of 20 liters. The centrate was stored in refrigerators at the temperature of 4 °C.

Four industrial wastewaters were selected for this study: potato processing wastewater from Simplot Foods Ltd in Portage la Prairie, MB, Ca; canola processing and oil refining wastewater from Bunge Canada in Harrowby, MB, Ca; glycerol, a byproduct of biodiesel industry; and glycol, the main contaminant in the deicing wastewater from the Winnipeg International Airport. Among them, the wastewaters from the potato processing and canola oil refining factories were delivered, as requested, by couriers directly from the producing facilities to the environmental engineering labs, and were also kept refrigerated at 4 °C. The wastewaters from the biodiesel industry and the airport were synthetically produced with the commercial-brand glycerol and glycol solutions in lieu of the real wastewaters. For the purpose of comparison, the most commonly used external carbon sources, methanol and ethanol, were also tested in this study. All of the commercial-brand chemicals were from Fisher Scientific.

The feedings to the bioreactors were prepared using raw centrate mixed with different carbon sources and were added to all three SBRs at the beginning of each cycle. The influent to each SBR was 2 L. With potato processing and canola processing wastewaters as carbon sources, the ratio of the volume of raw centrate to the volume of these two industrial wastes in the feedings was changed periodically; with glycerol,

glycol, ethanol and methanol as carbon sources, the influent consisted of 1.4 L of raw centrate and 0.6 L of chemical solutions, i.e. mixtures of chemical and tap water. For each investigation performed on one specific chemical, different volumes of this chemical were used to make different dosages in the feedings. The volumes of chemicals which should be applied were determined based on ammonia concentrations of raw centrate, preferred dosages and bsCOD strength of each milliliter of chemical (Table 3-2). An example of calculation is presented in Appendix F. Therefore, the influent NH_3-N concentration varies with the fluctuation of NH_3-N in raw centrate.

Table 3-2 bsCOD equivalent of glycerol, glycol, ethanol and methanol

Chemical	bsCOD equivalent (mg bsCOD/ mL chemical)
Glycerol	1360
Glycol	1415
Ethanol	1641
Methanol	1187

Each reactor had 5 liters of working volume filled with mixed liquor of activated sludge. The ammonia and nitrite oxidizing bacteria are autotrophic and slow-growing, therefore a long SRT should be kept in a biological system containing nitrification process to avoid the washout of these functioning bacteria. The SRT in the SBRs set up in this study was 10 days, i.e. about one tenth of the total volume, 500 mL, was wasted every day. The bioreactors were fed once per day with 2 liters of feedings, therefore the operation hydraulic retention time (HRT) was 2.5 days. To keep the balance of flows, except for the 500 mL of wastage each day, 1.5 L of the supernatant in each reactor was drawn per day as decanting wastewater. The decant became the effluents from the SBRs.

Nitrification is an alkalinity-consuming process. On the contrary, denitrification is an alkalinity-producing process. Denitrification can only compensate for about one third of the alkalinity utilized by nitrifiers. Therefore the pH values in bioreactors will decrease with the consumption of the alkalinity. To maintain a beneficial pH level, the sodium bicarbonate solution, with a concentration of 60 mg/L, was added to all three bioreactors to elevate the alkalinity capacity. The addition of $NaHCO_3$ (sodium bicarbonate) solution was automatically controlled by pH meters (Eutech Instruments Pte Ltd.; αlpha pH 200 Series). Since the pH control value of 7.1 was set for the pH meters, the addition would start when the pH in bioreactors reached 7.1. The pH value of 0.2 was chosen as the buffer range, which meant the addition would stop when the pH was increased to 7.3.

The SBRs were operated in the open area of the laboratories, thus the room temperature was maintained year round at 21±2 °C.

3.2 Bioreactor maintenance

Bacteria are prone to attach onto the surfaces which they are in contact with. For SBRs, the biomass always accumulated on the interior walls of the bioreactors both above and under the mixed liquor surface. It also gathered around the exterior surfaces of the pipes for feeding, WAS, decant, and air supply, as well as pH probes. For the optimum performance of the bioreactors, especially the accurate control of SRT, this attached biomass was manually scraped back to the mixed liquor using spatulas several times per day.

The growth of bacteria was noted in the pipes and jars used for transporting and holding feedings, WAS, and decant as well, so the tubings were cleaned or changed periodically. The biomass could also grow around the air diffusers and block them thereby interfering with air transfer. To minimize these negative effects, the diffusers were changed every week and cleaned in bleach solutions. In addition, pH control was important in terms of the proper operation of the bioreactors, therefore the pH meters were calibrated each week using the standard pH solutions of 7.0 and 10.0.

In addition to the above maintenance work, the following parameters were recorded daily in order to monitor the conditions and performance of the bioreactors: consumption of $NaHCO_3$ solution, pH value in bioreactor, and three temperatures of room, feeding, and mixed liquor.

3.3 Sample collection

To thoroughly monitor the overall performance of the centrate treatment systems with different external carbon sources, samples were collected three times per week at three different locations of the systems: feedings, WAS, and decant. The volume of each sample was 20 mL.

The concentrations of total suspended solids (TSS), volatile suspended solids (VSS), ammonia (NH_3-N), nitrites (NO_2-N) and nitrates (NO_3-N) were measured for all of the three samples collected weekly from feedings and decant, with the exception that the

concentrations of total chemical oxygen demand (TCOD) and soluble chemical oxygen demand (sCOD) were measured for two out of the three samples. For the samples collected from the wastage lines, only the mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) were tested for all of the 3 samples.

Among these measured characteristics, the TSS, VSS, TCOD, sCOD, MLSS and MLVSS were analyzed immediately after the samples were collected. For the NH_3-N, NO_2-N and NO_3-N analysis, the samples were preserved using a drop of phosphoric acid and stored in refrigerators at the temperature of 4 °C. The preserved samples were analyzed once a week. The sampling and analysis schedules are summarized in Table 3-3.

Table 3-3 The schedule of sampling and analysis

Items to be tested	Collection times	Analysis times	Sample ports
NH_3-N, NO_2-N, NO_3-N	3 times/week	1 times/week	Feedings, decant
TCOD, SCOD		2times/week	
TSS, VSS		3times/week	Feedings, WAS, decant

After the delivery of centrate, potato processing wastewater, and canola processing and oil refining wastewater to the environmental labs, samples of 50 mL for each were taken and analyzed for NH_3-N, TSS, VSS, TCOD and sCOD. In addition, alkalinity concentration was measured only for the samples of the raw centrate.

3.4 Ammonia uptake rate (AUR) tests

The addition of carbon sources into bioreactors has a potential effect of inhibition on the nitrification process. The hindering impact is probably because the extra growth of

heterotrophic bacteria with the additional gain of biodegradable carbons out-competed the nitrifiers, which were autotrophic bacteria, and the relative low DO concentration created by aerobic consumption of biodegradable carbons. The degree of this hampering effectiveness was determined in the AUR tests. To perform the AUR tests, the procedures outlined in Kristensen et al. (1992) were followed.

The biomass utilized to perform the AUR tests need to be nitrifying biomass. Therefore the wastage from the aerobic R1 was used as the seed biomass because the nitrifiers in alternating aerobic/anoxic R2 and R3 may be inhibited by additional carbon sources. To prepare the tests, after the wastage was settled, the clear supernatant was dumped into sinks leaving the concentrated biomass within the jar in which the wastage was contained. The biomass was then divided into two equal parts and poured into two 400 mL beakers. Meanwhile, the solutions of raw centrate mixed with deionized water and the to-be-tested carbon source were prepared separately. The solution of centrate mixed with deionized water was used as the blank for reference. The basis of the tests is the degree of the influence can be clearly shown by the difference between the AURs obtained with these two different solutions. The total mixed liquor volume in the test beakers was 350 mL, and according to the main SBR volume and the initial NH_3-N concentration in the SBR, the amount of centrate which should be used in the preparation of the two solutions was calculated and was then applied.

To start the AUR tests, the prepared solutions were poured into the two beakers which contained the concentrated biomass from the aerobic SBR to the level of 350 mL of

mixed liquor. In the meantime, the mixed liquors were aerated. In addition, extra magnetic stirrers were needed to maintain the uniform mixed liquor in the beakers. These two beakers were operated in parallel with the mouths covered by Parafilm leaving only a small opening for ventilation. This way the effect of evaporation could be minimized and the bubbles, whose surfaces had attached biomass resulting from the aeration, were retained in the beakers. After a 10 minute stabilization period, the sampling step began. The samples, each of which was 3 mL, were taken every 30 minutes for about 9 hours until the ammonia in the mixed liquors was depleted. The pH values were monitored to ensure there was no alkalinity-limiting conditions occurring. The collected samples were then filtered through Filter Paper #1 (Cat No. 1001 125, Whatman® International Ltd.), and analyzed for ammonia. The concentrations of MLVSS in beakers were also measured. The volumetric nitrification rate, which is the change of ammonia concentration versus time, was received from the slope of the curve of the decreasing concentration of ammonia. According to the Eq. (3-1), the specific nitrification rate (SNR) was calculated from the volumetric nitrification rate divided by the tested MLVSS concentration.

$$SNR(mgNH_3 - N / gVSS \cdot h) = \frac{AUR(mgNH_3 - N / L \cdot h)}{MLVSS(mgVSS / L)} \times 1000 \qquad \text{Eq. (3-1)}$$

With the performance of AUR tests, both SNRs were obtained for the blank solution and the solution mixed with the specific carbon source. Based on these results, the effect of such carbon source on the nitrification process was reported.

3.5 Nitrate uptake rate (NUR) Tests

Because of its essential importance to this study, specific denitrification rate (SDNR) was tested for each carbon source. The values of SDNR were the parameters of indication for the applicability of industrial wastewater as an external carbon source and the degree of elevation of the denitrification process. The nitrate uptake rate (NUR) tests were carried out to measure the SDNRs following the method provided by Kristensen et al. (1992).

The biomass from the two alternating aerobic/anoxic reactors (R2 and R3), which contained a large amount of nitrate reduction bacteria – denitrifiers, was the seed biomass utilized in the NUR tests, and it was taken out of the wastage jars of these bioreactors in a concentrated form as the sedimentation of the wastage after a period of settlement. Then the biomass was separated into two 400 mL beakers with an equal amount in each. The decant from the R1 was used as the feed solution for the NUR tests, because this liquid was rich in nitrates resulting from its exposure only to the nitrification process and was ready for the nitrate reduction. For each batch of NUR tests, different amounts of carbon sources were added to this decant to make the feed solutions with various C/N (Carbon/Nitrogen) ratios. The ratio where the maximum SDNR was obtained was the optimum dosage of carbon sources. The 350 mL was the initial mixed liquor volume in the testing beakers, and the initial nitrates concentration and MLSS in them was calculated and maintained similar to those in the main SBRs.

The NUR tests began with pouring the decant from R1 mixed with different amounts of carbon sources into the biomass-containing beakers to the level of 350 mL. Meanwhile compressed nitrogen gas was introduced into the mixed liquor to strip off the dissolved oxygen. Therefore the anoxic condition was more easily maintained in the beakers to facilitate the denitrification process than without the introduction of nitrogen gas. The mixed liquor was also mixed constantly by extra magnetic stirrers for uniform mixing. In addition, Parafilm was used to cover part of the openings of these two beakers, which were operated in parallel, leaving small openings for ventilation. In this way, the effect of evaporation could be minimized and the bubbles, whose surfaces had attached biomass resulting from the introducing of nitrogen gas, were held in the beakers. The first sample was taken after a stabilization period of 10 minutes. The following samples were then taken at a 30 minutes interval for about 6 hours until the consumption of nitrates in the mixed liquor was complete. Each sample was 3 mL of mixed liquor to minimize the influence of the loss of biomass. The filter papers (Whatman® International Ltd.; Filter Paper #1 Cat No. 1001 125) were used to remove the solids from the samples before they were analyzed for nitrogen concentration. Along these concentrations, the MLVSS concentration in mixed liquor was also tested. Based on the recorded data, the volumetric denitrification rate (DNR) was obtained by observing the change in nitrate concentration versus time. Microsoft Office Excel® program was used to acquire this value and it was the slope of the curve of decreasing concentration of nitrate. Then the specific nitrification rate (SNR) was calculated from the volumetric rate divided by the concentration of MLVSS according to Eq. (3-2).

$$SDNR(mgNO_3 - N / gVSS \cdot h) = \frac{DNR(mgNO_3 - N / L \cdot h)}{MLVSS(mgVSS / L)} \times 1000 \qquad \text{Eq. (3-2)}$$

The obtained SDNR values formed the foundation on which the analysis of the potential for industrial wastewaters to be added to the denitrification process as external carbon sources for intensification was based.

3.6 Analysis of characteristic parameters

The characteristic parameters which were analyzed in this study included TSS, VSS, TCOD, SCOD, NH_3-N, NO_3-N and NO_2-N, and alkalinity. The analysis methods made reference to the Standard Methods (APHA et al, 1998).

Methods 2540D and 2540E were used to measure TSS and VSS, respectively. The samples were filtered through the Whatman® Glass Microfibre filters (934-AHTM, Cat. No. 1827 037) at first, then dried in Fisher Isotemp® oven (200 Series Model 230F) at 104 °C for the analysis of TSS. The residual was then ignited in Fisher Scientific Isotemp® Muffle furnace at 550 °C for VSS.

TCOD and sCOD were analyzed by the closed efflux, colorimetric method (5220D). Samples were filtered through Mandel® 0.45 μm Nylon membrane filer (W-Man-25N45/500) to get ready for the sCOD analysis.

Before the analysis of NH_3-N, NO_3-N and NO_2-N, the samples were all filtered by Whatman filter papers #1 (Cat No. 1001 125). The automated phenate method (4500-

NH_3 G) was applied to measure ammonia concentration and NO_3-N and NO_2-N was measured by the automated cadmium reduction method (4500-NO_3^- F).

Alkalinity analysis was done according to the titration method 2320B. Because of the high alkalinity concentration in centrate, the samples were diluted before adding the utilized standard acid, which was 0.02N sulfuric acid (H_2SO_4). During the tests, the amount of the standard acid used was recorded, and then the alkalinity concentration was calculated based on the Eq. (3-3) showed below:

$$Alkalinity, mgCaCO_3 / L = \frac{A \times N \times 50{,}000}{mLsample} \qquad \text{Eq.(3-3)}$$

where:
A = *mL standard acid used and*
N = *normality of standard acid*

3.7 Achievability of the objectives

Briefly, the objectives of this study were to identity a suitable industrial wastewater as an external carbon source and to determine the optimum dosage, the degree of the increasing, and the time to activate the biomass to new carbon. The performance of the established SBRs was the indication of the applicability of the selected industrial wastewaters as a denitrification booster in form of carbon; the NUR test results were the bases for the analysis of the increasing degrees; the optimum dosages were analyzed based on both the performance of SBRs and NUR test results.

4.0 RESULTS AND DISCUSSION

4.1 Characteristics of centrate and industrial wastewaters

The quality of the centrate utilized in the research was largely dependent on the performance of the anaerobic digesters and the centrifuges at the NEWPCC where the centrate was collected. In addition, in the plant, there is a water flushing line running occasionally to prevent struvite from building-up in the pipes. Sometimes the centrate was collected when the flushing water was still running. As a result, the centrate was diluted at that time. Therefore the quality of the centrate used through out this study varied very significantly. The characteristics of the centrate were analyzed when it was received at the environmental engineering labs and presented in Table 4-1.

Table 4-1 Characteristics of the centrate from NEWPCC

Parameters	Units	Minimum	Average	Maximum	St. Dev.
NH_3-N	mg/L	350	690	1200	215
TCOD	mg/L	125	802	1604	286
sCOD	mg/L	81	356	803	143
TSS	mg/L	165	307	810	162
VSS	mg/L	55	175	390	93
Alkalinity	mg $CaCO_3$/L	1960	2600	3400	354

The potato processing, canola processing and oil refining wastewaters were delivered from the original plants to the environmental labs as required. In particular, there is a

dissolved air flotation (DAF) unit at the canola processing and oil refining plant to treat the wastewater preliminarily, mostly removing solids and COD from the wastewater. Therefore the wastewaters from this plant originated from two sources: before the DAF unit and after the DAF unit. The contaminants concentrations in these two industrial wastewaters including NH_3-N, TSS, VSS, TCOD and sCOD were analyzed after delivery to the environmental labs. Based on the results of analysis, the NH_3-N concentration in these wastewaters was inferred to be undetectable and negligible. The results of the average concentration are presented in Table 4-2 along with the comparison to the characteristics of the centrate.

Table 4-2 Comparison of the characteristics of centrate and industrial wastewaters

	NH_3-N (mg/L)	TSS (mg/L)	VSS (mg/L)	TCOD (mg/L)	sCOD (mg/L)
Centrate	690	307	175	802	356
Potato processing wastewater	0	2387	2110	5600	2728
Raw canola oil refining wastewater	0	101	94	1929	1440
DAF treated canola oil refining wastewater	0	45	42	1325	1186

As the above tables demonstrate, it was concluded that the centrate from the NEWPCC had a very high NH_3-N concentration, as high as 1200 mg/L, and the average value was still at the level of 690 mg/L, which was typical for a centrate stream as Caffaz et al. (2005) stated that the NH_3-N concentration in centrate was typically in the range of 403 mg/L to 997 mg/L. It was noticed that the peak NH_3-N concentration in this centrate stream was higher than this common range probably because NEWPCC treated the

primary sludge and WAS from the three WWTPs in the City of Winnipeg. There were more nitrogen loads entering the anaerobic digesters from satellite plants and thus more exited via centrate stream than would be a case if only own sludge (NEWPCC) was digested. The centrate stream contributes up to 30% of the total nitrogen into NEWPCC.

In contrast to this high NH_3-N concentration, the TCOD concentration in the centrate, as in Table 4-1, was particularly low, which was only 802 mg/L in average. Even though the TCOD concentration was higher than the reported values of 119-650 mg/L (Carrio et al, 2003; Caffaz et al, 2005), it was still just over the average NH_3-N concentration. Among this TCOD, there were four fractions: soluble (readily) biodegradable COD, soluble unbiodegradable COD, particulate biodegradable and particulate unbiodegradable COD. sCOD represents mostly the soluble biodegradable carbon. In the centrate from the NEWPCC, sCOD was only 356 mg/L in average, which was less than half of the TCOD. In terms of the particulate biodegradable substrate, it can be estimated according to the concentration of VSS, which were presumed to be organic matter, around 60-70 percent of which was considered as biodegradable COD. The VSS in the centrate used in this research was at a mean value of 175 mg/L, which only occupied a small portion of the TCOD of the centrate. Combining the low soluble and particulate biodegradable CODs produced very limited carbon sources for bacteria to uptake, in particular, the ratio of bCOD/TN was below 1, which was far less than the recommended C/N ratio of 5:1 (Reyes-Avila, 2004; Labelle, 2005; Punal, 2000) to facilitate the total nitrogen removal process. When centrate was treated directly without any addition of external carbon,

nitrates could not be removed because of the lack of adequate biodegradable COD as shown by Eilersen et al. (1995).

The industrial wastewaters, on the contrary, were very high in bsCOD, especially agro-food wastewaters are characterized by very low ratio of N/COD (De Lucas et al., 2005). The potato wastewater used in this study had an average TCOD of 5600 mg/L and sCOD of 2728 mg/L (Table 4-2), which were much higher than the COD concentrations in the digested sludge centrate. The COD would mostly be residual starch from potatoes, which is biodegradable.

In terms of the canola processing and oil refining wastewater, it contained COD only half as high as in the potato wastewater. However even the DAF-treated canola processing and oil refining wastewater had a high value of COD at 1186 mg/L (Table 4-2) – much higher than that in raw centrate. The COD in this wastewater would be contributed largely by oil and grease. These substances are not easily hydrolyzed and withstand biological degradation because of their low solubility (Metcalf and Eddy, 2003). Besides being slowly biodegradable, they have the potential to inhibit bacteria. As a result, their use as an external carbon source is questionable.

Both potato wastewater and canola processing and oil refining wastewater had very low nitrogen concentrations in the form of NH_3-N, which were undetectable and negligible. However, potato processing wastewater has been reported to contain total Kjeldahl nitrogen (TKN) (Kadlec et al., 1997), which was not measured in this research. Yoomin

Lee (personal communication) stated that potato processing wastewater contained approximately 200 mg/L of TKN, but the ratio of COD/TKN in the tested potato processing wastewater was still as high as 13. The high ratio of COD/N in these two industrial wastewaters is a great advantage which can be taken in terms of the enhancement of denitrification by external carbon sources. Therefore they were chosen as candidates for potential industrial wastewaters to increase denitrification rate.

The wastewater from the biodiesel industry contains glycerol, which is the main by-product of biodiesel production. It is well known that glycerol is a readily biodegradable compound (Akunna et al., 1993; Grabinska-Loniewska et al., 1985). Therefore, this wastewater was also a good candidate to be used as a booster to the denitrification process. Besides, at airports, glycol is the substance used to deice airplanes. Since glycol can be easily utilized by bacteria as a substrate (Trela 1998), the wastewater containing glycol from the Winnipeg International Airport was also a potential substitute to external carbon sources. In this study, the solutions of commercial-brand glycerol and glycol were used as synthetic replacements of these two wastewaters.

From the above analysis, the individual characteristics of COD/N ratio in the centrate from NEWPCC and industrial wastewaters were in the opposite positions, one being very low and the other being extremely high, respectively. The combination of the centrate and industrial wastewaters at various ratios can produce a mixture which has a well-balanced C/N ratio. Because of this increased C/N ratio in the centrate, denitrification can proceed at an elevated rate without any addition of exogenous chemicals as carbon

sources. The use of industrial wastewaters as external substrates will let the plant avoid purchasing chemicals.

4.2 Addition of industrial wastewaters as carbon sources

4.2.1 Addition of canola processing and oil refining wastewater as a carbon source

4.2.1.1 Effects on the nitrification process

There were two sources of the canola processing and oil refining wastewaters: the original raw wastewater and the treated wastewater after the DAF process where some solids and COD were removed from the raw wastewater. Both sources were utilized in this study. After different amounts of canola processing and oil refining wastewaters were taken and mixed with raw centrate to make solutions, the mixtures were then added into the three SBRs as the feedings. Figure 4-1 presents the NH_3-N concentration in influents and the amounts of ammonia leaving the three parallel SBRs.

The testing of canola processing and oil refining wastewaters was divided into three stages. First, the raw canola processing and oil refining wastewater was added into R2 and R3 only. An immediate increase in NH_3-N concentration in the effluents from R2 and R3 was observed. In the meantime, the NH_3-N concentration leaving R1 remained unaffected since R1 did not receive this canola wastewater. Given that the consumption

of NH_3-N was the responsibility of nitrification process, this industrial wastewater had an inhibitory effect on this process.

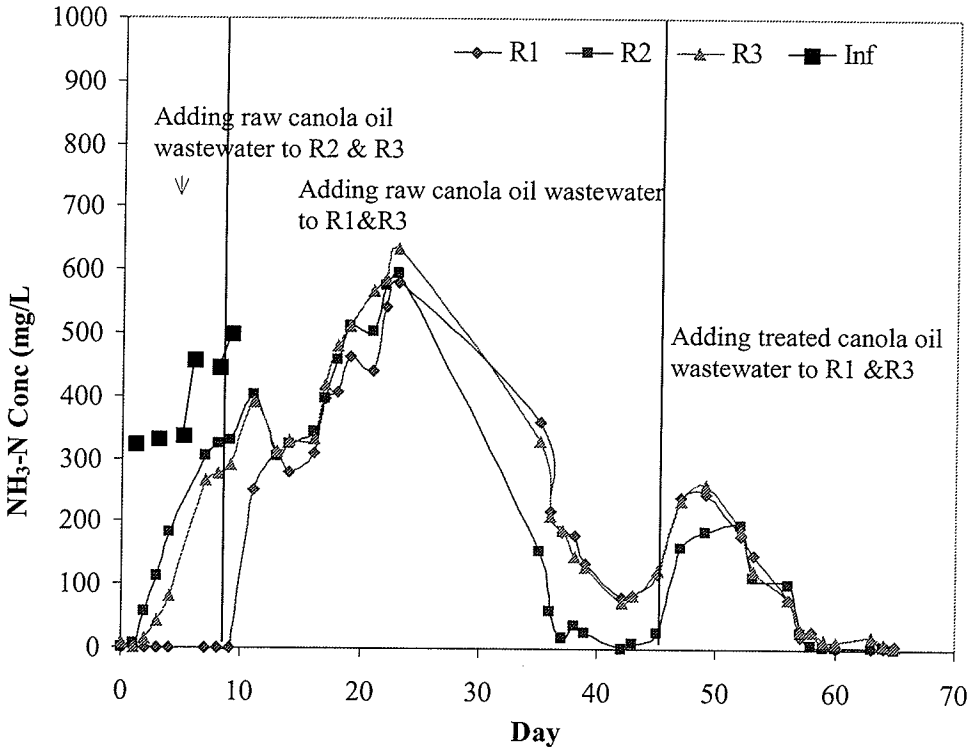


Figure 4-1 NH_3-N Conc. in influent and effluent with canola oil wastewater added
(R1 – aerobic; R2 & R3 – alternating aerobic/anoxic)

On Day 9, the addition of raw canola processing and oil refining wastewater to R2 was stopped and switched to R1. In just two days, the effluent NH_3-N concentration from R1 rose to the same level as that from R3, which continued to obtain raw canola oil wastewater. However the NH_3-N contents in R2 effluent did not decrease even though the addition of this wastewater ceased. The effluent NH_3-N concentrations from all three SBRs increased to as high as 600 mg/L, which was close to the concentration in the influent. After about 24 days, the concentration of NH_3-N started to drop and the declining was quicker in R2 than in R1 and R3. Not until then did the less negative effect

of the canola processing and oil refining wastewater on nitrification process show in R2, which had not received this industrial wastewater since the 9th day. It can be inferred that the shock of the canola oil wastewater was hard for nitrifying bacteria to overcome and it took 24 days for the microorganism to respond to this wastewater.

Last, on the 44th day, the feedings to R1 and R3 were switched to the mixture of centrate mixed with the DAF-treated canola processing and oil refining wastewater, the NH_3-N concentration in effluents still increased even though the biomass had spent some time to acclimate to the untreated industrial wastewater. For the treated canola processing waste, however, less time, about one week compared to the 24 days for the raw canola oil wastewater, was required for the nitrifiers to acclimate to it, and the shorter acclimation time was probably because the biomass had already acclimated to the raw canola oil wastewater. It was also noticed that the NH_3-N concentration in the effluent from R2 was also increased even though DAF-treated canola oil wastewater was not added into it, most likely resulted from the changes in the characteristics of the raw centrate.

4.2.1.2 Effects on the denitrification process

Simultaneously, corresponding to the changes of NH_3-N concentration in the effluents, the concentration of NO_3-N in effluents fluctuated accordingly (Figure 4-2) because of the incomplete nitrification process.

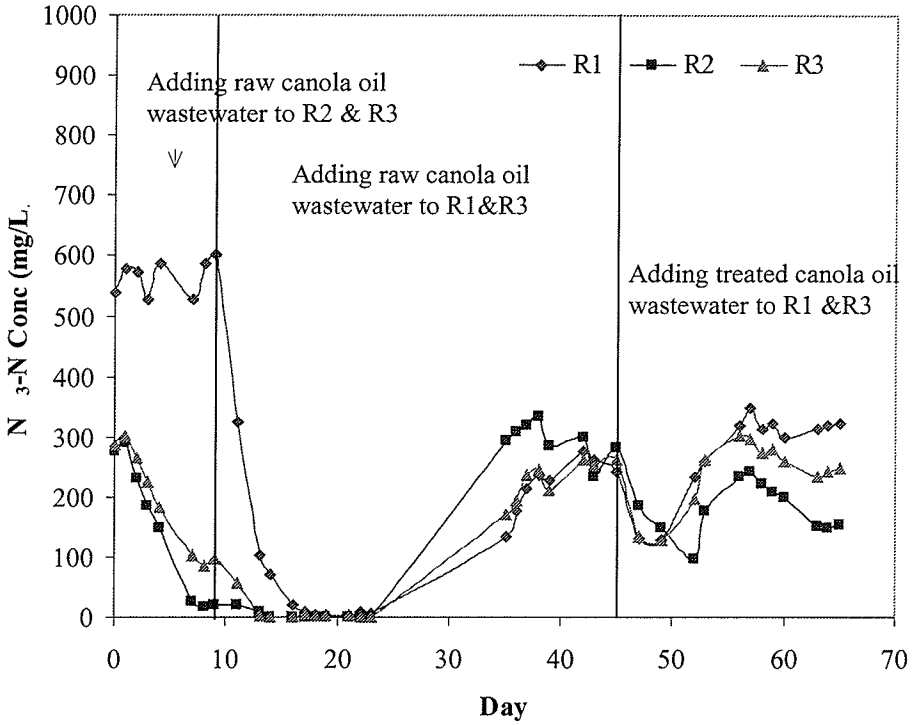


Figure 4-2 NO_3-N Conc. in effluent with canola oil wastewater added
(R1 – aerobic; R2 & R3 – alternating aerobic/anoxic)

During the first stage of adding canola processing and oil refining wastewaters when only R2 and R3 had received this raw industrial wastewater, the NO_3-N concentration leaving these two bioreactors decreased quickly as the nitrification process was restrained by the canola oil wastewater but that leaving R1 remained constant. When the experiment was turned to the second stage, R1 began to receive raw canola oil wastewater and the nitrification process in it was suppressed by the wastewater, resulting in a significant decrease in effluent NO_3-N concentration thereafter. As in Figure 4-2, the NO_3-N contents in effluents from all three SBRs decreased to zero around the 17^{th} day, so the activities of nitrifiers stopped completely. On Day 24, the NO_3-N concentration in effluents started to increase, and it was at this time that the nitrifiers became to show acclimation to this industrial wastewater. The full acclimation was reached on Day 40,

on which the maximum TN removal of 37% was achieved. The dosage used was 4.1 mg bsCOD/mg NH_3-N *d.

When the tested canola processing and oil refining wastewater was switched to the wastewater after the DAF process, there were still decreases and increases in NO_3-N concentration in effluents, and turning point from decrease to increase was on Day 50. All of the changes in NO_3-N concentration followed the same pattern as the fluctuation in NH_3-N concentration in effluents but in reverse order.

This phenomenon supported that the performance of nitrification process influences the denitrification process since they are two integral parts of TN removal process (Hall et al., 1980; Carley et al., 1991). The inhibition of nitrification by canola processing and oil refining wastewater could be explained by the detrimental impact on the oxygen transfer. It was demonstrated that oil and grease could form a lipid coat around the activated sludge flocs and interfere in the oxygen transfer (Chao and Yang, 1981; Becker et al., 1999). Since nitrifiers are DO sensitive (Ruiz et al., 2003), their activities were negatively influenced by the decreased DO transfer rate caused by oil and grease contained in the canola oil wastewater. In addition, oil and grease have a higher melting temperature than ambient temperature and a low solubility in water, therefore they are hard to hydrolyze (Becker et al., 1999). As a result, they could not be accessed easily by microorganisms.

In conclusion, canola processing and oil refining wastewater was not a good substitute to the purchased chemicals as an external carbon sources because the COD from this industrial wastewater cannot boost denitrification process owing to its inhibitory effect on the nitrification process and low accessibility to active denitrifying bacteria. Furthermore, the figures (Figure 4-1 and Figure 4-2) indicate that the nitrifiers need a long full acclimation period of 40 days to adapt to canola processing and oil refining wastewater and this carbon source cannot be used on an ad hoc basis.

However, canola processing wastewater had a potential to be used as an external carbon source in the post-denitrification process, i.e. after full nitrification had been completed. As indicated in the later section, the specific denitrification rate with canola processing wastewater was only 5 $mgNO_3$-N/gVSS*h, and presented no improvement on denitrification comparing to the SDNR of 0.3-6 $mgNO_3$-N/gVSS*h with raw municipal wastewater reported in literature (Peng et al., 2007; Kraume et al., 2005; Henze 1989). Therefore canola processing wastewater can not be applied during the post-denitrification phase to enhance TN removal.

4.2.1.3 Effects on specific nitrification rate

In the above discuss, it was clearly demonstrated that the nitrification process in the three SBRs was inhibited by the addition of canola processing and oil refining wastewater. To determine how much the nitrification process was impacted, the AUR tests were carried out in small beakers.

The specific nitrification rates (SNRs) of the original centrate (Cen) mixed with water (W) as blank for reference, raw canola oil refining wastewater (R.C.), and treated canola oil wastewater (T.C) were tested, respectively. Figure 4-3 shows the relationship of NH_3-N concentration in beakers versus time. It was obvious that the line for centrate mixed with water had the deepest slope and the line for centrate mixed with raw canola oil wastewater had the flattest slope, just as predicted. It meant the highest NH_3-N utilization rate (AUR) was achieved in the beaker where centrate mixed with water was tested and the lowest in the beaker containing centrate mixed with the raw industrial wastewater.

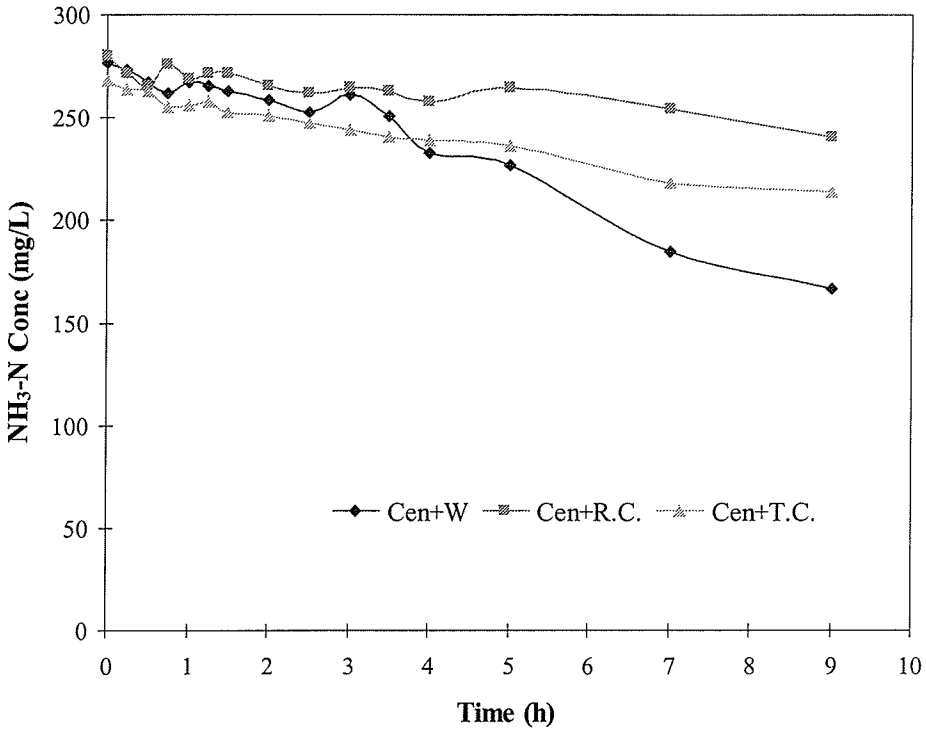


Figure 4-3 SNR tests with canola oil wastewater added

Note: R.C.=Raw canola oil refining wastewater W=Water
T.C.=Treated canola oil refining wastewater Cen=Centrate

SNRs were calculated based on the results from the AUR tests. The reference test, where centrate was mixed with tap water, achieved the highest SNR, which was 36 $mgNH_3$-N/gVSS*h. The lower SNRs than that of the reference test were foreseeable in the other two beakers, which were with the addition of centrate mixed with R.C. and T.C., respectively. The SNR decreased to 7 $mgNH_3$-N/gVSS*h when centrate was mixed with raw canola oil refining wastewater, while the SNR was 13 $mgNH_3$-N/gVSS·h when centrate was mixed with treated canola oil refining wastewater (Figure 4-4). These results strongly support the conclusion that canola oil wastewater has a negative effect on the nitrification process.

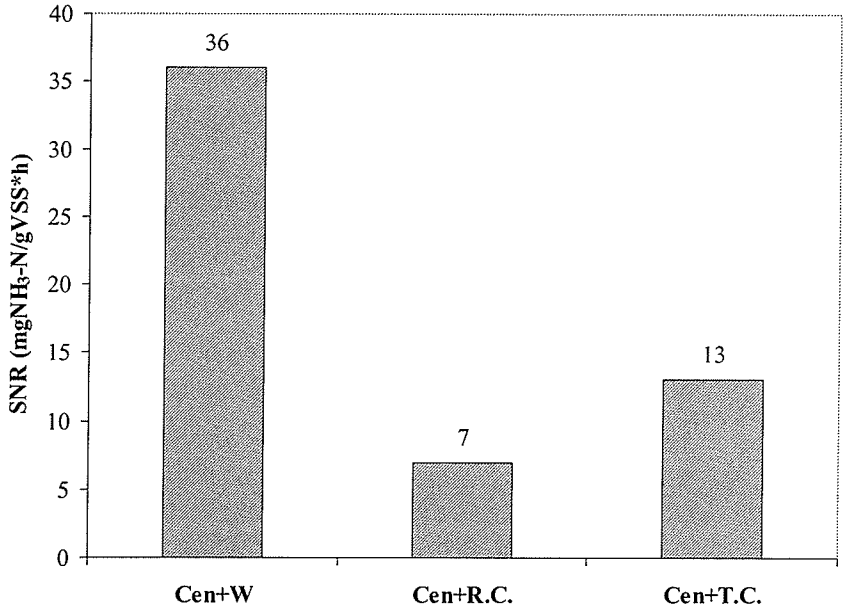


Figure 4-4 SNR values with canola oil wastewater added

Note: R.C.=Raw canola oil refining wastewater W=Water
T.C.=Treated canola oil refining wastewater Cen=Centrate

The full nitrogen removal is a two-step process: first nitrification followed by denitrification. Because of the prohibitive influence of the canola processing and oil

refining wastewater on the nitrification process, the full nitrogen process cannot proceed to completion. This industrial wastewater was removed from the candidate list.

4.2.2 Addition of potato processing wastewater as a carbon source

4.2.2.1 Effects on the nitrification process

Potato wastewater, used as an external carbon substrate, was mixed with centrate in various proportions and then the mixtures were added into all of the three SBRs as feedings. After the addition, the NH_3-N concentrations in effluents from the bioreactors remained constant around 1 mg/L, which were much lower compared to the mean NH_3-N concentration of 420 mg/L in influents (Figure 4-5).

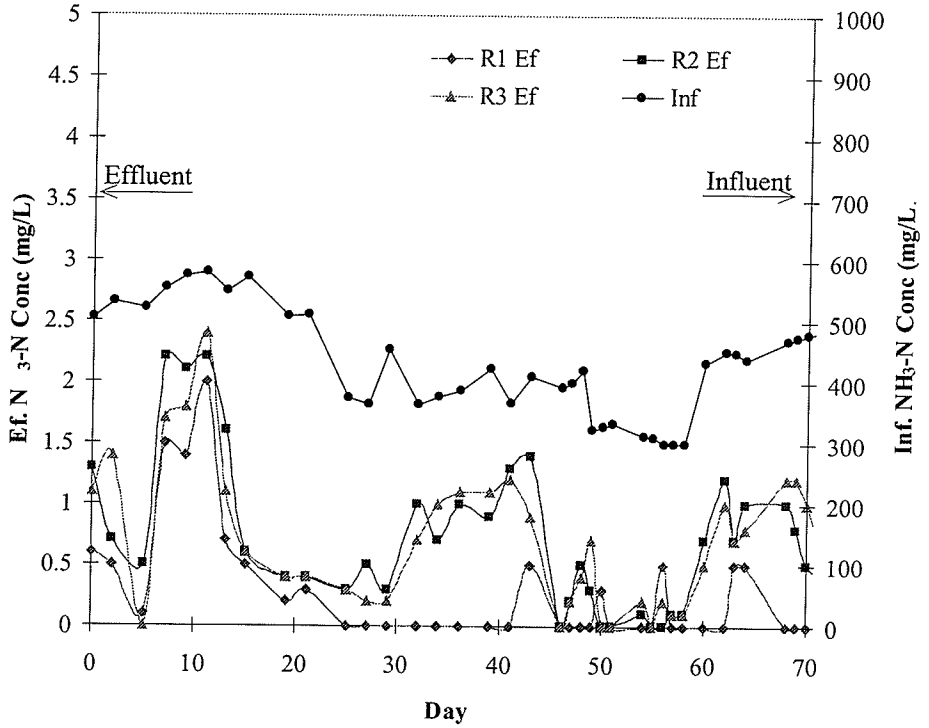


Figure 4-5 NH_3-N Conc. in influent and effluents with potato wastewater added
(R1 – aerobic; R2 & R3 – alternating aerobic/anoxic)

The near zero NH_3-N concentration in effluents indicated that the nitrification process had proceeded to near 100% completion. Therefore, this industrial wastewater had no harmful influence on nitrifiers. It was noted that there were fluctuations of effluent NH_3-N concentration available, which maybe was due to the quality of the centrate from NEWPCC.

4.2.2.2 Effects on the denitrification process

In addition to the constant effluent NH_3-N concentration, little fluctuation in effluent nitrates concentration can be seen in Figure 4-6. The NO_3-N concentrations in R2 and R3, in which potato wastewater was added, were around 170 mg/L in average, which were much lower than the mean NO_3-N concentration of 400 mg/L in R1, which had no anoxic period, and the average NH_3-N of 420 mg/L in influents. Therefore there were about 60% of TN removal in R2 and R3 with the addition of potato wastewater. Since the raw centrate from sludge processing facilities in WWTP had no positive influence on the denitrification process (Eilersen et al., 1995), the conclusion could be drawn from the stimulated TN removal in R2 and R3 that the denitrification process was enhanced by the addition of potato processing wastewater. Potato wastewater, thus, is a very good alternative to the purchased chemicals as an external carbon source.

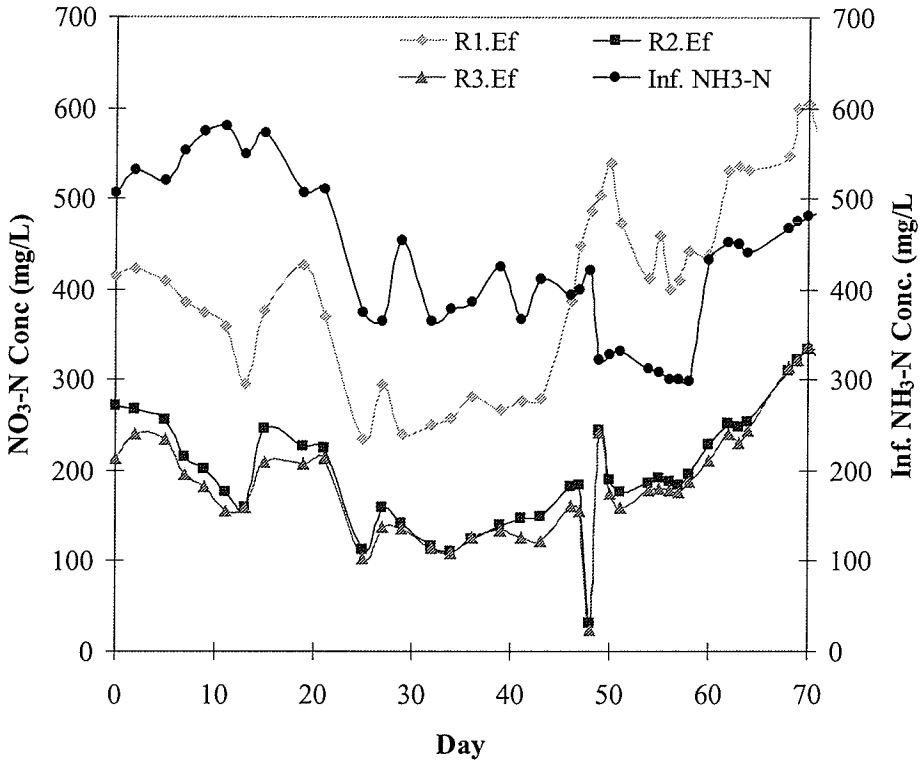


Figure 4-6 NO_3-N Conc. in effluents with potato wastewater added
(R1 – aerobic; R2 & R3 – alternating aerobic/anoxic)

Figure 4-6 also shows that the NO_3-N concentration in effluent from R3 started to drop only two days after the addition of potato wastewater, which indicated that the bacterial response to this wastewater was prompt. 71%, the maximum removal of TN, was achieved on Day 11 at the dosage of 4.5 bsCOD/mg NH_3-N*d. Therefore, the full acclimation time needed to utilize potato processing wastewater was 11 days and it can be used on as-needed basis.

The figure also showed there was large amount NO_3-N residual remained in effluent. The residual was the result of the conversion of ammonia to nitrate after the anoxic periods ended. Therefore the average of only 60 % of TN removal was achieved. The residual of nitrate can be reduced and the TN removal percentage can be increased by

extending the total aeration time before the end of anoxic period. All NO_3-N concentration in effluents presented an increasing trend at the end of experiment, which was the result of the increased ammonia concentration in influent. It was found that NO_3-N concentration in the effluent from R1 was higher than NH_3-N concentration in influent, which may be caused by a larger portion of organic nitrogen compound in influent than the previous period. After the addition of potato processing wastewater to R1, a pure nitrification reactor, the NO_3-N concentration also decreased, which indicated that simultaneous nitrification and denitrification happened. The improved SND in R1 with addition of potato processing wastewater was also the evidence that this industrial wastewater can enhance denitrification.

The enhancement of denitrification process by wastewater from potato processing industry was also demonstrated by De Lucas et al. (2005) and Eilersen et al. (1995). The increased TN removal by the addition of potato wastewater could be explained in terms of the characteristics and composition of this industrial wastewater. The main pollutant in potato wastewater was starch, which can easily decompose in water and become soluble. Therefore starch can be readily utilized by denitrifying bacteria as a carbon source. In addition to starch, there may be other soluble and particulate biodegradable COD available in the potato wastewater, as a result, the potato wastewater was accessible to microorganism.

4.2.3 Addition of glycerol as a carbon source

4.2.3.1 Effects on the nitrification process

In this study, the actual wastewater from the biodiesel industry, which contained mostly glycerol, was replaced with the synthetic solution made of tap water mixed with the commercial-brand glycerin. After the glycerol solution was dosed as an external carbon source to the reactors R2 and R3 to boost nitrates removal, the NH_3-N concentration around 2.2 mg/L was maintained in the effluents from these two reactors. When this average value was compared to that of 520 mg/L in the feedings, 99% of the original ammonia was removed. It was also noticed that the remaining NH_3-N was not as constant as that existing in the effluent from R1, where pure aerobic condition was kept. This situation indicated that glycerol might have a negative impact on nitrification since organic substrates can influence the conversion of ammonia to nitrates (Dytczak et al., 2007; Hanaki et al., 1990 a). However the effect was negligible as the fluctuation was only from 1 mg/L to 5 mg/L. In addition, the higher frequency of variation in R2 effluents than in R3 may have contributed to a smaller mass of nitrifying bacteria present in R2 in view of the fact that the less healthy microorganisms are, the less they can withstand changes. Based on the above observations, the nitrification process was almost fully completed and it was nearly not affected by the addition of glycerol solutions.

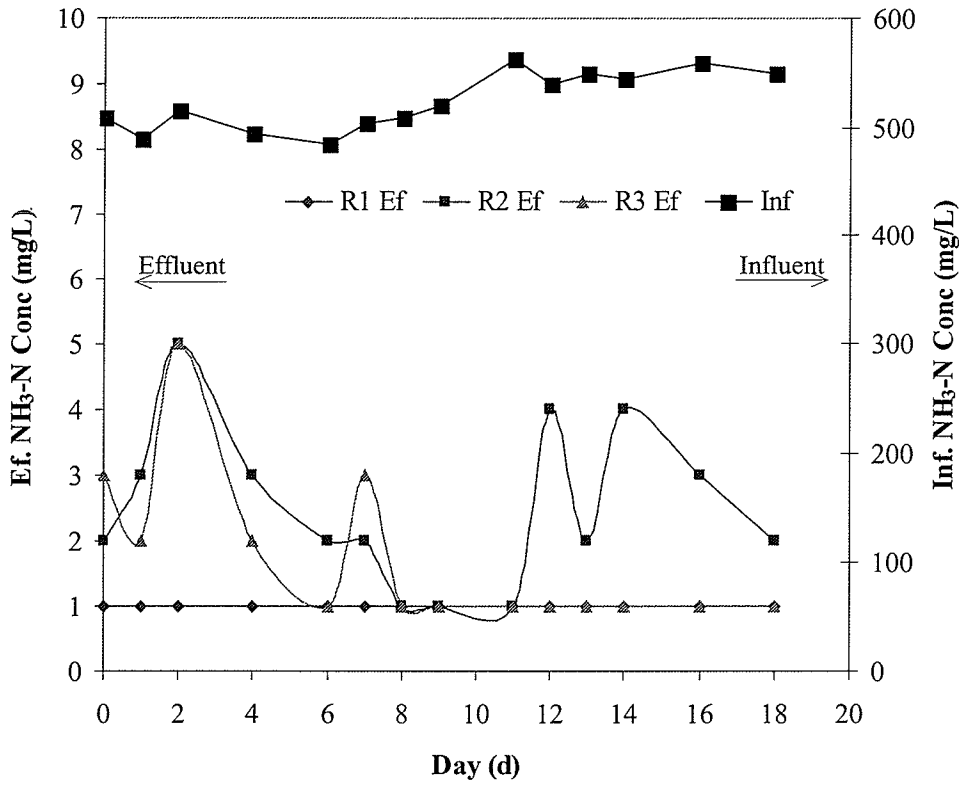


Figure 4-7 NH_3-N Conc. in influent and effluents with glycerol added
(R1 – aerobic; R2 & R3 – alternating)

4.2.3.2 Effects on the denitrification process

In terms of the denitrification process, the effluent concentration of NO_3-N declined with the addition of glycerol (Figure 4-8). The dose of glycerol added to R2 was steadily increased from 0.2 mg bsCOD/mg NH_3-N*d by daily addition of 0.4 mg bsCOD/mg NH_3-N *d more, so the effluent NO_3-N concentration gradually decreased correspondingly until a maximum TN removal was reached, where the dosage was 5.4 mg bsCOD/mg NH_3-N *d, the same as the constant dosage added to R3. By adding the constant amount of glycerol to R3, the insufficiency of carbon sources at the beginning was eliminated. Consequently, the effluent NO_3-N concentration from R3 dropped very

significantly after only four days, which was the response time of denitrifiers to glycerol. On the 11th day, the concentration in effluent from R3 reached the point where the best nitrogen removal level was achieved and remained constant afterward. Therefore the denitrifiers needed the full acclimation time of 11 days to utilize glycerol. The best removal result in R2 was obtained on Day 16, which was 5 days later than in R3 because sufficient carbon was not provided to R2 until Day 13. 90% of TN removal was achieved at the most with the addition of glycerol solution in both R2 and R3 with the dosage of 5.4 mg bsCOD/mg NH_3-N *d. Therefore the denitrification was intensified by glycerol.

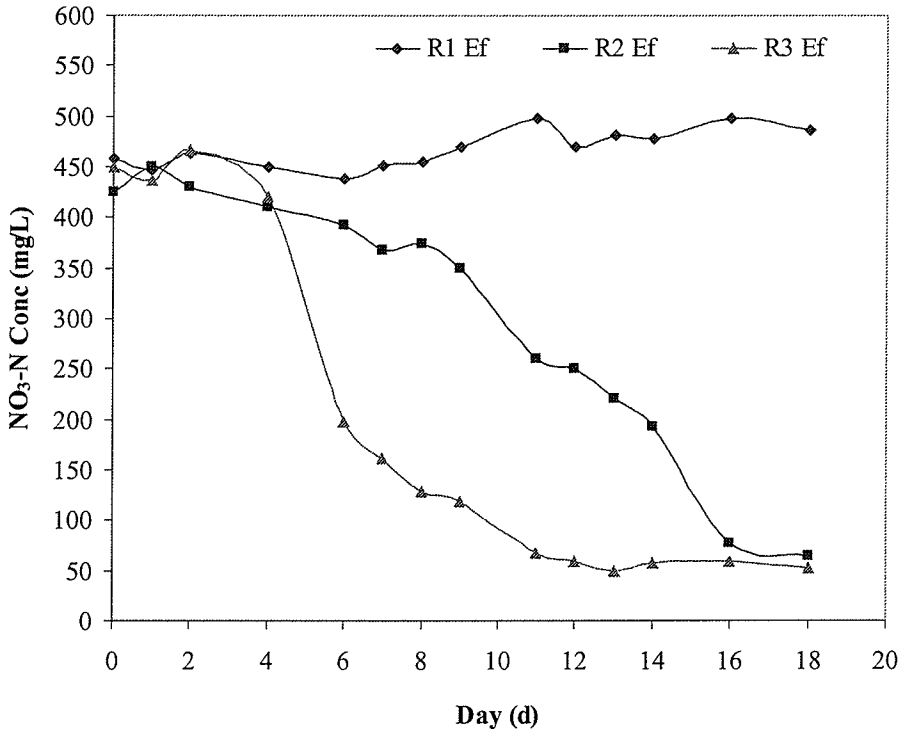


Figure 4-8 NO_3-N Conc. in effluents with glycerol added
(R1 – aerobic; R2 & R3 – alternating aerobic/anoxic)

The same result was demonstrated by Grabinska-Loniewska et al. (1985) and Akunna et al. (1993), who stated that glycerol can act as a hydrogen donor in the nitrates reduction

process. Laurin et al. (2006) discovered that the denitrifying activity of microbial communities can recover more quickly with the aid of glycerol after the biomass was preserved for a period in a frozen state. Since the constituents in the wastewater from biodiesel production industries are mostly glycerol (methanol and salts are minimal), which can be easily assimilated by denitrifying bacteria without the negative effects on nitrifiers, this industrial wastewater was a good candidate for an external carbon source. More importantly, only four days of response time and 11 days of acclimation time were needed for bacteria to utilize glycerol. As demonstrated in the later section, the times needed for bacteria to acclimate to ethanol and methanol were much longer than that needed for glycerol, bacteria can utilize COD provided by glycerol quicker than COD provided by methanol and ethanol, the potential for the application of biodiesel wastewater on as-needed basis is promising.

4.2.4 Addition of glycol as a carbon source

4.2.4.1 Effects on the nitrification process

The solution of glycol was used in this research to replace the glycol-containing wastewater from the Winnipeg International Airport. Glycol solution was supplied to R2 as the external carbon substrate to stimulate the process of nitrate reduction. Before the addition, the biomass in the bioreactor was prepared for the test by discontinuing any addition of external carbon sources, therefore the biomass was not acclimated to any substrate. The effluent NH_3-N concentration from R2, was compared to the NH_3-N

concentration in the decant from R1 - the pure nitrification reactor, and the influent NH_3-N concentration, as shown in Figure 4-9. The figure shows that the NH_3-N contents leaving R2 were well comparable with the R1 effluent NH_3-N contents since both of the NH_3-N concentrations were close at around 3 mg/L. It was noticed that there were three spikes available in the effluent from R2, but they persisted just for one day or so. As a result, they can be treated as outliers and their effects were insignificant.

Comparing the effluent NH_3-N to that in the influent, the removal of ammonia was around 98 percent, which suggested that the nitrification process proceeded to almost complete and was not negatively influenced by the glycol solution.

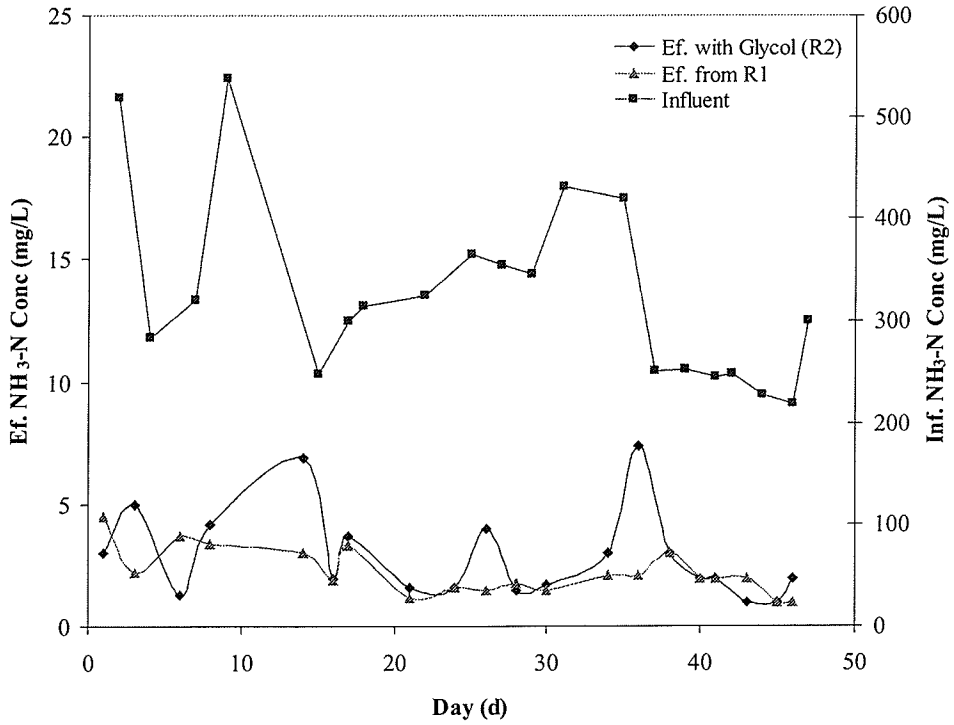


Figure 4-9 NH_3-N Conc. in influent and effluents with glycol added
(R1 – aerobic; R2 – alternating aerobic/anoxic)

4.2.4.2 Effects on the denitrification process

Since no additional readily biodegradable organic carbon was available in the bioreactor during the preparation period, the denitrification process did not proceed well. As in Figure 4-10, the initial NO_3-N concentration in the effluent from R2 was as high as 340 mg/L, which was close to that from R1 with no enhanced denitrification.

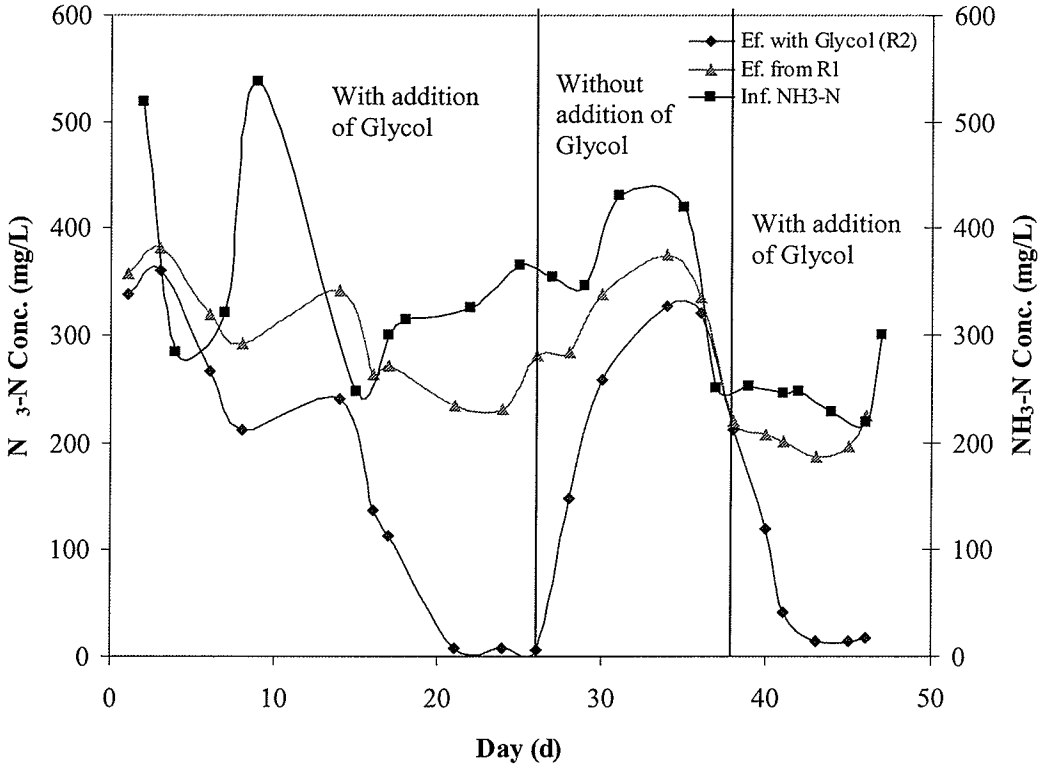


Figure 4-10 NO_3-N Conc. in effluents with glycol added
(R1 – aerobic; R2 – alternating aerobic/anoxic)

To verify if the denitrification could be enhanced by glycol solutions and how quickly the denitrifying bacteria responded to the changes, there were three periods when the glycol solution was added (Figure 4-10). At the beginning, the glycol solution was added at the dosage of 0.2 mg bsCOD/mg NH_3-N *d and increased gradually by 0.2 mg bsCOD/mg

NH_3-N *d more each day until the 21st day, on which the optimal TN removal was achieved. As a result, the optimum dosage for glycol was 4.6 mg bsCOD/mg NH_3-N *d. During this period, the effluent NO_3-N concentration started to decrease after only 3 days, which meant that the denitrifiers responded to glycol very fast. Since the dosage of glycol added was enlarged progressively, the NO_3-N concentration leaving R2 lessened step by step in response to this change. Even though on Day 14 the NO_3-N concentration arose, which may be caused by the centrate quality in the influent as the effluent from R1 also presented this variation, the gap between the NO_3-N concentrations in effluents from R1 and R2 widened in comparison to the previous days. This observation implied that there was more TN removed from the influent. The denitrification process was being enhanced and still in the middle of the development to the optimum TN removal condition. This condition, i.e. full acclimation of denitrifiers to glycol, was achieved on the 21st day. This optimal state was kept steady for 5 days, which was characterized by the constant MLSS in R2 and stable properties of effluents out of R2.

On Day 26, the second stage of the study on glycol was initiated by discontinuing adding glycol to R2. Because of the lack of biodegradable carbon, the denitrification process was hindered, resulting in a significant increase in the effluent NO_3-N concentration from R2. This concentration eventually reached a similar level as that from R1. The second period lasted for 12 days, which was longer than the system SRT of 10 days. At this time, it was considered that the denitrifying bacteria using glycol as a carbon source were washed out of the system. During this period, the NO_3-N concentration from both R1 and R2 decreased after Day 35 because of the decrease in influent NH_3-N concentration.

On the 38th day, the addition of glycol solution was resumed at the dosage of 4.6 mg bsCOD/mg NH_3-N *d to provide sufficient carbon. Surprisingly the bacteria responded to this change immediately as the NO_3-N concentration from R2 dropped promptly. In only five days, the optimal nitrates removal was attained. The denitrifiers using glycol as a carbon source can achieve a full recovery in very short time after the wash-out. It was concluded that the denitrification could be enhanced by the glycol solution without obvious delay.

In conclusion, the glycol-containing wastewater from WIA was a promising substitute to the commonly used chemical external carbon sources, which due mainly to the fact that glycol had no noticeable inhibition on nitrification process and boosted denitrification rapidly after the addition. The bacterial response to glycol took only three days and reached a full acclimation in 20 days. The similar results were also demonstrated by Trela (1998), who discovered an even shorter acclimation time for glycol as the TN removal percentage can reach 80% only 20 hours after glycol was introduced to bioreactors.

4.3 Addition of commonly purchased carbon sources for comparison

For a better understanding on the applicability of the four above-examined industrial wastewaters, i.e. canola processing and oil refining, potato processing, biodiesel industry and Winnipeg International Airport wastewaters, as external carbon sources to boost denitrification process, two of the purchased carbon substrates in the form of single

chemical compound, i.e. methanol and ethanol, which are utilized most often in the wastewater treatment industry, were tested to determine their impact on the conversion of nitrates to nitrogen gas. The performances of the enhanced denitrification process by these two chemicals were compared with those obtained with the four industrial wastewaters.

4.3.1 Addition of ethanol as a carbon source

4.3.1.1 Steps of the addition of ethanol

The experiment performed on the influence of ethanol on denitrification was paired with that on glycol with ethanol being dosed into R3 and glycol into R2. Therefore the addition of ethanol had the same pattern as glycol. The span of 46 days during which ethanol was utilized as an external carbon source was also divided into three periods, with the separating points on Day 26 and Day 38.

For the duration of the first interval, ethanol was applied with the starting dosage of 0.23 mg bsCOD/mg NH_3-N *d, and the dosage was increased by 0.23 mg bsCOD/mg NH_3-N *d each day. The enlargement of the amount of ethanol supplied into R3 was continued until the 21st day, on which the dosage became fixed at 5.34 mg bsCOD/mg NH_3-N *d. Five days later, the second phase started. The addition of the external carbon source was suspended during this period and the discontinuation lasted for 12 days, which was two days more than the 10-day SBR SRT. And then the third period followed. Throughout

this stage, the amount of ethanol applied into R3 was optimum at 5.34 mg bsCOD/mg NH_3-N *d. Therefore the carbon-limiting condition was avoided in order to enhance the denitrification to full strength.

4.3.1.2 Effects on the nitrification process

After ethanol solution was added into R3, the NH_3-N concentration exiting the bioreactor remained fairly constant at 3 mg/L, which was comparable with that in the effluent from the aerobic reactor R1 (Figure 4-11). In addition, less than 1% of the ammonia contained in the influent remained in the effluent – i.e. the nitrification process was practically complete.

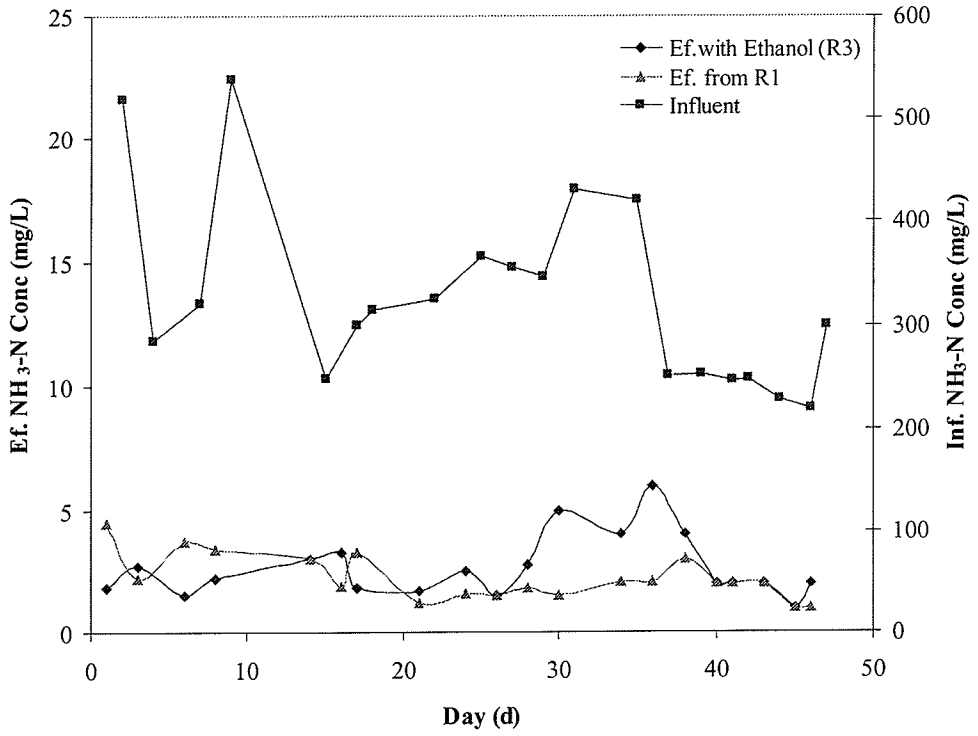


Figure 4-11 NH_3-N Conc. in influent and effluents with ethanol added
(R1 – aerobic; R3 – alternating aerobic/anoxic)

It was also observed that there was a peaking period during which the NH_3-N concentration from R3 was higher than that from R1 and this condition occurred between Day 26 and 38. However it was worth mentioning that ethanol was not added during this interlude. Therefore the increase in NH_3-N concentration was not caused by ethanol. It could be attributed to the influent quality since the peaks matched well with the high rises of NH_3-N concentration in influent. In summary, ethanol has no adverse effects on the first step of TN removal, and almost all of the ammonia in raw wastewater was oxidized to nitrates without any setback, even with the presence of ethanol.

4.3.1.3 Effects on the denitrification process

With reference to the impact of ethanol on denitrification, the NO_3-N concentration in effluents from R3 was employed as the indicating factor. Figure 4-12 presents the measured results of NO_3-N concentrations.

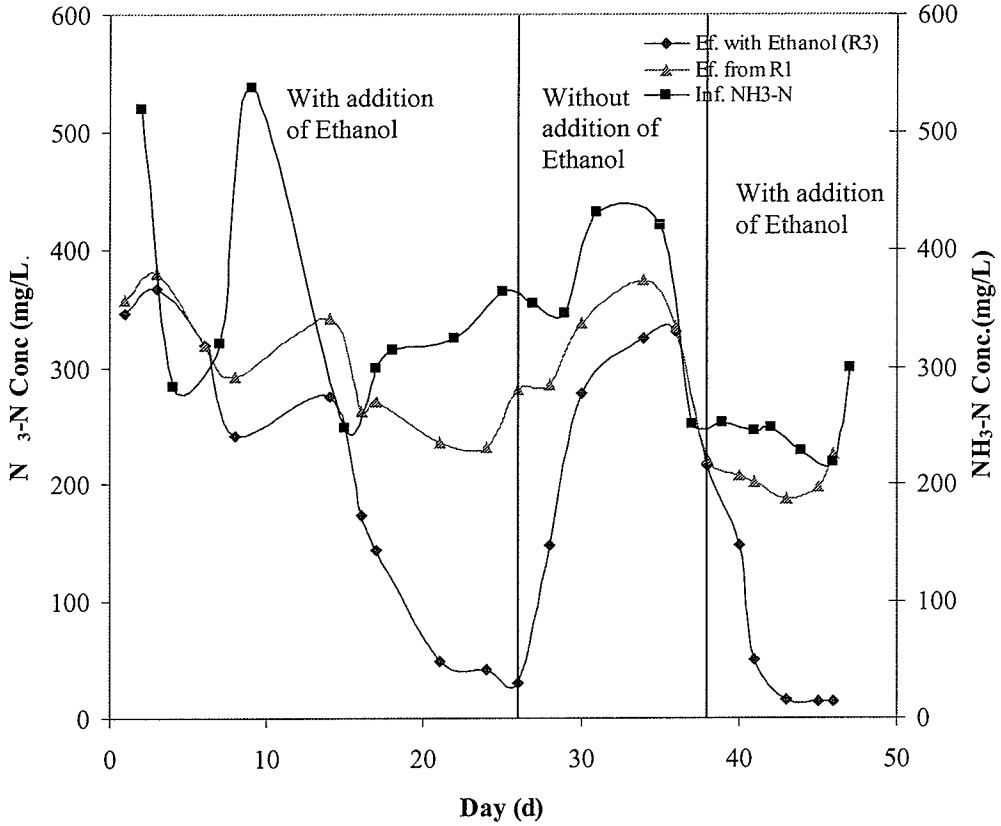


Figure 4-12 NO_3-N Conc. in effluents with ethanol added
(R1 – aerobic; R3 – alternating aerobic/anoxic)

From Figure 4-12, the decrease of the effluent NO_3-N concentration from R3 appeared within only 6 days after the addition of ethanol. This quick occurrence of decline indicated that the denitrifying biomass could start to utilize ethanol as their carbon source for the reduction of NO_3-N in a very short time. Hence the response time needed for bacteria to adapt to ethanol was only 6 days. This observation was in agreement with the demonstrations of other researchers (Andersson et al., 1995; Spevackova et al., 2005; Hallin et al., 1996a).

The decrease of NO_3-N concentration in R3 effluents continued after the 6^{th} day until the 26^{th} day on which the application of ethanol was stopped. This phenomenon showed the

denitrification rate was persistently improved by ethanol. The enhancement of denitrification by ethanol was also proved by the augment of the difference between the NO_3-N concentrations out of R1 and R3 even though there was a spike in the effluent NO_3-N concentration from R3 on Day 14. The bump could be caused by the influent quality as the NO_3-N concentration from R1 also increased at that time. On Day 21, the dosage of ethanol added into R3 remained unchanged at 5.34 mg bsCOD/mg NH_3-N *d, and about 89% of TN removal was maintained. Denitrifiers reached a full acclimation to ethanol in 21 days.

On Day 26, the addition of ethanol stopped. The effluent NO_3-N concentration from R3 ascended significantly to as high as that from R1 thereafter. This fact was also an indicator for the intensifying effect of ethanol on denitrification. When ethanol was provided again to R3 at 5.34 mg bsCOD/mg NH_3-N *d after 12 days (two days longer than the 10 d system SRT) of the absence of ethanol, the denitrifying bacteria responded to it immediately even though some the denitrifiers which had been adapted to ethanol were washed out. The maximum TN removal was achieved in five days.

Comparing the effluent NO_3-N concentration with glycol and ethanol applied as external carbon sources (Figure 4-10 and Figure 4-12), a similar pattern was presented. The same centrate was used during the investigation on these two carbon sources and the fluctuation of NH_3-N concentration in raw centrate had a significant impact on the effluent NO_3-N concentration, so the pattern of NO_3-N in effluent was similar. The dosage of glycol was 0.2 mg bsCOD/mg NH_3-N *d at the beginning and increased by

daily increment of 0.2 mg bsCOD/mg NH_3-N *d, while the dosage of ethanol was 0.23 mg bsCOD/mg NH_3-N *d at the beginning and increased by daily addition of 0.23 mg bsCOD/mg NH_3-N *d. The similar dosage utilized could be another factor resulting in the similar effluent NO_3-N pattern. However, the denitrifiers spent 4 days to respond to glycol and 6 days to ethanol. The glycol-utilizing denitrifiers have a higher growth rate than ethanol-utilizing ones. The effluent NO_3-N concentration decreased faster with glycol than with ethanol, and a higher TN removal was achieved. When they were added again at the optimum dosages on Day 38, glycol and ethanol presented the almost same pattern. This phenomenon could be explained that the advantage of higher growth rate of glycol-utilization bacteria could not be shown in a short time of only 5 days. The similar NO_3-N pattern may be an indication that ethanol can be replaced by glycol.

Ethanol can be used in ad hoc situations to provide a prompt boost to denitrification process, which takes advantage of the short acclimation time of denitrifiers to ethanol. This conclusion was comparable with the statement of Hasselblad and Hallin (1998), who demonstrated that ethanol can be employed as an external carbon source in an intermittent strategy to enhance denitrification in activated sludge, resulting from the short acclimation time.

Based on the above discussion, the commonly-used ethanol was an excellent external carbon source for TN removal from wastewater and a short acclimation time was needed for denitrifying bacteria. However, ethanol has drawbacks, such as toxic, flammable and expensive. These disadvantages necessitate the search for its substitutes.

4.3.2 Addition of methanol as a carbon source

4.3.2.1 Steps of the addition of methanol

Besides ethanol, methanol is the most often used external carbon source to assist TN removal in the wastewater treatment industry. To study its impact on denitrification process, methanol was dosed into R2 and R3, the two alternating aerobic/anoxic reactors. The aerobic R1 did not receive methanol. The addition of methanol was in two different approaches. For R2, the dosage of supplied methanol was increased gradually from 0.34 mg bsCOD/mg NH_3-N *d and by daily enlargement of 0.17 mg bsCOD/mg NH_3-N *d for the first 28 days until the dose reached 4.9 mg bsCOD/mg NH_3-N *d. R3 received 4.9 mg bsCOD/mg NH_3-N *d all the time through the study to eliminate the potential carbon-limiting condition.

4.3.2.2 Effects with methanol addition by gradually increased dosage

After the addition of methanol into R2 in a manner of gradually increased dosage, the NH_3-N concentration in effluent rose gradually from 6 mg/L, a value close to that in the effluent from R1, at the beginning correspondingly (Figure 4-13). On Day 12, it reached a relatively steady level and remained at around 200 mg/L. The nitrification process was hindered by the addition of methanol.

During the same time, the NO_3-N concentration in the effluent from R2 dropped step by step because of the incomplete nitrification process (Figure 4-14). There was no enhanced denitrification happening, which was indicated in Figure 4-15 and Figure 4-16 as the remaining total amount of NH_3-N and NO_3-N and TN removal percentage remained similar to those in the effluent from R1. The conversion of NO_3-N to nitrogen gas by denitrifiers using the biodegradable COD available in centrate as electron donors could be the reason for the removal of TN during this period.

For R1, a pure aerobic SBR, the TN reduction in it was believed to be initiated by the SND with the low bulk liquid DO of 0.5 mg/L at the beginning of the aeration cycle since SND at low DO concentrations has been demonstrated by various researchers before (Pochana and Keller, 1999; Zeng et al., 2003; Ju et al., 2007).

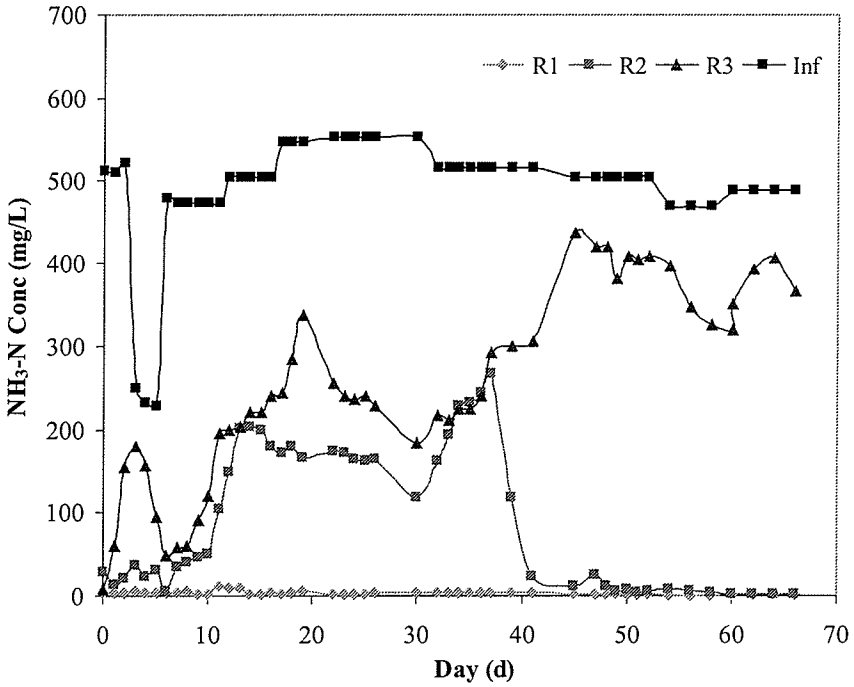


Figure 4-13 NH_3-N Conc. in influent and effluents with methanol added
(R1 – aerobic; R2 & R3 – alternating aerobic/anoxic)

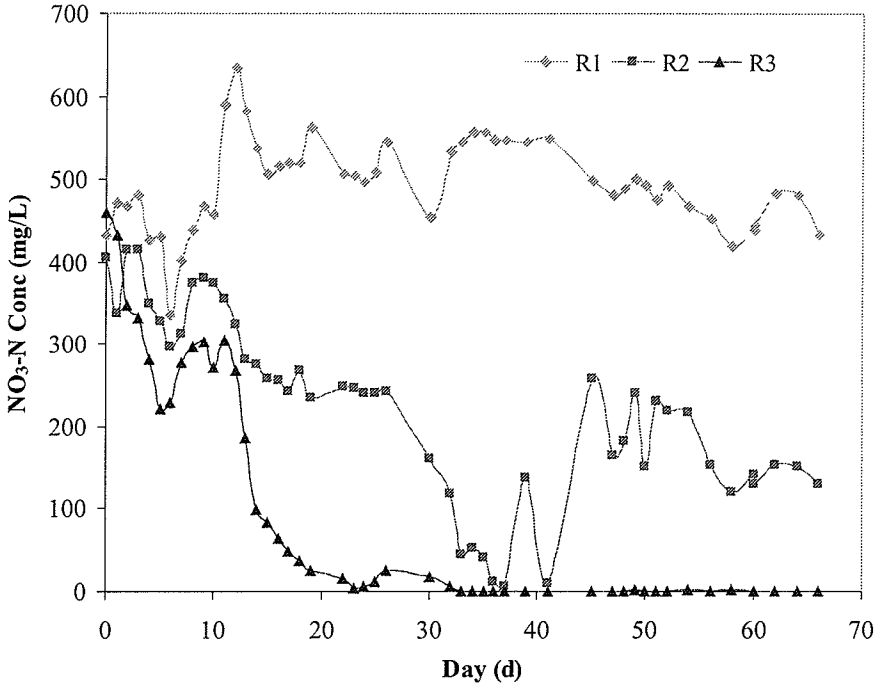


Figure 4-14 NO_3-N Conc. in effluents with methanol added
(R1 – aerobic; R2 & R3 – alternating aerobic/anoxic)

The methanol-enhanced reduction of NO_3-N did not show a noticeable boost until the 26th day, on which the dosage of methanol was 4.76 mg bsCOD/mg NH_3-N *d. Since then, the remaining sum of NH_3-N and NO_3-N decreased and the TN removal percentage increased significantly to 52 % until the 30th day, on which a fairly steady state reached and the optimum dosage was 4.9 mg bsCOD/mg NH_3-N *d. The denitrifiers needed at least 26 days (more than two times the 10 d system SRT) to respond to methanol, and 30 days – three times SRT – for a full acclimation. This statement was consistent with the results demonstrated by Akunna et al. (1993) and Nyberg et al. (1992 & 1996), who reported that 2-3 times SRT were needed to reach a stable nitrate reduction rates with methanol.

The long acclimation time which was required for denitrifiers to acclimate to methanol could attribute to the fact that there was only one special single group of denitrifying bacteria which can utilize methanol as their carbon source and this specialized denitrifying population had a low growth rate, as demonstrated by Andersson et al. (1995), Hallin et al. (1996b) and Akunna et al. (1993). The toxicity of methanol could be another factor resulting in the prolonged acclimation time for methanol (Chen et al., 2006).

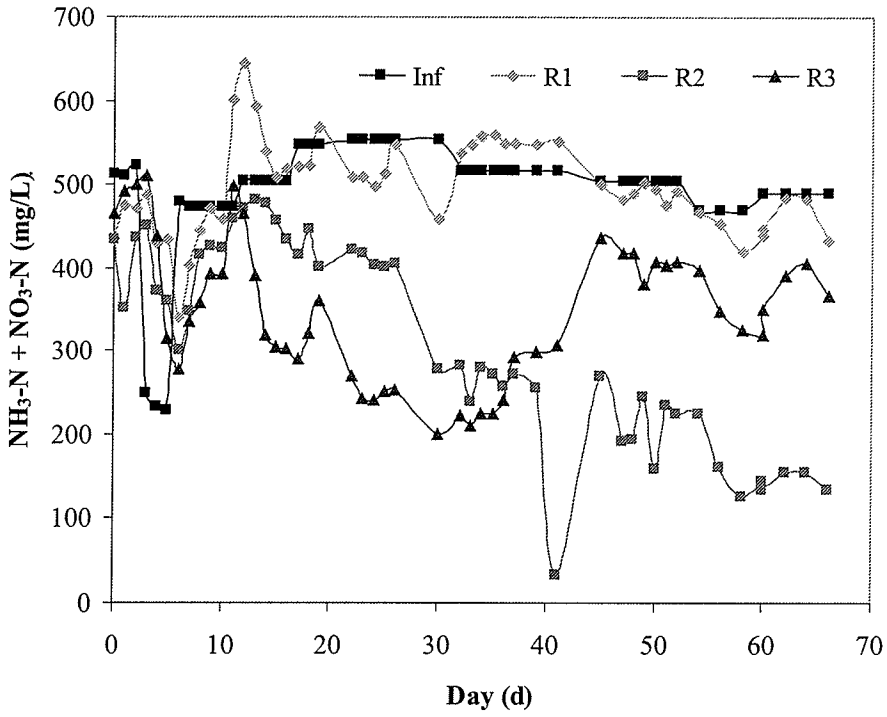


Figure 4-15 Sum of NH_3-N and NO_3-N Conc. in influent and effluents with methanol added
(R1 – aerobic; R2 & R3 – alternating aerobic/anoxic)

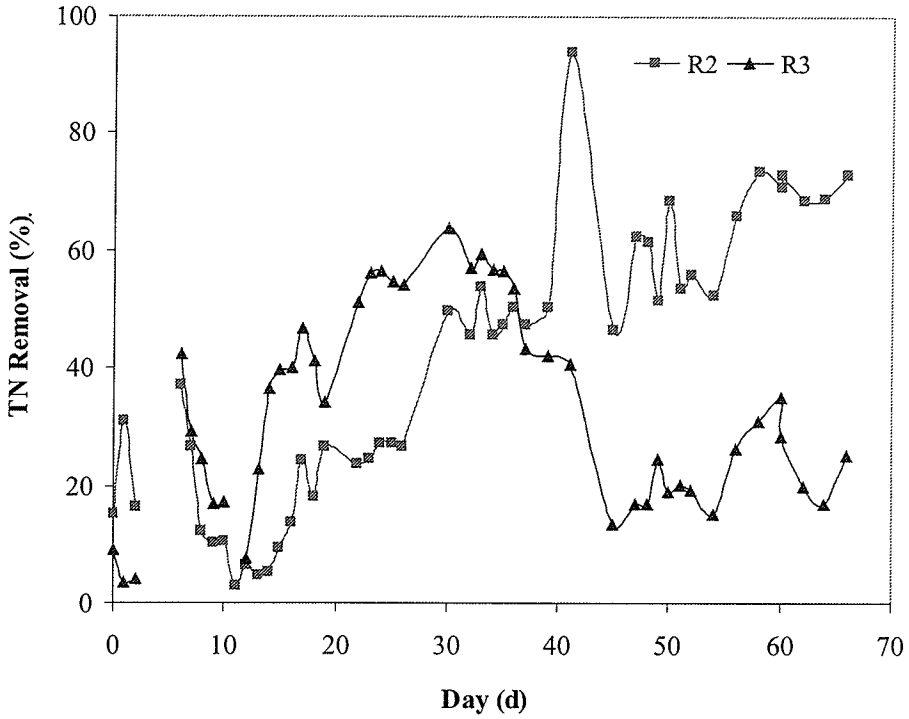


Figure 4-16 TN removal efficiency in reactors with methanol added
(R1 – aerobic; R2 & R3 – alternating aerobic/anoxic)

The NH_3-N concentration in the effluent from R2 declined after the 26th day, which could be caused by the improved SNR by the increased pH in R2 (the impact of pH on SNR is discussed in detail later). The rising pH could be explained by the fact that the more and more alkalinity was produced by methanol-boosted denitrification and was not completely consumed by nitrifiers since the nitrification process had not improved at this time yet. On the 30th day, the optimum pH for SNR of 7.7 was reached. After this day, the pH sustained the increasing trend. However from then on, the negative influence of pH on nitrification began to occur, resulting in the NH_3-N concentration increased steadily. It was the inhibition of NH_3-N oxidation by pH that caused the effluent NO_3-N concentration continued to decrease from the 30th day till the 37th day.

On Day 37, a sudden drop in the effluent NH_3-N concentration from R2 was observed. The concentration decreased to 10 mg/L, a level close to the NH_3-N in R1 effluent, in just 3 days. It was until this time that the nitrifiers became accumulated to the change of the addition of methanol. The acclimation of nitrifiers to methanol took 40 days, four times the 10 d SRT. On the other hand, because the denitrification process had developed to its optimum condition, it could not uptake the excessive NO_3-N produced by the acclimated nitrification, resulting in an increase in NO_3-N concentration in R2 effluent to around 150 mg/L and a slightly improved TN removal of 65% from the influent. Methanol can not elevate SDNR to a very high level and the HRT for the anoxic phase needed to be extended if more NO_3-N was required to be removed.

4.3.2.3 Effects with methanol addition by constant optimum dosage

The nitrification process in R3 was affected by methanol more notably at the beginning in R3 than in R2 because R3 received a constant dosage of 4.9 mg bsCOD/mg NH_3-N *d, which was larger than the dosage added to R2 during this period. The NH_3-N concentration leaving R3 ascended quickly to as high as 350 mg/L 20 days after the addition of methanol, as in Figure 4-13. Methanol had an inhibitory effect on nitrification, resulting in the decrease of the NO_3-N concentration out of R3 during the same period (Figure 4-14).

From Figure 4-15 and Figure 4-16, an increase of TN removal from R3 started to appear on the 12^{th} day, and then the TN removal reached 55% on Day 23. The approach to the

high TN removal percentage was at a faster pace than in R2 comparing to the 27% of TN removal in R2 at the same time. Denitrification process could be enhanced by methanol in a shorter time when adequate methanol was provided than when methanol was added in gradually increased dosage. The response time needed was 12 days and a full acclimation could be reached in 23 days with constant dosage of methanol, comparing to 26 days of response time and 30 days of full acclimation time with gradually increased methanol.

Since denitrifiers had reached a full acclimation on Day 23 but the nitrifiers, more and more alkalinity produced by denitrification accumulated after Day 23, resulting in an increased pH. On Day 30, pH reached 7.7, below which pH had a positive impact on SNR and above which a negative impact. After Day 30, pH continued to increase and an inhibition on SNR started to show. In turn, hinder nitrification increased the accumulation of alkalinity, resulting in pH to increase. On Day 40, pH reached 9.3, at which there was almost no NH_3-N in influent being oxidized. The removal of NH_3-N after the 45^{th} day could be explained by the stripping of the free ammonia gas out of the mixed liquor. Therefore, nitrifiers never recovered after the shock of the addition of methanol at the optimum dosage, even after 4 SRTs. The pH inhibition and free ammonia toxicity on nitrification was behind this observation.

Nitrifying bacteria can acclimate to methanol after 37 days (more than three time 10 d SRT) when methanol was added in incremental dosage, while the harm done on nitrification by the constant addition of high dosage of methanol had never recovered even after four times SRT. Therefore gradually increased dosage was preferred over the

constant optimum dosage when methanol was applied as an external carbon substrate even though shorter acclimation time was required for denitrifying bacteria when methanol was added in constant dosage and SDNR was increased more quickly. It was inferred that the ubiquitously used methanol is not the best carbon substrate in conditions of variable nitrate loads as the build-up of methanol-utilizing biomass took over three solids residence times (SRT). It is necessary to find substitutes to methanol.

4.3.2.4 Influence of pH on nitrification process

The inhibitory effect of pH on nitrification was observed during the course of the experiments performed on the influence of methanol on denitrification. The influence of pH on SNR was studied in detail. The experiment was performed in separate batch beakers. The results are shown in Figure 4-17.

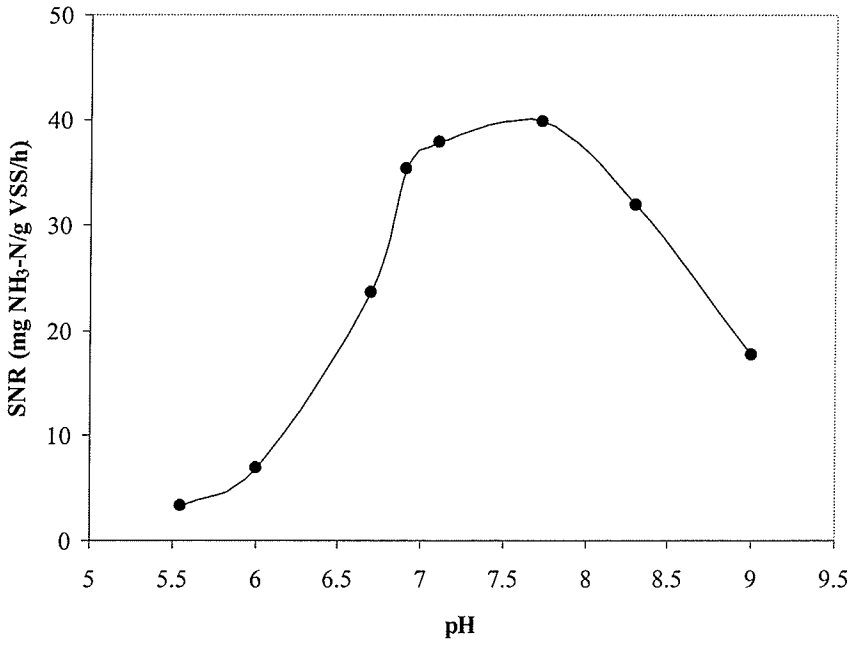


Figure 4-17 Effects of pH on SNR

From Figure 4-17, the optimum pH range which had the highest SNRs was from 6.9 to 8 with the acme of SNR occurring at the pH of 7.7. This range was within the pH extent of 6.45-8.95, under which total nitrification process could happen, reported by Ruiz et al. (2003) and comparable to the results presented by Groeneweg et al. (1994), who demonstrated that the pH between 7.5-8 could produce the maximum ammonia oxidation rate. Figure 4-17 also indicates that SNR decreased very significantly when pH became below 6.9 as the similar value was reported by Metcalf & Eddy (2003). When pH reached 5.6, the SNR was only 8% of the highest one. For the alkali range of pH, the SNR did not drop as fast as the acidic side of pH. It could be predicted that the SNR with pH of 9.3 was below one fourth of the highest SNR by extending the line describing the relationship of pH versus SNR. Since AOB is less sensitive than NOB to pH, there would be an accumulation of NO_2-N with pH lower than 6.9. The buildup of NO_2-N could produce un-ionized nitrous acid (HNO_2), which is a toxic compound on nitrification as reported by Yoo et al. (1999) and Zheng et al. (1994). In addition, the higher pH can produce a higher concentration of free ammonia, which also inhibits the nitrifiers' activities.

Since the increases of both pH and temperature can cause the free ammonia concentration to ascend and centrate is characterized by the high ammonia concentration and high temperature, the pH should be properly maintained when TN is removed from centrate.

4.4 Performance of intensified denitrification with external carbon sources

4.4.1 Enhanced SDNRs with different carbon sources

To determine the degree of boost of denitrification with different carbon sources, specific denitrification rate (SDNR) was used as the indicator. SDNR was measured by conducting NUR tests on the carbon sources after the biomass had acclimated to each carbon source and a steady state had been achieved. Therefore the measured SDNRs were the optimum values for each carbon source. Even though canola processing and oil refining wastewater inhibited the nitrification process, the SDNR with this substrate was still measured and compared with other carbon sources. To obtain a clear perspective of the rise in the denitrification rate with industrial wastewaters, the SDNR with methanol and ethanol, were also measured.

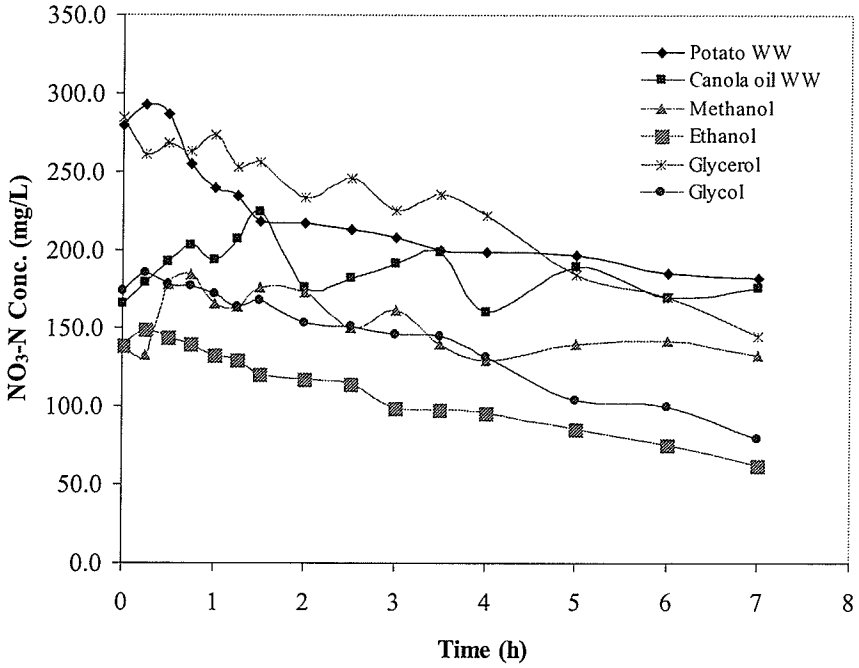


Figure 4-18 SDNR tests with different carbon sources

As in Figure 4-18, which presents the relationship of the decrease in NO_3-N concentrations versus time when the NUR tests were performed with each of the six carbon sources studied in this research, the lines for potato processing wastewater and glycerol solution had the deepest slopes, which were an indication that these two carbon sources had the highest SDNRs, while the line representing canola processing and oil refining wastewater had the flattest slope, therefore it could have the lowest SDNR. It was also noticed that the declining trends of NO_3-N concentrations for methanol and glycol had the similar pattern and the MLSS were also close when the NUR tests were conducted on them, thus they should have the similar SDNRs. In addition, the line for ethanol was the steadiest one and had a similar slope as methanol and glycol, but the MLSS for the test on ethanol was lower than the other two carbon sources. Consequently, a higher SDNR with ethanol than those with methanol and glycol could be resulted. The SDNRs were calculated according to Eq. (3-2), and they are summarized and compared in Figure 4-19.

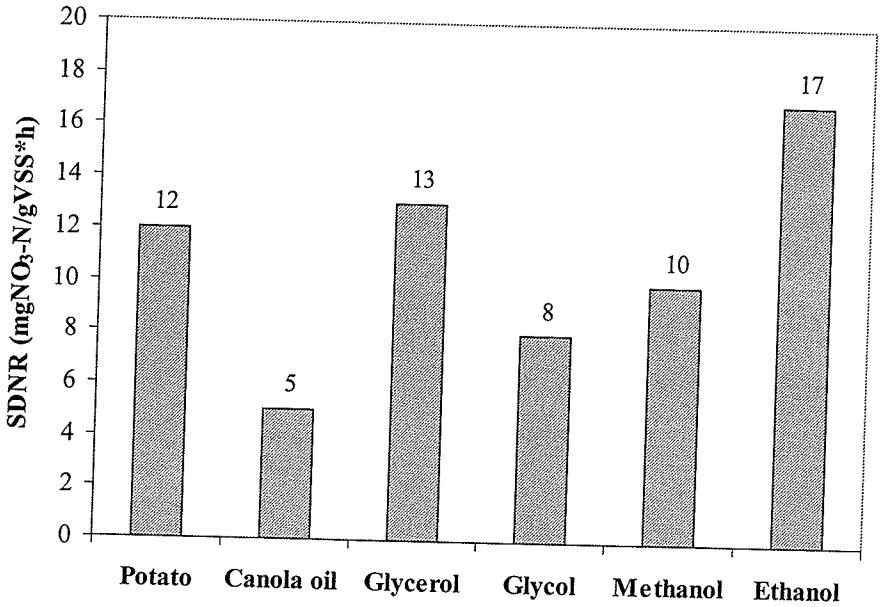


Figure 4-19 Values of SDNR with different carbon sources

As anticipated, the canola processing and oil refining wastewater had the lower SDNR than the other five carbon sources: the three industrial wastewaters of potato processing, the glycerol solution for biodiesel production, and glycol solution for the wastewater from WIA; and the two commonly purchased carbon sources in the form of single chemical of methanol and ethanol. The highest average SDNR with industrial wastewaters was 13 mgNO_3-N/gVSS*h, which was received when glycerol was used as a carbon source. The next highest average SDNR of 12 mgNO_3-N/gVSS*h was for potato processing wastewater. Glycol could enhance the SDNR to 8 mgNO_3-N/gVSS*h, which was not as good as the above two carbon sources. The SDNR with canola oil wastewater was the lowest one, which was only 5 mgNO_3-N/gVSS*h. In terms of the two purchased chemicals, methanol and ethanol had 10 mgNO_3-N/gVSS*h and 17 mgNO_3-N/gVSS*h respectively. It was clearly shown that the SDNR with ethanol was the highest among all of the six carbon sources. In comparison, the potato wastewater and glycerol as carbon sources had better SDNRs than methanol but worse than ethanol. The canola oil wastewater had the worst SDNR of all carbon substrates. The SDNR with glycol was also lower than both methanol and ethanol. The evaluated SDNRs were compared with those reported in various published literatures. Table 4-3 summarized the comparison.

Table 4-3 Comparison of denitrification rates (SDNR) with different carbon sources

Source	Wastewater Type	SDNR (mgNO_3-N/gVSS*h)					
		Potato WW	Canola WW	Glycerol	Glycol	Methanol	Ethanol
This study	Centrate	12	5	13	8	10	17
Literature	Municipal WW	Raw WW		5-25	6.5-8.4	1.8-10	6-26
		0.3-6.0					

Note: Reference to the Table 2-5

As in the above table, the obtained SDNRs for methanol, ethanol, glycol and glycerol were well in the range of the reported typical values. When the potato wastewater was used as an external carbon source, the SDNR could be increased by more than two times the rate with raw municipal wastewater, while canola processing and oil refining wastewater did not show any enhancement of the denitrification process.

Glycerol and glycol could boost the SDNR of raw municipal wastewater, hence the wastewaters from biodiesel industry and WIA could be good replacements to methanol and ethanol in BNR plants. The large variation in SDNRs found in this study could be explained that the combination of biomass in each test was different. Some of the tests may have larger amount of active bacteria, while others may have less. The diverse characteristics of each carbon source, especially the fractions of readily and slowly biodegradable COD, could be another factor influencing the SDNRs as the yield of each substrate differed, resulting in different substrate uptake rate by bacteria. The electron donors had different chemical structure, so the carbon requirement for each varied, resulting in a various degree of assimilability by denitrifiers. The species of denitrifying bacteria using different carbon sources had different growth rates. All of these reasons could have contributed to the varying values of SDNRs.

4.4.2 Acclimation time and optimum dosage for each carbon source

The time needed for a bacterial response to each external carbon source varied from 2 days up to 26 days. Denitrifiers reached a full acclimation to each carbon source in the

time range of 11 to 40 days. Among the four tested industrial wastewaters, potato processing wastewater had the shortest response time, while canola processing and oil refining wastewater had the longest. Figure 4-20 illustrates the relationship between TN removal and the days with the addition of each carbon source. In this figure, all of the carbon sources were added on the second day.

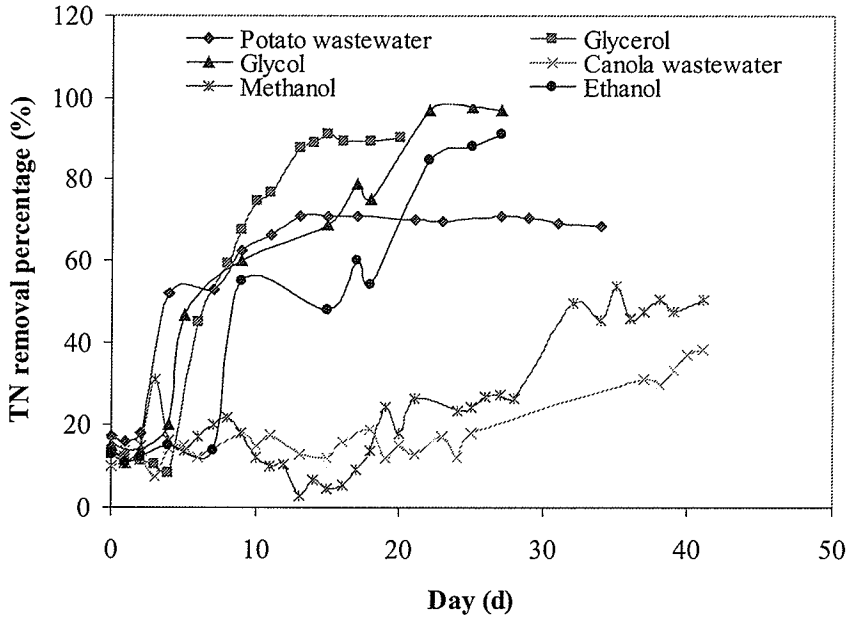


Figure 4-20 Time required to reach a full acclimation and TN removal achieved with different carbon sources

The response time was 2 days for potato processing wastewater and 4 days for glycerol. A full acclimation of denitrifiers using both of the two carbon sources was achieved in 11 days. Both substrates boosted SDNR to a level higher than that when methanol was utilized as an external carbon source. Under full acclimation, the optimum dosage of potato processing wastewater at 4.5 mg bsCOD/mg NH_3-N*d can achieve 71 % of TN removal, while 90 % of TN can be removed when glycerol was added at the optimum dosage of 5.4 mg bsCOD/mg NH_3-N*d. Therefore the potato wastewater and glycerol-

containing wastewater from biodiesel industry could be the most appropriate substitutes to the methanol and ethanol to enhance denitrification.

As for glycol, it could not produce a SDNR higher than methanol, but the response and acclimation time needed for glycol were only 3 days and 21 days, respectively. It could give denitrification an immediate boost in situations such as high flow and load conditions. At its optimum dosage of 4.6 mg bsCOD/mg NH_3-N*d, glycol can enhance the TN removal to 97 %.

The canola processing and oil refining wastewater cannot be used to improve denitrification. The bacteria took a very long 24 days to respond and 40 days to acclimate. Only 37 % of TN can be removed when this wastewater was dosed at 4.1 mg bsCOD/mg NH_3-N*d as the optimum. Thus its usage as a biodegradable substrate to replace methanol and ethanol was questionable.

In this study, a long response time of 26 days and acclimation time of 30 days were required for methanol, however it elevated the SDNR to a high level, resulting in 52 % of TN removal at the optimum dosage of 4.9 mg bsCOD/mg NH_3-N*d. Due to its relatively lower cost than ethanol, it is the most common external carbon source used in BNR plants – but not on as-need basis. Ethanol, on the other hand, not only produced a higher SDNR than methanol, but also required a much shorter response time of 6 days and a shorter acclimation time of 21 days, so it can be used either on regular or as-needed basis. With full acclimation, 89 % of TN can be removed with ethanol was applied at the

optimum dosage of 5.34 mg bsCOD/mg NH_3-N*d. The application of ethanol is not as extensive as methanol in the industry because it is more expensive than methanol.

Table 4-4 summarizes the performance of each carbon source.

Table 4-4 Summary of performance of each carbon source

Carbon sources	SDNR (mg NO_3-N /gVSS*h)	Response Time (d)	Acclimation Time (d)	TN removal (%)	Optimum dosage (mg bsCOD /mgTN)
Glycerol	13	4	11	90	5.40
Potato WW	12	2	11	71	4.50
Glycol	8	3	20	97	4.60
Canola WW	5	24	40	37	4.10
Ethanol	17	6	20	89	5.34
Methanol	10	26	30	52	4.90

The results obtained in this study were similar to those of De Lucas et al. (2005), who studied several agro-food wastewaters from slaughter-house, potato processing industries, cheese industries, milk bottling industries, beet sugar processing industries, winery industries and tomato processing industries. The same authors concluded that potato and tomato processing wastewaters had the highest denitrification potential (DNP) while the DNP was the lowest for slaughter-house wastewater, which contained oil and grease.

4.5 Impact of tested carbon sources on nitrification rate

The fact that organic compounds have an inhibitory effect on nitrification has been reported previously (Hanaki et al., 1990 a; Takach 1999; Dytczak et al., 2007). The main

reason for the inhibition on nitrifiers is the limited dissolved oxygen (DO) concentration resulted from the accelerated oxygen consumption rate by aerobic heterotrophic bacteria with increased organic substrate.

Among the six external carbon sources tested in this study, only canola processing and oil refining wastewater and methanol exerted inhibition on nitrification rate. The inhibition of canola processing wastewater was mainly due to the blocked passage of DO to activated sludge flocs by the oil and grease contained in canola processing wastewater. The low DO in the flocs limited the activities of nitrifiers. The inhibition of methanol on nitrification process was due to its toxic nature.

Other four external carbon sources, i.e. potato processing wastewater, glycerol, glycol, ethanol and methanol, did not present the inhibitory impact on nitrification rate, which was opposite to the literature-reported result. This observation in this study can attribute to the fact that adequate aeration was provided in the relatively small bioreactors during the research. No DO-limiting condition happened with this four carbon sources.

5.0 SUMMARY AND CONCLUSIONS

Three sequencing batch reactors (SBRs) were operated with different alternative external carbon sources to enhance the denitrification rate of digested sludge centrate. The sources included industrial wastewaters: potato processing wastewater, canola processing and oil refining wastewater, glycerol – a byproduct of biodiesel industry, and glycol – the main contaminant in the wastewater from Winnipeg International Airport, in comparison to methanol and ethanol. Their influence on the nitrification and denitrification was analyzed. The ratio of bsCOD to nitrogen was the basis to select the optimum dosage. The specific denitrification rate (SDNR) was specified for each carbon source by performing nitrate utilization rate (NUR) tests on each after a steady state was achieved.

The study found that:

1. No measurable harmful influence on nitrification rate was observed when potato processing wastewater, glycerol, glycol, and ethanol were applied.
2. Methanol was toxic and had a restraining impact on the nitrification process. Nitrifiers needed 37 days to respond to methanol when its dosage was increased gradually, and nitrifiers never recovered, even after four solids retention times (SRTs), when methanol was provided with the constant optimum dosage.

3. Canola processing and oil refining wastewater as a carbon source hindered the specific nitrification rate down to 7 mgNH_3-N/gVSS*h from 36 mgNH_3-N/gVSS*h without the carbon source.
4. Glycerol as an external carbon source produced an SDNR of 13 mgNO_3-N/gVSS*h which was better than methanol (10 mgNO_3-N/gVSS*h) but not as good as ethanol (17 mgNO_3-N/gVSS*h). A response time of 4 days and an acclimation time of 11 days were required. 90 % of TN removal can be reached at the optimum dosage of 5.4 mg bsCOD/mg NH_3-N*d.
5. Potato wastewater as a carbon source generated a SDNR of 12 mgNO_3-N/gVSS*h, it also had the shortest response time of 2 days and acclimation time of 11 days. Optimum dosage of 4.5 mg bsCOD/mg NH_3-N*d can remove 71 % of total nitrogen.
6. Glycol could boost the SDNR to 8 mgNO_3-N/gVSS*h. A shorter response time of 3 days was required than 26 days and 6 days for methanol and ethanol, respectively. The required acclimation time was 21 days, the same as ethanol but shorter than the acclimation time of 30 days required for methanol.
7. Glycol needed the optimum dosage of 4.6 mg bsCOD/mg NH_3-N*d and achieved the highest TN removal of 97 %.
8. Ethanol and methanol can remove 89% and 30 % of TN at the optimum dosage of 5.34 mg bsCOD/mg NH_3-N*d and 4.9 mg bsCOD/mg NH_3-N*d, respectively.
9. Canola processing and oil refining wastewater generated a SDNR of only 5 mgNO_3-N/gVSS*h, the lowest of all substrates. It needed a long response

time of 24 days and full acclimation time of 40 days. Only 37 % of TN can be removed at the most.

10. Both lower and higher pH had inhibitory effects on SNR. The high SNRs above 35 mgNH_3-N/gVSS*h could be obtained in the pH range of 6.9 to 8 with the highest SNR of 40 mgNH_3-N/gVSS*h occurring at the pH of 7.7.

It was concluded that potato processing wastewater and glycerol can be dependable alternatives to the purchased commercial chemicals because of the high denitrification efficiency and lack of any hindering effect on nitrification. In addition they had very short acclimation times and could exert an immediate boost of the denitrification rate.

Even though glycol can not enhance denitrification rate to the same level as potato wastewater or glycerol, it was still a good candidate to replace methanol and ethanol as it had a short acclimation time. On the other hand, canola processing and oil refining wastewater cannot be used as an external carbon source since it inhibited nitrification process, required a long acclimation time, and produced the lowest SDNR.

6.0 ENGINEERING SIGNIFICANCE

Throughout the world, millions of dollars are spent annually to purchase chemicals which enhance the denitrification process to achieve TN removal in large biological nutrient removal (BNR) wastewater treatment plants (WWTPs). The concept of replacing these costly chemicals with industrial wastewaters, which are rich in carbon and low in nitrogen, has tremendous sustainable development implications. The industrial waste is not treated at a significant cost to the industry but is picked up by the municipality as a valuable raw material for their wastewater treatment process. The municipality would have to cover the cost of transporting the material to its site, however this could be shared with the industries. Both parties would benefit from this merger of interests - the operating costs of nutrient removal are substantially lowered and at the same time the costs and environmental stress caused by treatment to industrial wastewaters are eliminated. The results obtained in this study were promising for the utilization of industrial wastewater as a source of carbon for enhanced denitrification, plus the initiation of adding industrial wastewater is a simple upgrade. This could save up to 2 million dollars annually in Winnipeg, MB, Canada alone. Adding some waste and observing the improvement in performance does not cause any disturbance in the normal operation of the plant, therefore the concept could be applicable and tested at other BNR plants, since almost all WWTPs face the same issue of insufficient carbon in raw wastewater. In addition, the enhanced denitrification can reduce the problems of filament proliferation caused by nitrate and nitrite.

7.0 RECOMMONDANCES FOR FUTURE WORK

For the purpose of this study, commercial glycerol substituted for biodiesel byproduct wastewater and while glycol substituted for wastewater from Winnipeg International Airport (WIA). Actual wastewaters produced in the biodiesel industry and from WIA are recommended in future studies to determine if there are any inhibiting substances existing in the wastewaters. City of New York is conducting trials on glycerol and collaboration is recommended by the Department of Environmental Protection there.

Other forms of industrial wastewaters available in Manitoba, such as wastewaters from fiber-board manufacturing, pulp mill, wet grain milling, and distillery plants, are recommended for testing to determine whether a higher intensification of denitrification can be achieved.

The major objective of this research was to determine the applicability of industrial wastewaters as external carbon sources. Therefore the effluent nitrogen concentration was not reduced to a very low level. Future study need to be conducted to search the optimum operating parameters to reduce the TN concentration in effluents.

This research has indicated that potato processing wastewater has a good potential to act as an external carbon substrate to augment denitrification process. The pilot-scale application of this wastewater should be the next step.

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APPENDIX A NITROGEN TESTS RECORDS

Table A-1 NH_3-N & NO_3-N concentrations in influents and effluents with canola processing and oil refining wastewater added

Date	NH_3 (mg/L)				$NO_3 + NO_2$ (mg/L)			NO_2 (mg/L)		
	In	R1	R2	R3	R1	R2	R3	R1	R2	R3
22.VIII.05	323.6	0	0	8.9	537.4	275.9	287.4	0	0	0
23.VIII.05		0	4.4	1	577.8	291.6	302.4	0	53.3	79
24.VIII.05	332.5	0	56.7	15	571.6	232	265.1	0	0	37.5
25.VIII.05		0	110.9	43	527.8	184.1	225.8	0	0	40.1
26.VIII.05	337.9	0	181.2	81.1	586.6	147.6	183.2	0	0	30.6
29.VIII.05	459.1	0	304.1	266.5	526.5	25.2	102.4	0	11.5	44.8
30.VIII.05		0	325.3	277.1	585.7	18	86.2	0	9.6	38
31.VIII.05	448.2	0	329.2	290.6	600.5	21.3	96.5	0	13	52.8
2.IX.05	499.5	250.2	401.2	391.2	326.2	20.5	57	0	10.8	27.4
4.IX.05		308.5	303.4	312.3	103.2	9.5	3.9	0	0.5	0.2
5.IX.05		279.5	323	328.5	71.4	0.5	1.1	0	0	0
7.IX.05		309.3	342.2	331.9	20	0	0	0	0	0
8.IX.05		397.5	396.8	420.3	7.5	0	4.8	0	0	0
9.IX.05		408.2	456.8	480.4	2.5	0	3.5	1.5	0	0
10.IX.05		464.4	510	510.8	4	0	1.6	0	0	0
12.IX.05		442.4	502.4	567.8	4	0	1.9	0	0	0
13.IX.05		542	575.4	585.3	8.5	0	1.4	0	0	0
14.IX.05		580	595.2	633.9	6.5	0	0	0	0	0
26.IX.05		361.4	154.3	330.6	132.9	293	169.9	29.7	77.4	36.4
27.IX.05		217.2	59.9	208.9	177	307.9	192.8	46.7	108.4	0
28.IX.05		184.6	16.7	187.2	215	317.9	235.7	55	129.8	59.3
29.IX.05		177.7	35.4	145.5	235.5	332.7	244.5	89.1	198.7	79.3
30.IX.05		135.4	24.3	129.5	226.9	285.3	209.6	80.9	85.6	111.3
3.X.05		82.4	0	72.9	275.6	300.4	261.4	194.8	118.8	77.8
4.X.05		83.7	8.77	83	262.9	232.9	253.1	112.6	0	129.7
6.X.05		116.7	26.4	124	243.5	281.1	260.9	110.6	102.8	43.9
8.X.05		240.5	159.1	234.7	130.3	184.1	134.1	0	0	0
10.X.05		245.1	184	260.5	128.9	146.9	128.3	9.2	19.8	5.8
13.X.05		178.7	194.5	187.3	233.9	97.9	197.4	67.7	0	31.7

Date	NH_3 (mg/L)				$NO_3 + NO_2$ (mg/L)			NO_2 (mg/L)		
	In	R1	R2	R3	R1	R2	R3	R1	R2	R3
14.X.05		149.2	111.8	124.2	259.1	176.1	262.9	78.6	27.6	0
17.X.05		78.5	99.2	78.2	319.4	233.5	301.3	183.7	57.2	152.4
18.X.05		20.1	25.5	31.3	347	243.3	295.9	26.6	45.3	67.5
19.X.05		29.2	5.1	28.8	313.7	222.1	274.6	117.6	86.5	126.9
20.X.05		5.8	2	15.2	322	207.7	278.9	102.4	47.2	98.8
21.X.05		3.3	1.6	10.7	300	198.8	260	58	97.1	35.4
24.X.05		3.2	2.5	19.9	313	150.2	233.1	36.9	41.3	62.4
25.X.05		3.4	2.3	7.4	318.6	146.8	241	60.7	42.3	76.6
26.X.05		5.5	1.1	5.4	323.3	153.6	247.8	149	44.4	85.7

Table A-2 NH_3-N & NO_3-N concentrations in influents and effluents with potato processing wastewater added

Date	NH_3 (mg/L)				$NO_3 + NO_2$ (mg/L)			NO_2 (mg/L)		
	IN	R1	R2	R3	R1	R2	R3	R1	R2	R3
8.VI.05	505.5	0.6	1.3	1.1	414.3	271.9	212.5	52.6	0	
10.VI.05	531.7	0.5	0.7	1.4	422.3	268.2	240	23.3	0	
13.VI.05	519.9	0.1	0.5	0	409.2	255.5	234.8	0	0	
15.VI.05	552.9	1.5	2.2	1.7	385.6	216	196.8	0	0	
17.VI.05	573.5	1.4	2.1	1.8	375	200.9	181.8	0	0	
19.VI.05	580	2	2.2	2.4	359.3	176.5	154.5	0	0	
21.VI.05	548.7	0.7	1.6	1.1	295	158.9	158.1	0	0	
23.VI.05	572.4	0.5	0.6	0.6	376.1	246	208.5	0	0	
27.VI.05	506.5	0.2	0.4	0.4	425.7	227.1	207.3	6.6	0	
29.VI.05	509.1	0.3	0.4	0.4	371.2	225.9	212.5	0	0	
3.VII.05	374.4	0	0.3	0.3	233.9	113.4	102.4	0	0	
5.VII.05	364	0	0.5	0.2	295.1	159.7	138.4	0	0	
7.VII.05	453.4	0	0.3	0.2	240.4	141.3	135.6	0	0	
10.VII.05	365	0	1	0.7	249.2	116.3	114.2	0	0	
12.VII.05	378.1	0	0.7	1	257.9	110.1	108.9	0	0	
14.VII.05	386.3	0	1	1.1	281.1	123.4	126.7	0	0	
17.VII.05	424	0	0.9	1.1	268.2	140.3	133.6	40.4	38	

Date	NH_3 (mg/L)				$NO_3 + NO_2$ (mg/L)			NO_2 (mg/L)		
	IN	R1	R2	R3	R1	R2	R3	R1	R2	R3
19.VII.05	366.5	0	1.3	1.2	277.7	147.3	126.6	0	0	
21.VII.05	410.3	0.5	1.4	0.9	278.6	148.6	122.8	0	0	
24.VII.05	393.2	0	0	0	387.5	181.9	160.1	0	0	
25.VII.05		0	0.2	0.2	448.6	184.2	154.2	0	0	0
26.VII.05		0	130.6	142.9	487.1	30.25	23.24	29.22	21.34	0
27.VII.05	322	0	0.3	0.7	503.2	243.8	241.8	0	0	5.2
28.VII.05		0.3	0	0	538.1	189.4	174.4	0	0	2
29.VII.05	332.2	0	0	0	473.7	176.7	159	0	0	2.5
1.VIII.05	311.6	0	0.1	0.2	413.8	186.1	178.6	0	0	2
2.VIII.05		0	0	0	460.4	191.3	180.4	0	0	0
3.VIII.05	301.2	15.2	0	0.2	399.1	188.9	178.6	0	0	0
4.VIII.05		0	0.1	0.1	410.8	184.6	176	0	0	0
5.VIIII.05	299.5	0	0.1	0.1	442	195.8	188.8	0	0	0
7.VIII.05	432.4	0	0.7	0.5	438.5	229.3	210.9	0	0	20
9.VIII.05	451.8	0	1.2	1	530.9	252.3	239.5	0	0	45
10.VIII.05		0.5	0.7	0.7	534.5	248.5	230	0	0	50
11.VIII.05	439.3	0.5	1	0.8	532.1	253.6	244.6	0	0	50
15.VIII.05	468	0	1	1.2	547.4	310.9	314.5	0	0	77.2
16.VIII.05		0	0.8	1.2	599	322.4	322.4	0	0	68.2
17.VIII.05	480	0	0.5	1	605.1	332.9	337.1	0	0	41
19.VIII.05	490	0	0.4	0.5	515.2	313.2	324.4	47.3	0	28.5

Table A-3 NH_3-N & NO_3-N concentrations in influents and effluents with glycerol added

Date	NH_3 (mg/L)				$NO_3 + NO_2$ (mg/L)			NO_2 (mg/L)		
	In	R1	R2	R3	R1	R2	R3	R1	R2	R3
16.XI.06	510	1	2	3	458	426	450	0	0	0
17.XI.06	490	1	3	2	446	450	436	0	0	0
18.XI.06	515	1	5	5	463	431	466	0	0	0
20.XI.06	495	1	3	2	450	410	420	3	0	0
22.XI.06	485	1	2	1	438	392	197	0	0	0

23.XI.06	505	1	2	3	452	367	161	0	8	0
24.XI.06	510	1	1	1	455	374	129	0	0	0
25.XI.06	520	1	1	1	470	350	119	0	0	0
27.XI.06	562	1	1	1	498	261	67	0	0	0
28.XI.06	540	1	4	1	470	250	59	4	0	0
29.XI.06	550	1	2	1	482	221	49	0	0	3
30.XI.06	545	1	4	1	478	193	58	0	0	0
2.XII.06	560	1	3	1	498	77	59	0	0	0
4.XII.06	550	1	2	1	486	65	52	0	0	0

Table A-4 NH_3-N & NO_3-N concentrations in influents and effluents with glycol and ethanol added

Date	NH_3 (mg/L)				$NO_3 + NO_2$ (mg/L)			NO_2 (mg/L)		
	IN	R1	R2	R3	R1	R2	R3	R1	R2	R3
14.VI.06	519	4.5	3	1.8	357.8	338.3	346.1	0	19.5	21.4
16.VI.06	284	2.2	5	2.7	380.6	360.7	367.8	0	19.9	22.5
19.VI.06	320	3.7	1.3	1.5	319.6	265.8	318.9	2	16.6	17.1
21.VI.06	538	3.4	4.2	2.2	292.3	212	240.9	0	10.1	15.2
27.VI.06	248	3	6.9	3	341.3	240.7	275.6	0	12.3	6.3
29.VI.06	300	1.9	2	3.3	262.6	137.6	173	0	9.3	7.1
30.VI.06	315	3.3	3.7	1.8	270.5	112.6	142.8	0	2	2.2
4.VII.06	325	1.2	1.6	1.7	235	8.3	47.9	4	5	0
7.VII.06	365	1.6	1.6	2.5	231	7.3	41.3	0	0	0
9.VII.06	355	1.5	4	1.5	281	7	30.1	0	0	0
11.VII.06	346	1.8	1.5	2.8	284.6	149.2	147.7	0	2	0
13.VII.06	431	1.5	1.7	5	338.2	258.7	277.6	5	0	3
17.VII.06	420	2.1	3	4	374.6	326.7	325.2	0	0	0
19.VII.06	251	2.1	7.4	6	335	321.4	330.5	3	0	0
21.VII.06	252	3	3	4	221	212	216	0	3	3
23.VII.06	246	2	2	2	207	120	148	0	0	5
24.VII.06	248	2	2	2	201	42	47	0	0	0
26.VII.06	228	2	1	2	187	14	5	0	0	0
28.VII.06	219	1	1	1	197	15	5	7	2	0
29.VII.06	300	1	2	2	225	18	5	0	0	0

Note: Glycol was added into R2; Ethanol was added into R3.

Table A-5 NH_3-N & NO_3-N concentrations in influents and effluents with methanol added

Date	NH_3-N (mg/L)				NO_3-N (mg/L)			NH_3-N + NO_3-N (mg/L)			Removal %		
	IN	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
21-II-07	512	3	6	7	432	405	459	435	411	466	15.0	19.7	9.0
22-II-07	510	3	13	60	472	338	432	475	351	492	6.8	31.1	3.5
23-II-07	522	3	22	154	468	414	347	471	436	501	9.7	16.5	4.0
24-II-07	500	6	38	180	481	414	331	487	452	511	0.0	9.7	0.0
25-II-07	490	4	24	157	427	349	282	431	373	439	0.0	23.9	10.4
26-II-07	490	4	32	94	430	328	222	434	360	316	0.0	26.6	35.5
27-II-07	480	4	5	49	336	296	228	340	301	277	29.1	37.3	42.3
28-II-07	474	3	35	58	401	313	278	404	348	336	14.7	26.6	29.1
1-III-07	474	5	41	60	439	374	297	444	415	357	6.3	12.3	24.6
2-III-07	474	3	46	90	468	380	303	471	426	393	0.7	10.1	17.1
3-III-07	474	3	51	121	457	374	272	460	425	393	3.0	10.4	17.2
4-III-07	474	12	105	195	589	355	304	601	460	499	0.0	3.0	
5-III-07	505	9	149	198	635	323	268	644	472	466	0.0	6.6	7.7
6-III-07	505	10	201	203	582	281	187	592	482	390	0.0	4.6	22.7
7-III-07	505	2	202	221	537	276	99	539	478	320	0.0	5.3	36.7
8-III-07	505	2	200	221	506	258	83	508	458	305	0.0	9.4	39.7
9-III-07	505	3	180	239	516	255	63	519	435	303	0.0	13.8	40.1
10-III-07	547	3	172	243	519	243	48	522	415	291	4.6	24.2	46.8
11-III-07	547	3	180	284	520	267	37	523	447	321	4.4	18.2	41.3
12-III-07	547	6	167	336	563	235	25	569	402	361	0.0	26.5	34.1
15-III-07	553	2	173	255	507	249	15	509	422	270	7.9	23.7	51.2
16-III-07	553	3	172	240	505	246	3	508	418	243	8.2	24.4	56.0
17-III-07	553	3	163	236	496	240	5	499	403	241	9.8	27.1	56.5
18-III-07	553	3	162	239	509	240	11	512	402	250	7.4	27.3	54.7
19-III-07	553	4	165	228	544	242	25	548	407	253	0.9	26.5	54.2
23-III-07	553	4	119	183	510	160	17	514	279	200	7.1	49.6	63.8
25-III-07	516	4	162	216	533	119	6	537	281	222	0.0	45.5	56.9
26-III-07	516	4	194	210	545	45	0	549	239	210	0.0	53.8	59.3
27-III-07	516	3	228	224	556	53	0	559	280	224	0.0	45.7	56.7
28-III-07	516	4	231	225	556	41	0	559	272	225	0.0	47.3	56.4
29-III-07	516	3	244	240	547	12	0	550	256	240	0.0	50.3	53.5
30-III-07	516	3	266	292	547	5	0	550	272	292	0.0	47.3	43.4

Date	NH_3-N (mg/L)				NO_3-N (mg/L)			NH_3-N + NO_3-N (mg/L)			Removal %		
	IN	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
1-IV-07	516	3	119	299	545	138	0	548	256	299	0.0	50.3	42.1
3-IV-07	516	3	23	306	548	238	0	551	261	306	0.0	49.4	40.6
7-IV-07	505	2	12	437	499	259	0	500	271	437	0.9	46.4	13.5
9-IV-07	505	2	26	419	480	165	0	482	191	419	4.5	62.2	17.1
10-IV-07	505	2	12	419	489	182	0	491	194	419	2.8	61.5	17.1
11-IV-07	505	2	6	380	500	240	1	503	246	381	0.4	51.4	24.5
12-IV-07	505	2	8	408	493	151	0	495	159	408	2.0	68.5	19.1
13-IV-07	505	1	5	403	475	230	0	476	235	403	5.7	53.5	20.1
14-IV-07	505	1	5	407	492	219	0	493	224	407	2.4	55.7	19.4
16-IV-07	470	1	7	397	467	217	1	467	224	398	0.5	52.4	15.3
18-IV-07	470	1	7	347	452	154	0	453	161	347	3.7	65.8	26.2
20-IV-07	470	1	4	324	419	121	1	420	125	325	10.7	73.4	30.9
22-IV-07	490	1	2	318	438	142	0	439	143	318	10.3	70.7	35.1
24-IV-07	490	2	2	351	444	131	0	446	133	351	9.0	72.9	28.4
26-IV-07	490	2	2	392	482	153	0	484	155	392	1.3	68.4	20.0
28-IV-07	490	1	2	406	481	152	0	482	154	406	1.6	68.6	17.1
30-IV-07	490	1	2	366	431	131	0	433	133	366	11.6	72.9	25.2

APPENDIX B COD TESTS RECORDS

Table B TCOD & sCOD concentrations in raw centrate, influents, and effluents from reactors with different carbon sources added

Date	Total COD (mg/L)					Soluble COD (mg/L)				
	IN_a	IN_b	R1	R2	R3	IN_a	IN_b	R1	R2	R3
7.VI.05		2137.6	159.9	149.8	128.6		1644.5	101.1	98.8	80.1
10.VI.05		1781.2	138	265.1	132		1010.4	115.6	187.3	129
13.VI.05		1400	127.5	230.7	100.6		1189.8	82.7	100.6	55.8
15.VI.05		1699	100.6	167.9	78.2		840	82.7	70.7	55.8
17.VI.05		2184.8	99.1	145.5	57.3		916.2	70.7	67.7	39.3
21.VI.05		1997.9	84.2	160.4	123		844.5	55.8	70.7	85.7
23.VI.05		2894	175.4	227.7	169.4		1109.1	85.7	115.6	100.6
27.VI.05		2229.6	205.3	230.7	169.4		1064.2	100.6	145.5	102.1
29.VI.05		2386.5	235.2	205.3	160.4		1243.6	132	123	97.6
3.VII.05		2745.3	220.2	172.4	167.9		1472.3	145.5	115.6	115.6
5.VII.05		2715.4	176.9	170.9	147		1638.2	145.5	114.1	100.6
7.VII.05		2603.3	203.8	205.3	173.9		1512.6	144	142.5	132
10.VII.05		2371.6	175.4	190.3	160.4		1342.2	112.6	112.6	87.2
12.VII.05		2409	158.9	205.3	182.8		1342.2	100.6	109.6	100.6
14.VII.05	993.5	2438.9	130.5	220.2	212.7	308.4	1283.9	100.6	115.6	100.6
17.VII.05	829.1	1997.9	199.3	194.8	260.6	369.7	1153.9	175.4	160.4	163.4
19.VII.05		2072.6	115.6	182.8	157.4		1333.3	85.7	115.6	100.6
21.VII.05		2035.3	100.6	220.2	160.4		1198.7	85.7	102.1	100.6
24.VII.05	769.3	2110	115.6	145.5	145.5	339.8	1167.4	88.7	100.6	70.7
27.VII.05	708.8	1691.5	147	294.9	294.9	290.5	929.7	100.6	197.8	235.2
29.VII.05	449.4	1781.2	160.4	190.3	166.4	190.3	1028.3	109.6	90.2	81.2
1.VIII.05		1624.2	145.5	130.5	115.6		786.2	85.7	63.3	69.2
5.VIII.05	715.5	1400	145.5	108.1	100.6	447.4	804.1	70.7	49.8	55.8
7.VIII.05	978.5	1863.4	190.3	132	132	366.7	970	145.5	70.7	100.6
11.VIII.05	1038.3	1699	235.2	160.4	160.4	339.8	781.7	163.4	87.2	91.7
15.VIII.05		1474.7	209.7	184.3	220.2		884.8	173.9	100.6	108.1
17.VIII.05	649.7	1243	190.3	175.4	259.1	250.1	974.5	160.4	118.6	100.6
19.VIII.05		929.1	175.4	161.9	280		840	138	130.5	103.6
22.VIII.05		1011.4	160.4	115.6	145.5		642.7	127.5	103.6	100.6

Date	Total COD (mg/L)					Soluble COD (mg/L)				
	IN_a	IN_b	R1	R2	R3	IN_a	IN_b	R1	R2	R3
24.VIII.05	548	1175.8	182.8	444.4	309.9	223.2	745.8	85.7	160.4	220.2
26.VIII.05		1385.1	173.9	459.4	474.3		795.2	93.1	182.8	197.8
29.VIII.05	592.9	1332.7	190.3	549.1	519.2	247.1	799.6	114.1	224.7	235.2
31.VIII.05		1048.7	230.7	803.2	607.4		750.3	129	574.5	294.9
2.IX.05	574.9	876.8	638.7	530.1	637.2	309.9	799.6	407.1	278.5	335.3
1.XI.05		1720	590	409	515		1463	540	319	429
4.XI.05		1658	542	320	592		1328	510	220	372
8.XI.05		1210	537	320	464		1008	500	260	408
13.XI.05		1148	653	423	601		978	609	319	516
15.XI.05		1034	683	413	639		894	624	376	568
17.XI.05		906	698	492	681		872	649	459	600
20.XI.05		1056	685	458	715		840	640	426	636
21.XI.05		1130	660	445	744		852	642	431	633
23.XI.05		1368	557	494	743		1078	539	432	725
25.XI.05		1642	622	540	780		1538	583	516	754
28.XI.05		1740	380	368	720		1472	271	300	585
30.XI.05		2200	232	243	604		1874	179	234	575
3.XII.05		2050	305	326	595		1644	234	255	498
5.XII.05		2398	330	330	615		1930	212	258	473
7.XII.05		2354	372	330	480		1980	264	178	362
10.XII.05		2580	282	250	369		2114	204	191	260
3.I.06		2680	454	312	315		2298	265	154	189
5.I.06		2450	464	226	272		2056	360	178	187
9.I.06		2628	397	225	268		2316	254	185	205
11.I.06		2708	360	283	247		2306	278	163	177
13.I.06			324	280	260			237	170	185
16.I.06		2634	236	210	210		2174.0	157	156	162
20.I.06		2324	198	199	185		1990	151	150	144
23.I.06		2532	201	214	205		2208	172	157	160
27.I.06	440	2056	192	212	261	194	1938	136	140	110
31.I.06	125	2158	148	116	163	81	2020	143	94	115
4.II.06	940	2664	274	349	323	351	2184	180	139	213
13.II.06		2420	342	223	293		2140	253	178	237

Date	Total COD (mg/L)					Soluble COD (mg/L)				
	IN_a	IN_b	R1	R2	R3	IN_a	IN_b	R1	R2	R3
17.II.06	696	2132	236	212	246	383	1974	174	205	219
24.II.06	1540	2734	264	377	388	506	2048	177	197	192
28.II.06		3014	266	433	409		2152	173	183	195
4.III.06	1519	2806	213	395	402	512	2296	169	179	196
9.III.06		2978	171	205	207		2484	156	188	162
13.III.06	987	2970	270	293	294	438	2522	180	144	197
23.III.06	1604	3400	317	208	438	546	2818	230	122	246
17.IV.06		714	193	227	238		297	156	167	189
20.IV.06		567	171	236	245		302	149	182	158
2.V.06		587	246	245	239		318	195	184	193
4.V.06		624	307	284	229		268	176	183	171
18.V.06		432	182	193	180		271	120	120	125
24.V.06		650	197	218	232		227	146	167	138
30.V.06		538	198	213	240		405	172	151	121
5.VI.06		507	211	276	235		234	147	217	134
14.VI.06	1098		283	302	214	443		251	178	157
16.VI.06	589		229	297	280	230		189	204	183
19.VI.06			217	197	204			129	92	122
21.VI.06	1083		200	191	182	485		131	125	135
27.VI.06	360		330	278	340	170		240	250	230
29.VI.06	440		160	190	176	290		137	123	151
4.VII.06	370		125	120	105	190		100	90	85
7.VII.06	660		115	135	115	230		100	104	100
11.VII.06	600		207	260	144	245		110	145	125
13.VII.06	745		205	280	270	325		120	125	150
19.VII.06	449		250	320	330	186		124	128	150
21.VII.06	430		200	210	220	220		100	150	100
24.VII.06	415		175	185	170	165		120	148	106
26.VII.06	306		133	135	170	150		109	100	105
28.VII.06	295		120	136	141	145		90	85	95
31.VII.06	756		125	123	162	326		85	100	117
2.VIII.06	710		335	300	485	346		262	228	303
4.VIII.06	710		269	347	520	302		217	292	373

Date	Total COD (mg/L)					Soluble COD (mg/L)				
	IN_a	IN_b	R1	R2	R3	IN_a	IN_b	R1	R2	R3
7.VIII.06	787		182	248	1169	326		143	210	1059
9.VIII.06					1366					1185
11.VIII.06	1073		193	204	602	432		187	180	220
14.VIII.06	1077		259	299	386	420		172	193	254
16.VIII.06					315					250
17.VIII.06	550					298				
18.VIII.06	910		260	290	330	436		185	165	200
4.XII.06	810		171	468	218	280		165	454	211
6.XII.06			200	340	220			140	270	200
11.XII.06			209	310	225			184	240	160
13.XII.06	780		180	210	240	340		136	158	150
9.I.07	580		170	330	190	340		140	170	170

Note: INa: original centrate; INb: mixture of centrate with carbon sources
With Potato wastewater: 8.VI.05-19.VIII.05
With Canola oil wastewater: 22.VIII.05-26.X.05
With Glycol and Ethanol: 14.VI.06-29.VII.06
With Glycerol: 16.XI.06-4.XII.06

APPENDIX C SOLIDS TESTS RECORDS

Table C Solids concentrations in influents, effluents, and bioreactors

Date	Influent TSS (mg/L)	MLSS (mg/L)			Influent VSS (mg/L)	MLVSS (mg/L)			VSS/TSS				Effluent TSS (mg/L)		
		R1	R2	R3		R1	R2	R3	IN	R1	R2	R3	R1	R2	R3
8.VI.05	1440	3130	3250	4060	1160	2560	2700	3380	0.81	0.82	0.83	0.83	80	80	40
10.VI.05	1470	3410	3500	4610	1190	2750	2910	3680	0.81	0.81	0.83	0.80	45	87	20
13.VI.05	1590	3820	3940	4150	1270	3030	3030	3270	0.80	0.79	0.77	0.79	25	90	20
15.VI.05	1420	4030	4090	4320	1100	3170	3160	3250	0.77	0.79	0.77	0.75	45	100	33
17.VI.05	1520	4170	4130	4720	1270	3320	3290	3660	0.84	0.80	0.80	0.78	57	110	62
19.VI.05		3710	3840	3540		2990	2980	2820		0.81	0.78	0.80			
21.VI.05	1680	4110	3850	3730	1270	3180	2900	2760	0.76	0.77	0.75	0.74	42	102	50
23.VI.05	1830	4270	4120	3930	1330	3130	3120	2830	0.73	0.73	0.76	0.72	62	105	62
27.VI.05	1300	4250	3975	4100	950	3350	3075	3225	0.73	0.79	0.77	0.79	57	55	37
29.VI.05	1275	4225	4125	4300	1000	3575	3350	3500	0.78	0.85	0.81	0.81	62	67	37
3.VII.05		4175	4100	4050		3425	3375	3350		0.82	0.82	0.83	57	67	32
5.VII.05	1225	4300	4175	4200	1100	3350	3225	3325	0.90	0.78	0.77	0.79	27	75	30
7.VII.05	1075	4325	4250	4375	925	3400	3600	3775	0.86	0.79	0.85	0.86	27	50	20
10.VII.05	1050	3700	3750	3775	725	2900	2975	3075	0.69	0.78	0.79	0.81	47	75	25
12.VII.05	1600	4500	3975	4050	1100	3500	3100	3325	0.69	0.78	0.78	0.82	37	72	32
14.VII.05	920	3650	3200	3500	800	3050	2700	2950	0.87	0.84	0.84	0.84	20	50	25
17.VII.05	800	3325	3150	3200	725	3075	2850	2950	0.91	0.92	0.90	0.92	25	57	47
19.VII.05	950	3650	3125	3475	800	3050	2650	2875	0.84	0.84	0.85	0.83	32	55	52
21.VII.05	900	3400	2905	3175	800	2975	2575	2850	0.89	0.88	0.89	0.90	27	72	50
24.VII.05	850	3250	3075	3250	750	2725	2600	2750	0.88	0.84	0.85	0.85	42	62	42
27.VII.05	880	2860	2840	3100	700	2300	2460	2640	0.80	0.80	0.87	0.85	52	57	47
29.VII.05	640	2140	2600	2860	540	1820	2320	2500	0.84	0.85	0.89	0.87	30	55	37
1.VIII.05	650	1580	2440	2540	580	1460	2320	2320	0.89	0.92	0.95	0.91	70	50	50
3.VIII.05	980	2060	2440	2680	720	1480	2100	2180	0.73	0.72	0.86	0.81	117	60	67
5.VIII.05	720	1360	2600	2460	580	1100	2120	2080	0.81	0.81	0.82	0.85	130	42	50
7.VIII.05	680	1210	2200	2300	580	970	1890	1910	0.85	0.80	0.86	0.83	117	27	52
9.VIII.05	650	1490	2330	2380	600	1160	1980	1960	0.92	0.78	0.85	0.82	97	75	72
11.VIII.05	680	1400	2370	2420	540	1080	1960	2010	0.79	0.77	0.83	0.83	80	35	35
15.VIII.05	550	1500	2210	2190	440	1230	1940	1920	0.80	0.82	0.88	0.88	52	62	112

Date	Influent TSS (mg/L)	MLSS (mg/L)			Influent VSS (mg/L)	MLVSS (mg/L)			VSS/TSS				Effluent TSS (mg/L)		
		R1	R2	R3		R1	R2	R3	IN	R1	R2	R3	R1	R2	R3
17.VIII.05	340	1590	2370	2210	280	1280	2030	1940	0.82	0.81	0.86	0.88	60	57	65
19.VIII.05	360	1460	1980	1860	210	1120	1660	1650	0.58	0.77	0.84	0.89	62	65	90
22.VIII.05	210	1210	1890	1740	180	990	1620	1530	0.86	0.82	0.86	0.88	60	42	52
24.VIII.05	390	1320	1820	1750	240	1020	1550	1500	0.62	0.77	0.85	0.86	52	78	82
26.VIII.05	390	1310	1610	1570	270	980	1310	1290	0.69	0.75	0.81	0.82	62	170	112
29.VIII.05	300	1210	1190	1230	220	950	970	1040	0.73	0.79	0.82	0.85	75	137	127
31.VIII.05	380	1130	1010	1130	260	930	820	910	0.68	0.82	0.81	0.81	127	122	132
2.IX.05	380	890	1110	930	220	690	790	730	0.58	0.78	0.71	0.78	145	102	110
1.XI.05	440	1290	1650	1310	250	1020	1370	1040	0.57	0.79	0.83	0.79	30	22	47
4.XI.05	240	1120	1220	1290	150	960	1090	1090	0.63	0.86	0.89	0.84	32	40	35
8.XI.05	190	1040	1290	1240	150	880	1180	1060	0.79	0.85	0.91	0.85	30	52	47
13.XI.05	260	1060	1280	1130	100	870	1100	910	0.38	0.82	0.86	0.81			
15.XI.05	200	960	1290	1050	100	790	1140	920	0.50	0.82	0.88	0.88	50	50	65
17.XI.05	210	940	1360	1020	70	740	1150	850	0.33	0.79	0.85	0.83	57	47	47
23.XI.05	480	960	1590	840	220	750	1340	630	0.46	0.78	0.84	0.75	40	47	50
25.XI.05	280	830	1600	840	150	650	1400	670	0.54	0.78	0.88	0.80	45	37	60
28.XI.05	220	1150	1610	1030	160	1040	1420	860	0.73	0.90	0.88	0.83	45	37	50
30.XI.05	410	1210	1710	1070	210	1070	1440	910	0.51	0.88	0.84	0.85	50	55	70
3.XII.05	350	1790	1410	1320	200	1500	1260	1130	0.57	0.84	0.89	0.86	52	62	57
5.XII.05	530	1910	2080	1670	310	1580	1730	1360	0.58	0.83	0.83	0.81	80	97	117
7.XII.05	430	1980	1910	1940	240	1740	1630	1620	0.56	0.88	0.85	0.84	67	75	87
10.XII.05	430	2270	2320	2160	220	1920	1940	1780	0.51	0.85	0.84	0.82	75	82	105
3.I.06	660	2780	1940	3210	360	2430	1610	2560	0.55	0.87	0.83	0.80	95	97	30
5.I.06	420	2700	1970	3040	220	2400	1640	2300	0.52	0.89	0.83	0.76	75	85	52
8.I.06	410	2810	2040	3280	170	2440	1740	2410	0.41	0.87	0.85	0.73	80	85	52
11.I.06	400	2620	2230	3400	220	2260	1870	2520	0.55	0.86	0.84	0.74	85	67	37
13.I.06	450	2760	2150	3630	240	2320	1800	2640	0.53	0.84	0.84	0.73	90	87	50
16.I.06	340	2840	2230	3340	180	2360	1830	2630	0.53	0.83	0.82	0.79	80	62	35
18.I.06		3120	2040	3340		2540	1670	2710		0.81	0.82	0.81	72	65	32
20.I.06		2860	2090	3350		2400	1700	2770		0.84	0.81	0.83	40	50	27
23.I.06	340	2860	2030	3320	200	2440	1750	2880	0.59	0.85	0.86	0.87	52	65	27
25.I.06	260	2970	2180	3440	170	2420	1760	2890	0.65	0.81	0.81	0.84			
27.I.06		2770	1880	3200		2300	1560	2710		0.83	0.83	0.85	37	37	10

Date	Influent TSS (mg/L)	MLSS (mg/L)			Influent VSS (mg/L)	MLVSS (mg/L)			VSS/TSS				Effluent TSS (mg/L)		
		R1	R2	R3		R1	R2	R3	IN	R1	R2	R3	R1	R2	R3
31.I.06		2310	1640	3180		1980	1380	2770		0.86	0.84	0.87	57	57	35
4.II.06		3090	1560	3010		1880	1300	2780		0.61	0.83	0.92	27	87	25
13.II.06		2870	1940	3220		2490	1580	2460		0.87	0.81	0.76	102	75	52
15.II.06	360	2120	2810	2820	170	1750	2410	2210	0.47	0.83	0.86	0.78	80	90	45
17.II.06		2410	2010	2780		2000	1620	2220		0.83	0.81	0.80	60	62	55
4.III.06		3370	2770	3530		2930	2350	3090		0.87	0.85	0.88	77	110	125
7.III.06	430	3710	2680	3350	170	3100	2180	2880	0.40	0.84	0.81	0.86	70	97	82
9.III.06	490	3600	2440	3530	280	3170	2070	3140	0.57	0.88	0.85	0.89	72	117	57
13.III.06		3480	2770	3220		3010	2240	2760		0.86	0.81	0.86	87	97	82
15.III.06	330	3520	2330	2870	210	3120	2000	2550	0.64	0.89	0.86	0.89	57	55	70
17.III.06		3620	2860	2930		3030	1800	2500		0.84	0.63	0.85			
23.III.06	220	4170	1470	3340	160	3540	1290	2940	0.73	0.85	0.88	0.88	85	127	155
17.IV.06	510	1310	1010	1430	180	1000	760	1100	0.35	0.76	0.75	0.77	70	82	105
20.IV.06	380	1110	950	1290	220	1030	850	1080	0.58	0.93	0.89	0.84	50	75	85
2.V.06	360	950	860	880	180	790	690	740	0.50	0.83	0.80	0.84	62	65	75
5.V.06	400	900	850	940	160	660	670	750	0.40	0.73	0.79	0.80	80	87	87
18.V.06	370	760	640	900	210	650	560	770	0.57	0.86	0.88	0.86	62	75	40
24.V.06	390	600	580	910	270	530	550	830	0.69	0.88	0.95	0.91	72	107	62
30.V.06	240	760	690	1010	180	640	600	860	0.75	0.84	0.87	0.85	30	45	57
5.VI.06		870	800	1170		790	690	1000		0.91	0.86	0.85	52	45	52
14.VI.06	336	830	820	1050	189	690	660	900	0.56	0.83	0.80	0.86	92	107	125
16.VI.06	280	710	840	1020	252	690	780	900	0.90	0.97	0.93	0.88	77	87	87
19.VI.06		760	960	1030									87	72	85
21.VI.06	256	930	980	950	140	730	780	850	0.55	0.78	0.80	0.89	80	75	72
27.VI.06	77	950	1510	1280	53	710	1230	1040	0.68	0.75	0.81	0.81	87	77	92
29.VI.06	81	670	1460	1160	74	615	1295	1065	0.91	0.92	0.89	0.92	30	10	30
4.VII.06	123	550	1680	1365	70	440	1425	1195	0.57	0.80	0.85	0.88	47	34	50
7.VII.06	189	540	1850	1495	88	480	1595	1345	0.46	0.89	0.86	0.90	37	35	22
11.VII.06	172	550	1755	1565	98	465	1560	1390	0.57	0.85	0.89	0.89	65	55	15
13.VII.06	181	650	1540	1410	97	525	1325	1210	0.53	0.81	0.86	0.86	112	105	102
17.VII.06		620	1105	1080		510	930	930		0.82	0.84	0.86	125	170	157
19.VII.06	83	545	925	875	74	510	815	805	0.89	0.94	0.88	0.92	85	115	110
21.VII.06	88	470	710	730	77	425	660	670	0.88	0.90	0.93	0.92	70	75	75

Date	Influent TSS (mg/L)	MLSS (mg/L)			Influent VSS (mg/L)	MLVSS (mg/L)			VSS/TSS				Effluent TSS (mg/L)		
		R1	R2	R3		R1	R2	R3	IN	R1	R2	R3	R1	R2	R3
24.VII.06	112	400	955	1005	67	315	830	880	0.59	0.79	0.87	0.88	55	57	82
26.VII.06	119	435	1310	1190	63	280	1150	1060	0.53	0.64	0.88	0.89	62	47	50
28.VII.06	74	325	1350	1150	39	260	1185	1040	0.52	0.80	0.88	0.90	40	12	72
31.VII.06	287	300	1345	1070	130	225	1200	945	0.45	0.75	0.89	0.88	8	17	30
2.VIII.06	221	345	1180	925	133	330	1120	840	0.60	0.96	0.95	0.91	22	35	65
4.VIII.06	263	540	1175	1005	144	400	990	860	0.55	0.74	0.84	0.86	90	80	120
7.VIII.06	196	560	1160	840	116	450	980	690	0.59	0.80	0.84	0.82	85	87	130
9.VIII.06	218	685	995	750	133	565	865	665	0.61	0.82	0.87	0.89	70	65	77
11.VIII.06	287	695	990	945	181	530	815	785	0.63	0.76	0.82	0.83	75	82	110
14.VIII.06	406	855	1120	1250	247	695	915	1050	0.61	0.81	0.82	0.84	110	112	85
16.VIII.06	364	995	1060	1750	238	825	870	1450	0.65	0.83	0.82	0.83	97	82	62
18.VIII.06	120	965	1110	800	70	775	895	685	0.58	0.80	0.81	0.86	97	107	87
29.XI.06	216	805	1105	1690	97	600	940	1430	0.45	0.75	0.85	0.85	52	40	30
4.XII.06	247	885	1255	2245	140	650	1080	1955	0.57	0.73	0.86	0.87	80	55	57
6.XII.06	312	915	1615	2170	154	690	1400	1885	0.49	0.75	0.87	0.87	65	52	45
8.XII.06	189	830	1695	2380	83	660	1460	2030	0.44	0.80	0.86	0.85	70	52	35
11.XII.06		745	1840	2305		600	1590	1925		0.81	0.86	0.84	80	50	33
13.XII.06	244	685	1790	2275	135	560	1535	1915	0.55	0.82	0.86	0.84	72	40	55
15.XII.06	215	670	1690	1960	105	535	1440	1670	0.49	0.80	0.85	0.85	70	35	57
9.I.07	228	855	3070	2800	95	620	2460	2370	0.42	0.73	0.80	0.85	65	112	82

Note: With Potato wastewater: 8.VI.05-19.VIII.05
With Canola oil wastewater: 22.VIII.05-26.X.05
With Glycol and Ethanol: 14.VI.06-29.VII.06
With Glycerol: 16.XI.06-4.XII.06

APPENDIX D SPECIFIC NITRIFICATION RATE TESTS WITH CANOLA PROCESSING AND OIL REFINING WASTEWATER

Table D1 Recorded ammonia concentrations during tests

Time(h)	NH_3-N (mg/L)		
	Cen+W	Cen+R.C.	Cen+T.C.
0	276.6	280.4	268.1
0.25	273.6	271.3	264
0.5	267	265.6	262.9
0.75	262.1	275.8	255.6
1	267	269	256
1.25	265.8	271.3	257.7
1.5	263.3	271.3	252.5
2	258.5	265.6	251.4
2.5	252.5	262.2	247.3
3	260.9	264.5	244.1
3.5	251.3	263.3	241
4	233.2	257.6	239
5	227.1	264.5	236.8
7	184.9	254.2	218
9	166.8	240.6	213.9

Note: R.C.=Raw canola oil refining wastewater
T.C.=Treated canola oil refining wastewater
W=Water
Cen=Centrate

Table D2 Recorded MLVSS in the testing beakers

	MLSS(mg/L)		
	Cen+W	Cen+R.C.	Cen+T.C.
Total	430	645	640
Volatile	335	480	475

Based on the above recorded data, Eq. (3-1) was applied. For easy reference, the equation was presented below.

$$SNR(mgNH_3 - N / gVSS \cdot h) = \frac{AUR(mgNH_3 - N / L \cdot h)}{MLVSS(mgVSS / L)} \times 1000 \qquad \text{Eq. (3-1)}$$

Table D3 Calculated SNRs

SNR (mgNH3-N/gVSS·h)		
Cen+W	Cen+R.C.	Cen+T.C.
35.8	6.9	12.6

APPENDIX E SPECIFIC DENITRIFICATION RATE TESTS WITH SIX DIFFERENT CARBON SOURCES

Table E1 Recorded nitrate concentrations during tests

Time	NO_3-N (mg/L)					
	Potato WW	Canola oil WW	Glycerol	Glycol	Methanol	Ethanol
0	279.1	166.1	284.7	173.7	138.9	139.6
0.25	292.7	178.8	260.9	185.5	133.0	149.0
0.5	286.9	191.9	268.1	178.0	178.4	144.5
0.75	254.7	202.9	262.6	177.0	184.7	140.6
1	239.6	193.6	272.8	172.3	165.8	133.2
1.25	234.3	207.1	252.4	164.0	164.1	129.8
1.5	218.2	224.0	255.8	167.6	176.3	121.0
2	217.0	176.2	233.4	153.5	172.9	118.0
2.5	212.7	182.6	245.7	151.1	150.7	114.8
3	208.0	191.4	225.3	146.0	162.0	99.3
3.5	200.0	198.6	235.5	145.4	140.6	98.3
4	199.0	160.2	221.9	131.8	129.7	95.9
5	196.0	189.8	184.5	104.6	140.6	86.1
6	185.0	169.9	170.0	100.0	142.3	76.2
7	182.0	176.2	145.0	80.0	132.6	62.0

Table E2 Recorded MLVSS in the testing beakers

	Carbon sources					
	Potato WW	Canola oil WW	Glycerol	Glycol	Methanol	Ethanol
MLVSS(mg/L)	3100	495	1380	1807	890	710

Based on the above recorded data, Eq. (3-2) was used to calculate the SDNRs. For easy reference, the equation was re-presented below.

$$SDNR(mgNO_3 - N / gVSS \cdot h) = \frac{DNR(mgNO_3 - N / L \cdot h)}{MLVSS(mgVSS / L)} \times 1000 \qquad \text{Eq. (3-2)}$$

Table E3 Calculated SDNRs

	Carbon sources					
	Potato WW	Canola oil WW	Glycerol	Glycol	Methanol	Ethanol
SDNR ($mgNO_3$-N/gVSS*h)	12	5	13	8	10	17

APPENDIX F CALCULATIONS OF THE APPLIED VOLUMES OF EACH CHEMICAL IN INFLUENT PREPARATION

The volumes of chemicals which should be applied were determined based on ammonia concentrations of raw centrate, preferred dosages and bsCOD strength of each milliliter of chemical.

For example, if ethanol is used as the external carbon source and centrate contains 600 mg/L of NH_3-N, how much of ethanol should be used to make an influent with the dosage of 2 mg bsCOD/mg NH_3-N.

Solution:

From Table 3-2, each mL of ethanol equals 1641 mg of bsCOD

Volume of ethanol = 600 mg NH_3-N /L × 2 L of influent × 2 mg bsCOD/mg NH_3-N ÷ 1641 mg bsCOD / mL of ethanol

= 1.46 mL

So the required volume of ethanol is 1.46 mL.

APPENDIX G PHOTOGRAPHS OF THE SYSTEM