HYDROGENOTROPHIC DENITRIFICATION OF SALINE AQUACULTURE WASTEWATER USING HOLLOW FIBER MEMBRANE BIOREACTOR

by

Diep Dinh Phong

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Environmental Engineering and Management

Examination Committee: Prof. C. Visvanathan (Chairperson)
Dr. Nguyen Thi Kim Oanh
Dr. Oleg Shipin
Dr. Jega V. Jegatheesan

Nationality: Vietnamese
Previous Degree: Bachelor of Science in Biology
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Abstract

High nutrient loading of wastewater discharged from aquaculture ponds into the environment causes eutrophication and affects aquatic life in receiving water bodies. This study used hollow fiber membrane bioreactor including denitrification reactor for hydrogenotrophic denitrification to remove nitrate followed by aerobic reactor designed for organic carbon removal. In the denitrification reactor, hydrogen was used as electron donor and CO_2 was supplied to control pH and scour the hydrogen diffusing membrane. Inlet concentrations of nitrate and organic matters in terms of DOC were 50 mg/L and 20 mg/L. The experiment included the acclimatization of hydrogenotrophic bacteria to salinity of 10 ppt and three experimental runs with salinity concentrations in wastewater of 10, 20, and 30 ppt. In each run, optimum hydraulic retention time (HRT) and operating parameters was determined.

Direct acclimatization method which acclimatized hydrogenotrophic denitrifiers directly to salinity of 10 ppt was found more efficient than the stepwise acclimatization with gradual increase of salinity. Optimum hydraulic retention times in run 1, 2 and 3 were determined at 3 h, 5 h and 6 h respectively. At these HRTs, the nitrogen removal efficiency reached to more than 90% and denitrification rate of total system was 366.8, 226.2 and 193.2 g/m^3.day respectively. In the denitrification tank, biomass yield was from 0.42 to 0.48 g cells/g NO_3^--N and hydrogen utilization efficiency was from 61 to 84%. After denitrification stage, dissolved organic carbon (DOC) decreased by 45 – 63%, indicating that there was the involvement of hetrotrophic denitrification in nitrate removal process. The hydrogen diffusing membrane was operated for one month without reduction of denitrification performance caused by membrane fouling. At optimum HRTs, water quality of treated wastewater in terms of nitrate, nitrite, DOC, SS was very good. The study demonstrated that this system can treat saline aquaculture wastewater with high efficiency and good quality of treated wastewater which can be recycled to closed aquaculture ponds in practice to avoid discharge pollutants into the environment.

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List of Abbreviations

BER	Biofilm Electrode Reactor
BOD	Biochemical Oxygen Demand
DA	Denitrification-Aeration sequence
DNR	Denitrification Rate
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
MBR	Membrane Bioreactor
HLR	Hydraulic Loading Rate
HRT	Hydraulic Retention Time
MBBR	Moving Bed Biofilm Reactor
MLSS	Mixed Liquor Suspended Solids
MLVSS	Mixed Liquor Volatile Suspended Solids
N	Nitrogen
NUR	Nitrogen Utilization Rate
ORP	Oxidation Reduction Potential
P	Phosphorous
SBR	Sequencing Batch Reactor
SRT	Sludge Retention Time
TAN	Total Ammonium Nitrogen
TMP	Transmembrane Pressure
TN	Total Nitrogen
TOC	Total Organic carbon
VSS	Volatile Suspended Solids

Chapter 1

Introduction

1.1 Background

In recent years, aquaculture has developed worldwide to meet the increasing market demand. This development generates profit and income, but it also causes adverse impacts on the environment. The discharge of intensive culture systems is high in nitrogen and phosphorus, originating from surplus food, feces and excretions. That can exceed the assimilating capacity of receiving waters, creating deterioration of water quality, eutrophication, and consequently affecting aquatic life. Long-term operation of closed aquarium and aquaculture systems results in nitrate accumulation which causes toxicity problems for invertebrates and affects the immune system of fish (Grguric et al., 2000). Treating this type of wastewater is a big concern not only for the protection of the environment but also for the reduction of water use.

Denitrification is a biological process to remove nitrate in wastewater by reducing nitrate into gaseous nitrogen under anoxic condition. The electron donors for the reduction can either organic matters in heterotrophic denitrification or inorganic in autotrophic denitrification. In heterotrophic denitrification a variety of organic carbon sources are available. For wastewater low in C/N ratio such as aquaculture effluent, the organic electron donor like methanol is popularly used. This process must be carefully controlled as overdosing of the organic electron donor can lead to severe water quality problems (Ergas and Reuss, 2001). In autotrophic denitrification, reduced sulphate compouds can be used as electron donor for autotrophic denitrification, but has some disadvantages, such as consumption of alkalinity and production of sulphate by-product residue (Koenig and Liu, 1996). Alternately, hydrogenotrophic denitrification is a biological process in which hydrogen oxidation bacteria remove nitrates, using hydrogen as electron donor and nitrates as electron acceptor.

Hydrogen gas is an excellent electron donor for autotrophic denitrification. Its advantages include: lower unit cost of electron donor, elimination of added carbon electron donor to the efflulent, and less cell yiel resulting in less sludge production (Ergas and Reuss, 2001) . The main limitation of using hydrogenotrophic denitrification is the low solubility of hydrogen gas, leading to low-mass transfer rate into wastewater and possible hydrogen-accumulation and explosive envrironment in a closed head space(Mansell and Schroeder, 2002). With the growing interest in the potential application of this process, a variety of denitrification systems have been developed to safely dissolve sufficient amount of hydrogen into water. One such system used electrochemical cell which electrolysed water and generated hydrogen on the cathode allowing the formation of a hydrogenotrophic denitrifying biofilm (Kiss et al., 2000; Sakakibara and Nakayama, 2001) or denitrification in a subsequent reactor (Grommen et al., 2006). Another alterative is membrane-gas diffusers, which allows bubble-less dissolution of hydrogen into the water. Membrane-gas diffusers have been used to deliver hydrogen either to a biofilm (Ergas and Reuss, 2001; Lee and Rittman, 2002), or to suspended bacteria (Rezania et al., 2005; Mo et al., 2005). An innovative system has been developed recently, it applied pressure from hydrogen cylinder to transfer hydrogen gas to water in a saturator tank, and the supersaturated feed was released to the reactor where dissolved hydrogen was consumed by bacteria (Rezania et al, 2007)

Most of these studies focused on denitrification of drinking water. More recent researches have focussed on hydrogenotrophic denitrification of fresh aquaculture wastewater by

applying electrochemically generated hydrogen gas as electron donor for biological denitrification (Grommen et al., 2006), or gas-transfer membrane in membrane bioreactor to dissolve hydrongen in wastewater (Hung, 2006). This process remains challenging in treating saline aquaculture wastewater and accordingly further studies are required to address nitrate removal for recirculating aquaculture systems.

This study used hollow fiber membrane to diffuse hydrogen in wastewater in a system incoporating an anoxic period for hydrogenitrophic nitrate consumption and followed by an aerobic period designed for organic carbon removal The objective was to investigate the potential of hydrogen-oxidizing bacteria in denitrifying aquaculture wastewater. Lab-scale experiment was conducted in this study to identify optimum parameters the denitrification of synthetic aquaculture wastewater .

1.2 Objectives of study

The study focused on hydrogenotrophic denitrification using hollow fiber membrane bioreactor (MBR) and its application in treating aquaculture wastewater with different salinities. Specific objectives were to:

- Study the potential of autotrophic, hydrogen oxidizing bacteria in hollow fiber membrane bioreactor in order to denitrify saline aquaculture wastewater with three different salinities; and
- Optimize operating parameters and requirement for performance of the hydrogenotrophic denitrification system.

1.3 Scope of study

In this research, a laboratory scale system of gas diffusing membrane bioreactor was used to treat synthetic aquaculture wastewater by hydrogenotrophic denitrifiers. To find optimum operating conditions for the system, the scope of this research was as follows:

- Synthetic aquaculture wastewater with three salinities of 10, 20, and 30 ppt was used for the denitrification;
- The study determined operating conditions including HRT, pH, nitrate loading rate, MLVSS, and DO; and
- Parameters indicating efficiency of the system were analyzed, which included NO_3^--N, NO_2^--N, DOC, nitrate reduction rate (mg NO_3^--N/mg VSS.d), and oxidation reduction potential (ORP).

Chapter 2

Literature Review

2.1 Aquaculture wastewater and treatment

2.1.1 Characteristics of aquaculture wastewater

In aquaculture systems, intensive culture has showed as a good practice for its higher production in a shorter period of time. The majority of the nutrients added to aquaculture ponds in the form of fertilizer or pellet feed is not incorporated into the fish, but deposited in pond sediments or discharged as effluent. Only small amount of the feed is assimilated by cultured fish. It was found by Thakur and Lin (2003) that in intensive culture, shrimp could assimilate only 23 – 31% nitrogen and 10 – 13% phosphorous of total input, the remaining nutrient went to sediment and drainage water (Fig. 2.1)

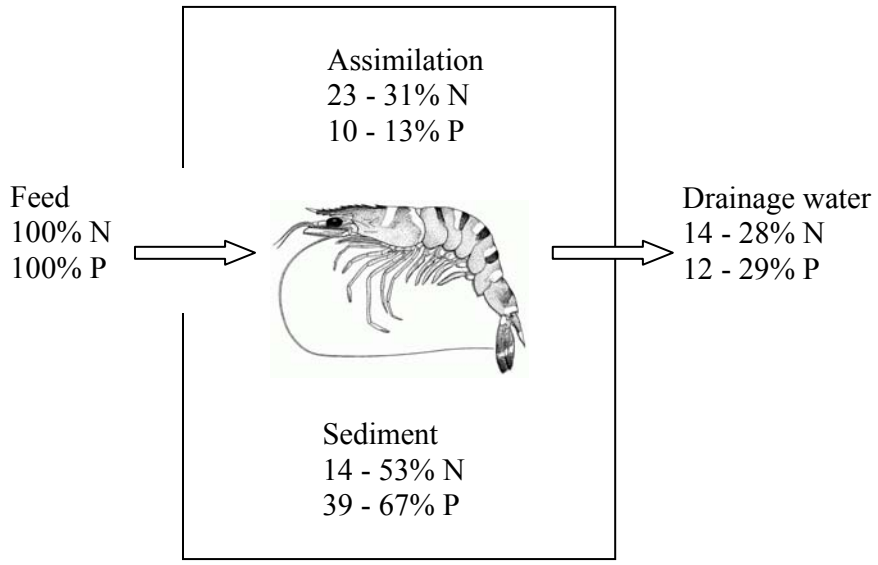

Figure 2.1 Nutrient budget in intensive shrimp culture

(Adapted from Thakur and Lin, 2003)

Studies on nutrient budgets have been carried out in many studies. Results are different depending on type of cultured fish, stock density and culture method but they all reported that the nutrient released in the environment was relatively high with 14 – 53% of total nitrogen input in sediment and 12 – 57% in drainage wastewater. These figures for phosphorous were 26 – 67% and 12 – 29% respectively (Sataporvanit, 1993**;** Yomjinda, 1993; Jackson et al., 2003; Thakur and Lin, 2003). More detailed about nitrogen fate in the aquaculture, Jackson et al. (2003) showed that in an intensive shrimp farm, nitrogen from artificial feed was the major part which reached to 90% of the total nitrogen input. Only 22% of the total nitrogen input was in the harvest and nitrogen released in the environment took considerable amount with 14% and 57 % of the input accumulated in sediment and discharged in wastewater respectively (Fig. 2.2)

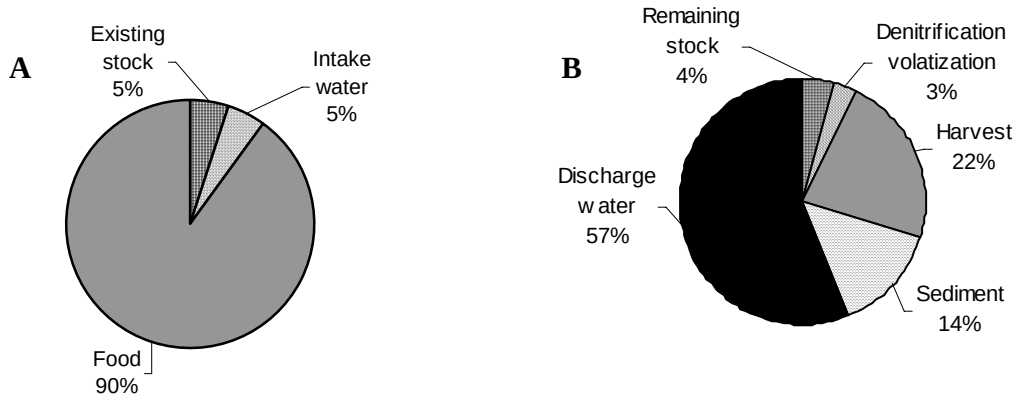

Figure 2.2 Nitrogen source (A) and fates (B) in shrimp pond (Jackson et al., 2003)

Pollutant load discharged into the environment has been calculated by many researchers. Suzuki et al. (2003) found that one ton of cultured fish released 0.8 kg of nitrogen and 0.1 kg of phosphorous per day. While Pillay (1992) reported that one kg of fish production discharged 577g of BOD, 90.4 g of nitrogen and 10.5 g of phosphorous. The result from study of Lin et al. (1993) showed that with shrimp stocking densities of 30-50/m^2, the average harvest of 5 tons to 6 tons/crop would require 10-12 tons of feed, assuming a food conversion ratio of 2. However, only about 20% of the feed was incorporated into shrimp biomass, so approximately 8-10 tons of feed ended up as uneaten food and excreted matter of shrimp. From studies on shrimp culture, nutrient budget in shrimp ponds is showed in Table 2.1.

Table 2.1 Nitrogen and phosphorous discharged from intensive shrimp farm

(adapted from Jackson et al., 2003)

Production (tons/ha/cycle)	Nitrogen discharged			Phosphorous discharged	References
	kg/ha/day	kg/ton	kg/ha/crop	kg/ha/crop	
2 –5	0.99	71.9	190	-	Jackson et al., 2003
4.3 – 4.6	1.4 – 1.5	38 – 44	175 - 194	-	Briggs and Funge-Smith, 1994
9	3.9	53.1	478	-	Phillips, 1994[a]
4.6	4.2	111	509	-	Robertson and Phillips, 1995[b]
-	-		455 - 668	238 - 321	Dierberg and Kiattisimkul, 1996[b]
-	-	-	478	154	Lin et al., 1993[b]

[a] assumes three cycles per year; [b]it is stated that pond sediment is also discharged in effluent, therefore nutrients reported as contained sediment are included.

Depending on stock density, pond effluent discharged from intensively operated farms usually contains variable concentration of nutrients, suspended solids, oxygen demanding substances. For open system, the concentration of nitrite, nitrate and organic matter is dependent of stock density (Table 2.2). Higher intensive culture leads to higher pollutant concentrations in the effluent. In a study of constructed wetland for treating effluent from an intensive shrimp culture in tank, Lin et al., 2005 found that with the stock density of 1000 – 2000 post larvae/m^2, NO_3^--N and PO_4^{3-}-P in the effluent could reach to 39.9 and 3.7 mg/L respectively.

Table 2.2 Characteristics of discharged water from intensive shrimp ponds

Parameters	Cowan et al., 1999	Páez-Osuna, 2001	Lin et al., 2005
Stocking, head/m^2	50 – 100	-	1000 – 2000
pH	7.2 - 7.7	-	7.8 - 8
NO_2^--N, mg/L	0.01 - 0.09	<0.1	0.1 - 0.26
NO_3^--N, mg/L	0.01 - 0.55	<0.1 - 0.44	5.88 - 39.9
TAN, mg/L	0.14 - 2.74	0.14 - 1.0	0.23 - 0.29
Total N, mg/L	3.3 - 5.58	-	-
PO_4^{3-}-P, mg/L	-	<0.1 - 0.12	1.06 - 3.7
Total P, mg/L	0.28 - 1.03	0.2 - 0.36	-
BOD_5, mg/L	6.5 - 10.5	0.4 - 9.9	3.1 - 7.1

Although aquaculture effluent has lower concentration of pollutants than domestic and industrial wastewaters, it is however discharged in a large amount that can exceed the assimilation capacity of receiving water bodies leading to environment problems. Suzuki et al. (2003) estimated that in Japan total fish production of inland aquaculture in 1999 was 63,000 tons. Hence, pollutant discharge from aquaculture corresponded to the waste generated by 5 million persons based on the assumption that human nitrogen load was equivalent to 11 g N per person per day. It was also reported that 40,000 ha of intensive shrimp ponds produced the waste equivalent of 3.1 – 3.6 million people for nitrogen and 4.6 – 7.3 million people for phosphorous, which was between 5 – 11% of the Thai population (Briggs and Funge-Smith, 1994 cited from Dierberg and Kiattisimkul, 1996).

To mitigate the environmental impacts of effluent discharge and to reduce the risk of disease contamination from externally polluted water supply, the intensive culture in recent years has been changed from open system with frequent water discharge to closed system with little or 'zero' water discharge. However, the major problem associated with closed system is the rapid eutrophication in ponds due to increasing concentrations of nutrients and organic matters over a period of time. That consequently damages fish and aquatic life in the system. Arbiv and van Rijn (1995) conducted a study on nitrogen removal in intensive aquaculture, in which one tank was treated by denitrification and the control tank without denitrification. The result showed an increase in concentration of nitrogen compound in the control tank over the time (Figure 2.3). Menasveta et al. (2001) found that in a recirculating system of black tiger shrimp without treatment, the nitrate concentration gradually increased and after 81 weeks it reached to more than 350 mg/L. These nitrogen concentration are much higher than safe level for many fish (table 2.3). In conclusion, wastewater in recirculating pond is characterized with high concentration of nitrate, especially in ponds without treatment or inefficient treatment.

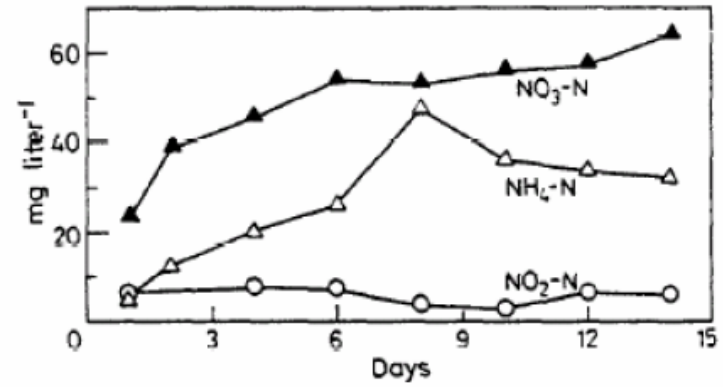

Figure 2.3 Increase in nitrogen concentrations in a fish culture unit without treatment (Arbiv and van Rijn, 1995)

Table 2.3 Recommended water quality for cultured fish

Species	NH_3-N, mg/L	NO_2^--N, mg/L	NO_3^-- N, mg/L	Reference
Shrimp	0.12	< 0.6	< 50	Lucas and Southgate, 2003
Channel casfish	0.1	< 9	< 130	Lucas and Southgate, 2003
SeaBass	<2	<2	<100	Blancheton, 2000
Surf clam	<0.0014	<0.14	<50	Lucas and Southgate, 2003

another parameter is salinity in the effluent of aquaculture pond, which is an important factor to decide the growth of fish. The salinity varies depending on cultured species, places, and period. For example, Marhaba et al. (2006) reported that shrimp farm effluents along the Bangpakong River, Thailand had salinity between 0.1 and 14.5 ppt. Cowan et al. (1999) conducted a study on two shrimp ponds in Thailand and found that salinity in the wet (monsoon) season was between 21 and 24 ppt. Meanwhile in dry season, the salinity was higher, between 30 and 31 ppt.

2.1.2 Effects of aquaculture wastewaters

Effluent from aquaculture ponds is high in organic matters, nitrogen and phosphorous which causes depletion of oxygen, eutrophication and algae blooming in water bodies. That severely reduces water quality and induces ecological stress on aquatic organisms. This is more severe when flash loading of wastewater especially during harvest when the entire contents of ponds are discharged (Pillay, 1991; Senrath and Visvanathan, 2001). For example, nutrient, BOD, and total suspended solids exported during harvest ranged from 23 to 71% of the loadings measured during several 4-months growth periods in a study on culture of black tiger shrimp (Dierberg and Kiattisimkul, 1996). The discharge can cause environmental hazards including mortality of fish (Pillay, 1991; Thakur and Liu, 2003).

The pollution is more problematic in inland aquaculture where small streams and irrigation canals have low assimilative capacity. Hence discharge of high-salt shrimp pond effluent into fresh water bodies during water exchange or at harvest induces adverse effects on surrounding water bodies, which are fresh surface waters. Similarly, drinking water can also be scare and contaminated by the wastewater intrusion causing threat to community health (Senrath and Visvanathan, 2001)

Coastal areas that have poor flushing characteristics, such as embayment, become eutrophic from farm discharges, that alters habitats (coral reef, sea grass) and community structure (e.g., eradication of demersal fisheries). Furthermore, ‘red tide’ outbreak, a common occurrence in many South East Asian countries, may be partially caused by shrimp pond effluent (Dierberg and Kiattisimkul, 1996).

Disease outbreaks can occur in intensive culture areas by spreading rapidly through aquaculture effluent from pond to pond. This has resulted in significant economic loss to farmers and it is the major constraint to sustainability of aquaculture industry. Water-born diseases from the effluent is also a concerned problems to people.

2.2 Denitrification of aquaculture wastewaters and saline wastewaters

Treatment of aquaculture wastewater is always a big concern of aquaculturists and researchers. To date, several methods have been developed from simple to complex systems to mitigate the environmental impacts of aquaculture discharge and to reduce the risk of disease contamination on fish cultured in ponds. Since the main problem of aquaculture ponds is algal growth due to high concentration of nitrogen, phosphorous, and organic compounds, in any given case only one is the critical or limiting factor (Jegatheesan, 2002). Accordingly, most of studies focused on nitrogen removal in recirculating systems rather than both nitrogen and phosphorous removals. Apart from the direct toxic effect on fish, nitrate removal is conducted for other reasons in recirculating systems: (1) environmental regulations associated with effluent discharge have permissible nitrate levels as low as 11.3 mg NO_3-N/l (European Council Directive, 1998); (2) prevention of high nitrite levels resulting from incomplete ‘‘passive’’ nitrate reduction; (3) stabilization of the buffering capacity; and (4) the concomitant elimination of organic carbon, orthophosphate and sulfide from the culture water during biological nitrate removal (van Rijn, 2006). Nitrate removal can be achieved using both physico- chemical and biological treatment processes. The conventional treatment processes used to remove nitrate are reverse osmosis, ion exchange, and activated carbon adsorption in conjunction with pH adjustment. Studies evaluating different denitrification strategies, such as the use of granular activated carbon (GAC), packed beds, and rotating biological contactors systems have been undertaken by various researchers.

2.2.1 Theory of denitrification

Denitrification is the dissimilatory reduction of nitrate or nitrite to nitrogen gas by microorganisms for protein synthesis under anoxic condition. In denitrification process nitrate is reduced sequentially to nitrogen gas through the following enzymatic reactions

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad \text{Eq. 2.1}$$

The overall reaction can be expressed as follows

$$NO_3^- + 6H^+ + 5e^- \rightarrow 0.5\, N_{2\,(g)} + 3H_2O \qquad \text{Eq. 2.2}$$

Denitrification is the second step in nitrification – denitrification process to remove nitrogen in water. It can be accomplished by autotrophic or heterotrophic bacteria. Those that utilize organic electron donor are heterotrophic denitrifiers. Meanwhile autotrophic denitrifiers use inorganic electron donors such as H_2 and reduced sulfur. Because of their great metabolic diversity, denitrifiers are commonly found in soils, sediments, surface waters, ground waters, and wastewater treatment plants (Rittmann and McCarty, 2001).

Heterotrophic denitrification

Under anoxic conditions, heterotrophic denitrifiers conduct denitrification using organic electron donors including:

- COD in the influent wastewater or COD added from other waste streams
- COD produced during endogenous decay
- External carbon sources such as acetate, methanol and ethanol.

Reaction stoichiometry for different electron donors is showed the following reactions. The term of $C_{10}H_9O_3N$ represents for the biodegradable organic maters in wastewater (Metcalf and Eddy, 2003).

COD in wastewater:

$$C_{10}H_{19}O_3N + 10NO_3^- \rightarrow 5\,N_{2\,(g)} + 3H_2O + NH_3 + 10CO_2 + 10OH^- \quad \text{Eq. 2.3}$$

Methanol:

$$6NO_3^- + 5CH_3OH \rightarrow 5CO_2 + 3N_2 + 7H_2O + 6OH^- \quad \text{Eq. 2.4}$$

Acetate:

$$8NO_3^- + 5CH_3COOH \rightarrow 10CO_2 + 4N_2 + 6H_2O + 8OH^- \quad \text{Eq. 2.5}$$

Almost any organic compound can be used as an exogenous electron donor. Methanol is often chosen for its economic benefits. In aquaculture wastewater treatment heterotrophic denitrification were applied by many studies (Arbiv and van Rijn, 1995; Sauthier et al., 1998; Menasveta et al., 2001; Suzuki et al., 2003). Advantages of these systems include low cost and high denitrification rates. Problems with these systems include carryover of added organic carbon and microbial biomass to the product water, especially for treatment of drinking water (Ergas and Reuss, 2001). Besides, concentrated organic wastes can be used as an inexpensive electron donor such as food processing, beverage industries which are very high in C/N ratio. However it releases little reduced nitrogen (Rittmann and McCarty, 2001). For most readily available organic carbon sources, a COD/NO3_-N (w/w) ratio from 3.0 to 6.0 enables complete nitrate reduction to elemental nitrogen (Narcis et al., 1979; Skinde and Bhagat, 1982). Carbon limitation will result in the accumulation of intermediate products, such as NO_2 and N_2O (van Rijn et al., 2006)

Biodegradable polymer is a solution to overcome the above disadvantages. In an experiment for denitrification in recirculated aquaculture system, biodegradable polymer pellets acted as a carbon source and biofilm carrier for denitrification. The system therefore did not require an exogenous carbon sources (Boley et al., 2000). This stoichiometric denitrification equation including biomass formation is given in Eq. 2.6

$$0.494C_4H_6O_2 + NO_3^- \rightarrow 0.13CO_2 + HCO_3^- + 0.415N_2 + 0.169C_5H_7NO_2 + 0.39H_2O \quad \text{Eq. 2.6}$$

Autotrophic denitrification

Autotrophic denitrifiers consume inorganic carbon compounds (e.g. CO_2, HCO_3^-) as their carbon sources instead of organic carbons. The denitrification uses inorganic electron donors including hydrogen, elemental sulfur or reduced sulfur (S^{2-}, $S_2O_3^{2-}$, SO_3^{3-}). Some advantages of autotrophic denitrification over heterotrophic denitrification include: (1) low biomass buildup (biofouling) and reduction of reactor clogging and (2) avoidance of organic carbon contamination of treated water (van Rijn et al., 2006). The most common source of reduced sulfur is elemental sulfur, which is oxidized to SO_4^{2-}. The sulfur normally is in a solid compound including a solid base such as $CaCO_3$, because the oxidation of $S_{(s)}$ generates strong acid. This reaction is expressed in Eq. 2.7 (Rittmann and McCarty, 2001)

$$S_{(s)} = \frac{6}{5}\,NO_3^- + \frac{2}{5}\,H_2O \rightarrow SO_4^{2-} + \frac{3}{5}\,N_2 + \frac{4}{5}\,H^+ \quad \text{Eq. 2.7}$$

This process was conducted by Koenig and Liu (1996) in a study on denitrification of landfill leachate. In this study autotrophic bacteria *Thiobaccillus denitrificans* oxidized elemental sulfur to sulphate while reducing nitrate to elemental nitrogen gas, thereby eliminating addition of organic carbon compounds.

Hydrogenotrophic denitrification is another autotrophic denitrification, which uses H_2 gas as electron donor. Its overall reaction is as follows:

$$2\,NO_3^- + 2H^+ + 5H_2 \rightarrow N_2 + 6H_2O \qquad \text{Eq. 2.8}$$

Its mechanism and advantages will be discussed in section 2.3.

2.2.2 Recent studies on denitrification of aquaculture wastewaters

Denitrification of fresh aquaculture wastewaters

Experimental systems with or without addition of external carbon sources were operated by a number of investigators with different freshwater fish (Knösche, 1994; Arbiv and van Rijn, 1995; and Shnel et al., 2002). In these studies, carbon compounds, released from the breakdown of endogenous carbon, were used for denitrification in an anoxic treatment step consisting of a digestion basin and a fluidized bed reactor. To treat wastewater from eel culture in recirculating system Knösche (1994) tested two systems including trickling filter and activated-sludge-biodisc-filter using endogenous carbon source. The result showed that the latter system was more effective with nitrification rate of 1.4 g N/m^2.d and 14 g N/kg MLSS. Arbiv and van Rijn (1995) by combining an aerobic, nitrifying trickling filter and an anoxic, denitrifying fluidized bed reactor treated wastewater from common carp in closed system. Carbon source for denitrification was the endogenous organic carbon. The maximum removal rate of ammonia by trickling filter was 430 mg/m^2.d and denitrification rate of 35.8 mg NO_3^--N/L.h was achieved by fluidized bed reactor. With methanol as an external carbon source. Suzuki et al. (2003) investigated the performance of a closed recirculating system with foam separation, nitrification and denitrification units for intensive culture of eel. About 90% of the total nitrogen in the system was removed by denitrification.

Biodegradable polymers as biofilm carrier for electron donor in denitrification in recirculating aquaculture system were investigated (Boley et al., 2000). In this system, removing of carbon substance and nitrification were accomplished via biofilter and nitrate was removed in denitrification reactor using biodegradable polymers as electron donor. Treated water was recirculated back to the aquarium. The denitrification rate varied depending on the type of polymer used, and the highest rate was 166 mg NO_3^--N/L.h. This method was more expensive than other methods using liquid substrate for biological nitrate removal however some positive points are: reduction of clean water requirement, reduction of wastewater production and reduction of energy consumption.

These studies concluded that with proper treatment, closed recirculating system without emission could be implemented for the intensive aquaculture of freshwater fish such as eel.

More recently, a new method in treatment of aquaculture wastewater has been applied and gained attention. Rather than organic carbons, hydrogen is used as electron donor for denitrification of aquaria effluent. Hydrogenotrophic denitrification of fresh aquaculture wastewater was mediated by applying electrochemically generated hydrogen gas as electron donor for biological denitrification (Grommen et al., 2006), or gas-transfer membrane in membrane bioreactor to dissolve hydrogen in wastewater (Hung, 2006). The comparison among denitrification systems is extremely difficult due to differences in operational parameters This method, hydrogen-dependent denitrification, however has its own advantages that will be discussed more detail in section 2.3. Various methods and their efficiency of removing nitrogen in aquaculture are presented in Table 2.4.

Denitrification of saline aquaculture wastewaters

Saline aquaculture wastewaters are generated from aquaculture located in coastal areas or inland shrimp ponds. Salt present in wastewater at high concentration can cause inhibition on biological process creating difficulties in treating this type of wastewater. Many studies have been carried out to find solution for this problems and popular systems used are moving bed and packed bed reactors.

A submerged moving bed biofilm reactor (MBBR) was applied for the denitrification of closed circuit mesocosm (Labelle et al., 2005). In this study methanol was used as a carbon source at various C/N ratios. The finding showed that optimum C/N ratio was 4.2 – 4.3 and at this range NO_3^--N reduction was from 53 to as low as 1.77mg/L and a maximum denitrification rate of 737.5 mg NO_3^--N/m^2.h was achieved. Fig. 2.4 is diagram of the system.

Similarly, Dupla et al. (2006) also used MBBR to treat saline wastewater and accomplished high denitrification rates up to 1125 mg NO_3^--N/m^2.h. The improvement in liquid circulation and the maintenance of a thin biofilm were the reasons to explain for this high rate. It showed that increasing the overall liquid velocity profile led to an increase of up to 30% in the denitrification rate in conditions with a 1-month-old biofilm. Both two studies concluded that MBBR design could easily be scaled up to denitrify saline wastewater.

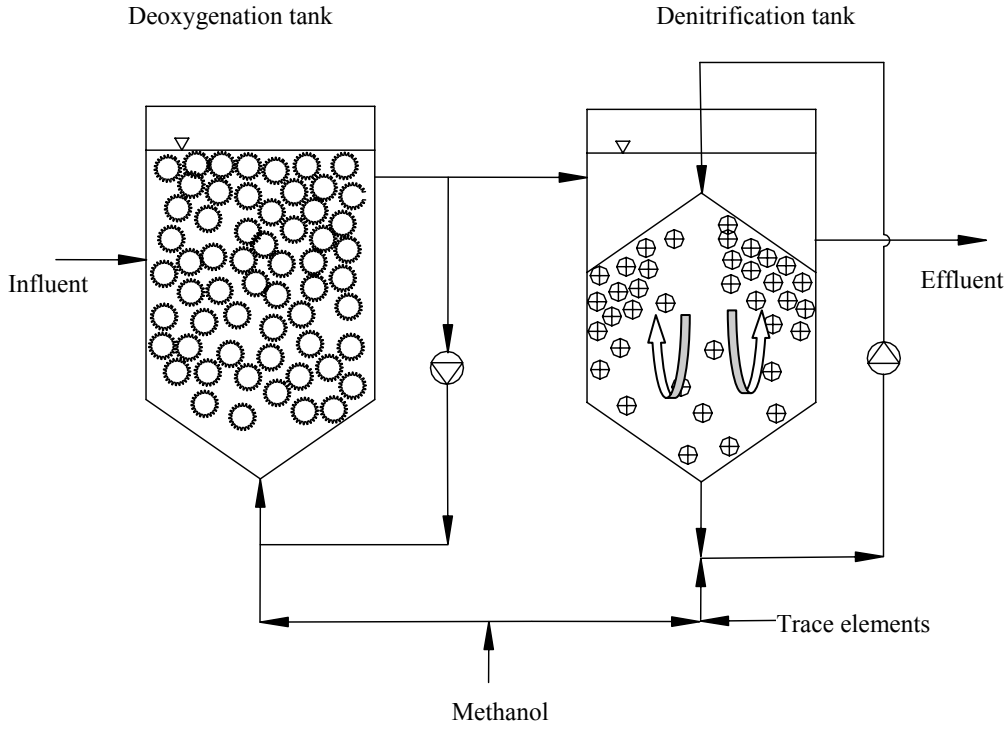

Figure 2.4 Moving bed biofilm reactor (Labelle et al., 2005)

Packed bed was used for denitrification of aquaculture recirculating systems in several studies (Sauthier et al., 1998; Grguric et al., 2000a,b; Menasveta et al., 2001). Different aspects of denitrification were identified. Sauthier et al. (1998) using brick granule as media and ethanol as electron donor for denitrification of marine closed system, found that the optimum TOC/NO_3^--N was 1g/g. Denitrification rate in this study was high up to 100 mg N/L.h and NO_3^--N concentration was low less than 1 mg/L in the effluent. For

recirculating seawater system of black tiger shrimp, Menasveta et al. (2001) used submerged filter with media of plastic balls/crushed oyster shells for denitrification. The result showed that changing carbon source from ethanol to methanol and increasing HRT resulted in significant nitrogen reduction. The denitrification rate reached to 16.6 mg NO_3^--N/L.d.

All above studies applied heterotrophic denitrification and used exogenous organic carbon sources (methanol, ethanol, and acetate). The feasibility of denitrification in a marine recirculating system for culture of gilthead seabream with endogenous carbon as the sole carbon source was demonstrated in a closed system comprising an anoxic digestion basin and fluidized bed reactor (Gelfand et al., 2003). It is interesting to note that nitrate removal in this system was mediated by both heterotrophic and autotrophic denitrification. Chemical analyses of the sulfur transformations and microbiological analyses of the bacterial populations in this treatment system revealed that sulfide, produced by sulfate reduction in the anaerobic parts of the digestion basin, was reoxidized by autotrophic denitrifiers (Cytryn et al., 2003 cited by van Rijn et al., 2006).

Although at high salinity the performance of denitrification was reduced, these systems showed that they could be practically applied for treating saline wastewater.

Table 2.4 Nitrogen removal by various process and the electron donors used (adapted from van Rijn et al., 2006)

Denitrifying reactor	Medium	Electron donor	Nitrate removal rate, mg NO_3^--N/L.h	Reference
Freshwater systems				
Fluidized bed	Sand	Endogenous	35.8	Arbiv and van Rijn, 1995
Packed bed	Biodegradable polymers	PHB $(C_4H_6O_2)_n$	7–41	Boley et al., 2000
Packed bed	Biodegradable polymers	PCL $(C_6H_{10}O_2)n$	21–166	Boley et al., 2000
Packed bed	Biodegradable polymers	Bionolle $(C_6H_8O_4)n$	1.5–77	Boley et al., 2000
Digestion basin	Sludge	Endogenous	5.9	Shnel et al., 2002
Fluidized bed	Sand	Endogenous	55.4	Shnel et al., 2002
Packed bed	Freeze-dried alginate beads	Starch	26.0	Tal et al., 2003
Digestion basin	Sludge	Endogenous	1.5	Gelfand et al., 2003
Fluidized bed	Sand	Endogenous	43.3	Gelfand et al., 2003
Packed bed	Polyethylene	Methanol	1.8	Suzuki et al., 2003
Moving bed	polyethylene	Methanol	-	Labelle et al., 2005
Moving bed	plastic	Methanol	-	Dupla et al., 2006
Submersed filter	-	H_2 (gas)	6.64	Grommen et al., 2006
MBR	-	H_2 (gas)	15.2	Hung, 2006
Marine systems				
Packed bed	Brick granules	Ethanol	100	Sauthier et al., 1998
Packed bed	Porous medium	Methanol	7.3–8.4	Grguric et al., 2000a,b
Packed bed	Polyvinyl	Alcohol/ Glucose	1.4	Park et al., 2001
Packed bed	Plastic balls/ oyster shells	Ethanol/ methanol	l 6.6	Menasveta et al., 2001
Packed bed	Freeze-dried alginate beads	Starch	2.6	Tal et al., 2003
Digestion basin	Sludge	Endogenous	2.5	Gelfand et al., 2003
Fluidized bed	Sand	Endogenous	72.6	Gelfand et al., 2003

2.3 Hydrogenotrophic denitrification

2.3.1 Theory of hydrogenotrophic denitrification

Hydrogenotrophic denitrification is a biological process in which hydrogen oxidizing bacteria remove nitrates and nitrites by autotrophic denitrification, using hydrogen as an energy source and inorganic carbon (CO_2, HCO_3^-) as carbon source (Kurt et al., 1987; Dries et al., 1988; Gros et al., 1988). In this reaction, nitrate or nitrite is the electron donor. Mansella and Schroederb (2002) reported that seven hydrogen oxidizing bacteria involved in this denitrification, including *Azospirillum brasilence, Rhizobium japonicum, Paracoccus denitrificans, Hydrogenophaga flava, Hydrogenophaga, pseudopflava, Hydrogenophaga taeniospiralis, and Alcaligenes eutrophus*.

The major pathway of denitrification is: $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$. It is simplified in following reactions:

Nitrate reduction

$$2NO_3^- + 2H_2 + \rightarrow H_2O + 2NO_2^- \qquad \text{Eq. 2.9}$$

Nitrite reduction

$$2NO_2^- + 3H_2 + 2H^+ \rightarrow 4H_2O + N_2 \qquad \text{Eq. 2.10}$$

The total reaction is

$$2\ NO_3^- + 2H^+ + 5H_2 \rightarrow N_2 + 6H_2O \qquad \text{Eq. 2.11}$$

Stoichiometric reaction among e^- donor, e^- acceptor, and biomass is

$$H_2 + 0.35\ NO_3^- + 0.35\ H^+ + 0.052CO_2 \rightarrow 0.17N_2 + 1.1\ H_2O + 0.010\ C_5H_7NO_2 \qquad \text{Eq. 2.12}$$

From Eq. 2.12, the cell yield is 0.24 g cells/g NO_3^--N theoretically, which is considerably lower than the 0.6 to 0.9 g cells/g NO_3^--N typically reported for heterotrophic denitrification (Ergas and Reuss, 2001). According to the Eq. 2.11, 1g of NO_3^--N converted to N_2 consumes 0.357 g of hydrogen gas and theoretically produces 3.57 g alkalinity (Ho et al., 2001). The pH will increase after the reaction, because 1 mole of H^+ is used when 1 mole of NO_2^--N is converted to nitrogen gas.

Advantages of hydrogenotrophic denitrification over heterotrophic denitrification include (Lee and Rittmann, 2000; Ergas and Reuss, 2001; Mo et al., 2005):

- Lower cell yield;
- Elimination of carryover of added organic electron donor to the product water;
- The relatively low solubility of H_2, which make it easy to remove from the product water by air stripping; and
- Low cost of H_2.

Disadvantages of hydrogenotrophic denitrification include:

- Hydrogen gas is explosive and flammable; and
- Hydrogen gas has low solubility so it is difficult to dissolve in water.

2.3.2 Hydrogenotrophic denitrification in water and wastewater treatment

To eliminate the explosibility and low dissolution of hydrogen in water, researchers have developed hydrogen delivery units, which safely diffuses sufficient hydrogen into water for the denitrification. Mixed culture or monoculture of bacteria has been applied for this denitrification and species of *Alcaligenes eutrophus* is often used in monoculture. A study on autotrophic membrane attached biofilm reactor to remove nitrate from drinking water was conducted by Ho et al. (2001). In this study hydrogen gas was diffused through a

silicon tube to feed *Alcaligenes eutrophus,* a hydrogenotrophic denitrifier, on the surface of the membrane. Instead of buffer solution, CO_2 was used and it proved the role of buffering alkalinity formation produced in the denitrification. Compared to sodium carbonate, carbon dioxide was better since pH in this case did not increase and no nitrite was found accumulated. However, in this study concentrations of carbon dioxide and hydrogen flowed together in silicone tube were inversely proportional inducing the difficulty in adjusting both concentrations independently. The specific nitrogen removal rate in this study ranged from 1.6 to 5.4 g N/m^2.d. This bioreactor was simple to operate and highly applicable in drinking water treatment.

Another device to diffuse hydrogen is diffusion plate which was used in hydrogenotrophic denitrification with immobilized *Alcaligenes eutrophus* for drinking water treatment (Chang et al., 1999). *Alcaligenes eutrophus* was immobilized in polyacrylamide and alginate copolymer to evaluate denitrification in continuous fluidized-bed reactor (Figure 2.5). The total nitrogen removal rate in this study was rather high and it increased with operation time and reached a maximum rate of 600 – 700 gN/m^3.d after 6 days of operation. The dissolved hydrogen concentration had a significant effect on denitrification. It was also found that nitrite reductase was inhibited when the dissolved hydrogen concentration fell below 0.2 mg/1, while nitrate reductase was inhibited at a concentration below 0.1 mg/l. This study also identified that phosphate had a significant effect on the accumulation of nitrite, but its effect on nitrate removal was small at a concentration greater than 0.5 mg PO_4^{3-}-P/L with the influent containing 22 – 25 mg NO_3^--N/L.

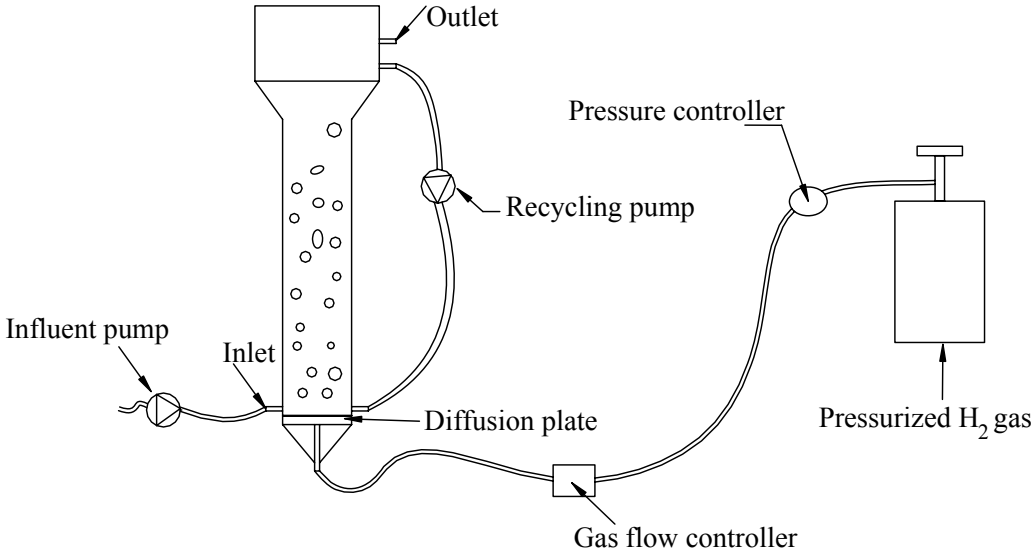

Figure 2.5 Hydrogenotrophic denitrification in fluidized bed with plate diffuser (Chang et al., 1999)

Although gas permeable silicon tubes and plate diffuser have been trialed, they do not efficiently transfer hydrogen for the denitrification. Recently, researchers have found other method for this challenge. One configuration, which addresses effective hydrogen delivery, is called a biofilm electrode reactor (Sakakibara and Kuroda, 1993; Flora et al., 1994; Islam and Suidan, 1998; Prosnansky et al., 2002; Kiss et al., 2000; Sakakibara and Nakayama, 2001). A biofilm electrode reactor is an electrochemical cell, in which water is electrolysed and hydrogen is generated. The hydrogen produced on the surface of the cathode allows for the formation of a hydrogenotrophic denitrifying biofilm. One of earlier studies on this process was conducted by Sakakibara and Kuroda (1993). An electric method was used for inducing and controlling the rates of denitrification. The rate of denitrification was found to be linearly related to the applied electric current with one mole

of electron reducing 0.2 mole of nitrate to nitrogen gas. The denitrification experiments were performed in a batch reactor with temperature between 25 and 30°C, and the pH controlled between 7.0 and 8.6. The denitrification rate in their system was reported to be 0.038 mg NO_3^--N/cm^2 of biofilm surface area/day. More recently, another system comprised of two steps: the water to be treated was first enriched with hydrogen (energy source) in the cathodic chamber of an electrochemical cell, and then denitrified in the bioreactor. The bioreactor was a packed bed of granulated activated carbon, and the water flow was directed in an upward continuous mode. The system was operated for one year, at various water velocities and current intensities. Denitrification rates up to 250 g/L.d was obtained at the hydraulic residence time of 1 h (Szekeres et al., 2001). This method effficiently generates hydrogen directly in water, however the main drawback of biofilm electrode reactors is gradual scale formation on the surface of the cathode, suppressing hydrogen production, which causes a dramatic decrease in denitrification rate (Kiss et al., 2000).

Hollow fibre gas transfer eradicates problems with low solubility as it provides gas fed directly to the biofilm or to the wastewater. Some research claiming that up to 100% gas transfer efficiency is possible (Mo et al, 2005). Hollow fiber membrane bioreactor (MBR) is the system that uses this type of membrane to diffuse efficiently hydrogen into the liquid hence making the denitrification rate higher than that of other devices. The MBR for hydrogen-dependent denitrification have been applied in studies recently (Lee and Rittmann, 2000; Ergas and Reuss, 2001; Ho et al., 2001; Hauge net al, 2002; Mo et al, 2005; Rezania et al., 2005; Hung, 2006). In a study of hollow fiber MBR to treat drinking water, Ergas and Reuss (2001) found that the denitrification rate reached to a maximum value of 770 g NO_3^--N/m^3.d at an influent concentration of 145 mg NO_3^--N/L. This denitrification rate was obviously high. It was explained that advantages of the hollow fiber MBR over systems that employed traditional gas sparging include higher gas transfer rates and bubbleless gas through membrane, which prevented the waste of excess H_2 and the accumulation of explosive gases in a confined space. Disadvantage of memrane is fouling. The biofilm grown on the surface of the membrane is usually thick and shearing of this biofilm requires high energy due to precipitation of inorganics inside the biofilm (Ergas and Reuss, 2001; Lee and Rittmann, 2003). When a membrane-gas diffuser is used to introduce hydrogen to a suspended culture, membrane fouling resulting from inorganic precipitation, water condensation inside the fiber, and biofilm formation may require frequent cleaning and replacement of membrane diffusers.

The concept of introducing gas-supersaturated feed water to a gas-consuming reactor can be used for efficient gas delivery. Rezania et al (2007) fed hydrogen gas into saturator tank, and high pressure of more than 8 bars in the tank made it possible for the gas to dissolve in wastewater. The hydrogen delivery system was efficient as almost 100% hydrogen transfer rate was observed. The transfer of hydrogen to the MBR effectively stimulated the growth of hydrogendependent denitrifiers, and complete nitrogen removal was achieved at a loading of 0.11 kgN/m^3.d and influent of 25mgNO_3^--N/L. The nitrogen gas produced during denitrification was recycled to achieve sufficient membrane scouring and the reactor mixing. The total organic carbon was similar to that of the incoming feed water, averaging approximately 6mg/L. (Rezania et al., 2007)

Most of studies on hydrogenotrophic denitrification focused on drinking water treatment. It is a good solution because drinking water always has very low concentration of organic carbon for heterotrophic denitrification and the adding of exogenous carbon source should be avoided for the safety to human. Whereas, hydrogenotrophic denitrification of wastewater has been neglected for a long time and there are a few studies on it. The most

recent study on hydrogenotrophic denitrification of wastewater was conducted by Grommen et al. (2006). In this study nitrate removal was carried out in aquaria by mean of electrochemically generated hydrogen gas as electron donor for biological denitrification. Electrochemical cell was used to generate hydrogen gas. During a 7 days aquarium test, a nitrate removal rate reached up to 18.5mg N/L.d at an influent NO_3^--N concentration of 20mg/L. Diagram of this system is presented in Fig. 2.6. The result of this experiment was not good as heterotrophic denitrification as well as other researches on hydrogenotrophic denitrification (Table 2.6). However, it is a pioneer study on this field and further studies are necessary to prove advantages of hydrogenotrophic denitrification in water and wastewater treatments as well.

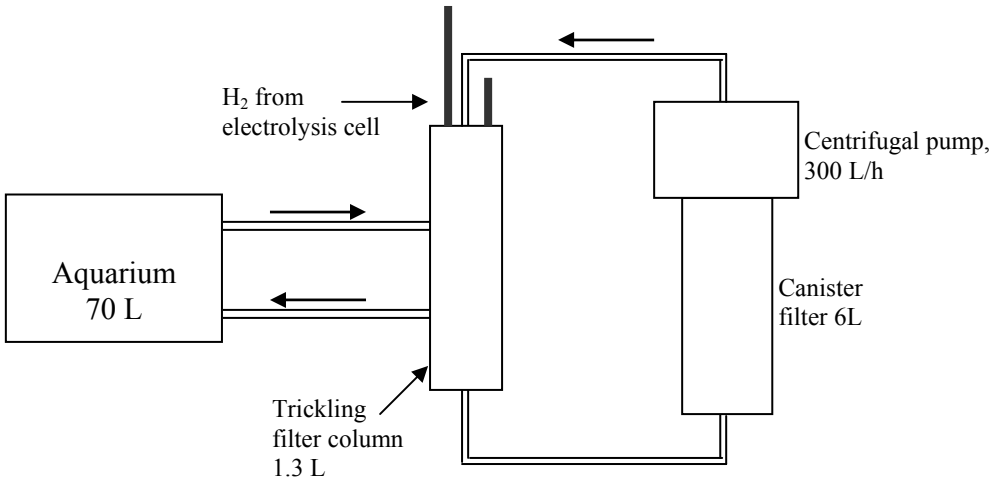

Figure 2.6 Hydrogenotrophic denitrification reactor to treat aquarium wastewater (Grommen et al., 2006)

Hydorogenotrophic denitrification using membrane bioreactor to treat aquaculture wastewater was carried out by Hung (2006). In the study, CO_2 was used to control pH. Average efficiency of nitrogen removal and denitrification rate of denitrification reactor were 88.3%; and 343 g/m^3.day at HRT of 3 hours respectively. For inlet nitrate nitrogen 50 mg/L, outlet was less than 10 mg/L. The study showed that using CO_2 to control pH was better than using mixture of buffer in term of P pollution in environment as well as efficiency of removal. In denitrification process, organic matter was not only removed but also added, this was due to soluble microbial products (SMP), amount of SMP added to the effluent is 10-15 mg/L expressed as COD.

Other researchers have investigated the hydrogenotrohic denitrification with different systems. The results of these studies are presented in Table 2.5.

Table 2.5 Results of studies on hydrogenotrophic denitrification

Denitrification reactor	Influent, mg NO_3^--N/L	HRT	Denitrification rate, mg NO_3^--N/L.d	Efficiency, %	Reference
Fluidized-bed sand reactor	25	4.5 h	130	-	Kurt et al., 1987
Polyurethane Carrier Reactor	50	353 min	200	80-100	Dries et al., 1988
Fixed bed reactor Plate diffuser	22-25	53 min	600 - 700	100	Chang et al., 1999
Hollow fiber membrane	12.5	42 min	370.6	86.5	Lee and Rittmann, 2000
Hollow fiber membrane	145	4.1 h	770	100	Ergas and Reuss, 2001
Electrochemical cell	21 - 27	1 h	250	85	Szekeres et al., 2001
Microporous membrane	40	-	-	92	Mansell et al., 2002
Hollow fiber membrane	48	12 h	96	100	Mo et al., 2005
Hollow fiber membrane	300	22h	800	-	Rezania et al., 2005
Trickling filter	20	-	18.5	-	Grommen et al., 2006
Hollow fiber membrane	50	3 h	363.7	91.4	Hung, 2006
Saturator	25	3 h	110	100	Rezania et al., 2007

2.4 Gas permeable membrane

2.4.1 Fundamentals of gas transfer

At steady state conditions, the rate of mass transfer of a gas through the gas film must be equal to the rate transfer through the liquid film. The mass flux for each phase for absorption is written as follows (Metcalf and Eddy, 2003)

$$r = k_g (P_G - P_i) = k_L (C_i - C_L) \qquad \text{Eq. 2.13}$$

Where: r: rate of mass transferred per unit of time

k_g : gas film mass transfer coefficient

k_L : liquid film mass transfer coefficient

P_G : partial pressure of constituent A in the bulk of gas phase

P_i : partial pressure of constituent A at interface in equilibrium with concentration C_i of constituent A in liquid

C_i : concentration of constituent A at the interface in equilibrium with partial pressure P_i of constituent A in the gas

C_L: concentration of constituent A in the bulk liquid phase

However, because it is difficult to measure the values of k_L and k_G at the interface it is common to use overall coefficient K_L and K_G, depending on whether the resistance to mass transfer is on the gas or liquid side. If it is assumed that all of the resistance to mass transfer is caused by the liquid film, then the rate mass transfer can be defined as follows in terms of the overall liquid mass transfer coefficient :

$$R = K_L(C_S - C_L) \qquad \text{Eq. 2.14}$$

Where r : rate of mass transferred per unit of area per unit time

K_L : overall liquid mass transfer coefficient

C_L : concentration of constituent A in the liquid phase

C_S : concentration of constituent A at the interface in equilibrium with the partial pressure of constituent A in bulk gas phase.

To estimate the flux of a slightly soluble gas from the gas to the liquid phase. The rate of mass transfer per unit volume per unit time can be calculated by multiplying Eq. 2.14 by the area and dividing by the volume

$$r_v = K_L \frac{A}{V}(C_S - C_L) = K_L a\,(C_S - C_L) \qquad \text{Eq. 2.15}$$

Where r_v : rate of mass transfer per unit volume per unit time, $ML^{-2}T^{-1}$

K_La : Volumetric mass transfer coefficient, LT^{-1}

A : area through which mass transfer, L^2

V : volume in which constituent concentration is increasing, L^3

a : interfacial for mass transfer per unit volume, L^{-1}

2.4.2 Hollow fiber membrane as hydrogen diffuser in hydrogenotrophic denitrification

Based on the Eq. 2.15, rate of mass transfer can be increased by increasing contact surface between gas and liquid phases. It is obvious that the smaller diameter of gas bubble is the higher contact surface area with water is achieved. Hence, to diffuse hydrogen gas in water efficiently hydrogen gas should pass through a diffuser which has small pore size. In studies on hydrogenotrophic denitrification, the pore size of hollow fiber membrane was from 0.02 to 0.05 μm. That makes it able to diffuse hydrogen in bubbleless form leading to high dissolution of hydrogen in water. Moreover, hollow fiber membrane has higher ratio of surface area per unit of volume than other membranes or conventional diffusers such as ceramic diffuser. Therefore, in hydrogenotrophic denitrification hollow fiber membrane is an excellent solution to overcome disadvantages of low solubility and explosibility of hydrogen (the explosive range for hydrogen is 4 to 74.5% in air). Many studies approved that nearly 100% efficiency of hydrogen use for denitrification could be accomplished (Lee and Rittmann, 2000; Ergas and Reuss, 2001; Mo et al., 2005). Membranes, which are hydrophobic, can have this capability because they are not wet when submerged in water and prevent water go inside the fiber. For this reason, hydrophobic materials such as polyurethane, polypropylene, polyethersulfone can be used to fabricate this type of membrane modules.

The membrane is also a carrier for microorganism to attach and create biofilm on the outer side of the membrane. Bacteria on the biofilm can directly uptake hydrogen gas and substrate for denitrification reactions (Figure 2.7).

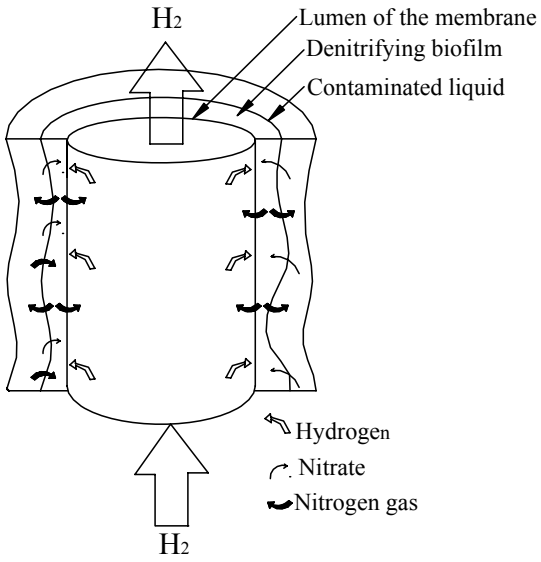

Figure 2.7 Hydrogen diffusion through membrane (Ergas and Reuss, 2001)

Typical hollow fiber membrane bioreactor for denitrification includes a cross-flow membrane module submerged in a reactor. Through the membrane hydrogen is supplied from a cylinder. Internal recycling rate is necessary and it is controlled in such level as to ensure a good mixing in the reactor.

2.5 Influencing factors of hydrogenotrophic denitrification in hollow fiber MBR

2.5.1 Effects of pH and temperature

The overall reaction of hydrogenotrophic can be expressed as follows (Rittmann and McCarty, 2001):

$$4\,H_2 + \frac{8}{5}\,NO_3^- \rightarrow \frac{4}{5}\,N_2 + \frac{16}{5}H_2O + \frac{8}{5}\,OH^- \qquad \text{Eq. 2.16}$$

From the equation 2.16, pH will increase in the hydrogenotrophic denitrification. pH is an important factor in denitrification because growth of bacteria and activity of enzymes are more enhanced in a favorable range of pH. The optimum range for heterotrophic denitrification appears to be 7 – 8. Meanwhile it was found that this value for autotrophic denitrification was from 7.7 to 8.6, with the maximum denitrification efficiency at pH 8.4. Increasing pH above 8.6 caused a significant decrease in nitrate removal and a dramatic increase in nitrite accumulation (Lee and Rittmann, 2003). This optimum range is even higher in another study, Rezania et al. (2005) found that the optimum pH was 9.5 at 25 ^oC and 8.5 at 12 ^oC. Nitrate reduction rates between 0.38 and 0.74 (g NO_3^--N/g VSS.d) and 0.21 and 0.28 (g NO_3^--N/g VSS.d) at 12 ^oC were obtained at pH ranging from 7.5 to 9.5. This result implies that temperature has effect on the denitrification, the denitrification efficiency at 25 ^oC was higher than that at 12 ^oC. Figure 2.8 shows the nitrogen concentrations in the effluent increases when pH is out of optimum range.

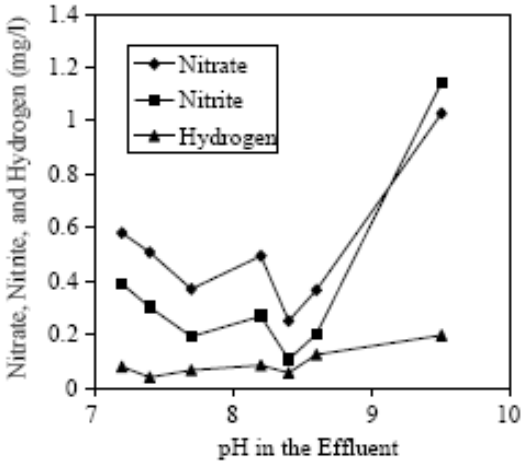

Figure 2.8 Effluent nitrate and nitrite versus effluent pH in a hollow fiber MBR (Lee and Rittmann, 2003)

2.5.2 Effects of hydrogen pressure and dissolution

Hydrogen in hydrogenotrophic denitrification is diffused into a denitrification reactor and consumed in four targets as follows:

- Hydrogen is consumed in the denitrification reaction:

  $$2\,NO_3^- + 2H^+ + 5H_2 \rightarrow N_2 + 6H_2O$$

  According to the above equation, 1g of NO_3^--N converted to N_2 consumes 0.357g of hydrogen gas (Ho et al., 2001);
- Hydrogen reacts with oxygen by the reaction: $2H_2 + O_2 = 2H_2O$. Theoretically, 1g of dissolved oxygen in water requires 0.125g of hydrogen gas;
- The undissolved hydrogen goes into the air as the gas phase; and
- Hydrogen which is dissolved in the water but not consumed remains in the effluent.

By using hollow fiber membrane Lee and Rittmann (2002) diffused considerable amount of hydrogen into wastewater. It reached to 1.1 – 1.4 mg H_2/L close to the saturation of dissolved hydrogen in water is (1.6 mg/L at 20^{o}C). Since hollow fiber membrane can diffuse efficiently hydrogen in the water, the hydrogen lost into the air is inconsiderable. The hydrogen consumption for reaction with oxygen can be high depending on DO of the influent, it reached to 40% of hydrogen input when DO and nitrate of the influent were 8 mg/L and 18.5mg NO_3^--N/L respectively (Grommen et al., 2006).

The dissolved hydrogen concentration has a significant effect on nitrate and nitrite reduction reactions. Of which nitrite removal is more sensitive because 1 mole of hydrogen is consumed per 1 mole of nitrate, whereas 1.5 mole of hydrogen is consumed per 1 mole of nitrite. Therefore, the accumulation of nitrite may occur if there is not sufficient hydrogen for the biomass. According to Chang et al. (1999) nitrite reductase was inhibited at a concentration lower than 0.2 mg/L and nitrate reductase was inhibited when the concentration is below 0.1 mg/L.

Rezania et al. (2005) found that nitrite accumulation occurred when hydrogen pressure was lower than 0.2 atm. Similarly, in a study of nitrate removal by hydrogenotrophic denitrification Lee and Rittmann (2000, 2002) applied hydrogen pressure from 0.2 atm to 0.56 atm and reported that at higher pressure of hydrogen the nitrate removal efficiency was higher but the concentration of dissolved hydrogen in the effluent was also higher. In

fact, the increase of hydrogen pressure is to increase hydrogen dissolution in the water that enhances the denitrification. However the hydrogen pressure or flow rate should be controlled properly to keep the optimum dissolved hydrogen concentration in the water otherwise less dissolved hydrogen will inhibit denitrification or too much hydrogen will increase the operation cost.

2.5.3 Effects of salinity

Hydrogenotrophic denitrification to treat wastewater is quite new and information on the effects of salinity on the denitrification performance has been not focused on. However, it is expected that the mechanism of salinity effects of hydrogenotrophic and heterotrophic denitrifications is similar. The information on effect of salinity on nitrification and heterotrophic denitrification is presented as follows:

High saline concentrations in wastewater have negative effects on nitrogen removal and it was reported that rapid shifts in salt concentration have more adverse effects than gradual shifts (Chen et al., 2003). A stepwise increase of NaCl concentration was implemented in a study of removing nitrogen in high-salinity wastewater by rotating biological contactor (Winday et al., 2005). The result showed that the reactor was not negatively affected by salt concentration up to 6 g NaCl/L but the nitrogen removal capacity was 31% lower at a salt level of 30 g NaCl/L compared to the reference period without salt addition. On contrast, the direct acclimatization to saline wastewater was found more efficient than stepwise acclimatization (Park et al., 2005). In this study fresh water denitrifiers were acclimatized to saline wastewater by two methods direct and stepwise acclimatization, the result showed that bacteria which acclimatized directly in wastewater with salinity of 30 ppt performed denitrification better than bacteria which were acclimatized to salinity of 30 ppt from wastewater with salinity of 7.5 and 15 ppt. Similarly, direct acclimatization with salinity of 15 ppt was more efficient than stepwise acclimatization from salinity of 7.5 ppt to 15 ppt.

From the above results, it is expected that either direct acclimatization or stepwise acclimatization could be suitable for hydrogenotrophic denitrification.

2.5.4 Membrane fouling

In any membrane bioreactor, membrane fouling always occurs after a period of operation and it is one of the main problems reducing efficiency of the reactor. Precipitation of mineral solids was found to have negative impact on the performance of hydrogen diffuser membranes as build-up inside microbial aggregates and on the surface of membranes (Lee and Rittmann, 2002). Multivalent cations present in waters and wastewaters can precipitate with basic anions such as carbonate (CO_3^{2-}), phosphate (PO_4^{3-}), monohydrogen phosphate (HPO_4^{2-}), dihydrogen phosphate ($H_2PO_4^-$), and hydroxide (HO^-) (Lee and Rittmann, 2003). Of which mineral have lower solubility such as $Ca_5(PO_4)_3OH$, $Ca_3(PO_4)_2$ and $CaCO_3$, with lower solubility therefore they are expected to contribute major precipitation. Solubility of the precipitated material is pH dependent, as higher precipitation of inorganic compounds is expected at higher pH (Rezania et al., 2005).

Lee and Rittmann (2003) reported that in short-term of operation mineral solids did not adversely affect hydrogen transfer and denitrification but for long-term the effects may occur leading to increase in mass-transport resistance for hydrogen diffusion out of the membrane. Whilst Ergas and Reuss (2001) noted that mass transfer limitations to the

extent that the introduction of a crossflow velocity was required to shear biofilm from the fibers. The contrast in findings indicates that much more work is needed to further understand biofilm formation and its influence on mass transfer. This requirement subsequently generated studies such as that undertaken by Lapsidou and Rittmann (2004). These authors developed a unified multi-component cellular automation (UMCCA) model, which predicted quantitatively the development of the biofilms composite density utilising three biofilm components: active bacteria, inert or dead biomass and extracellular polymeric substances (EPS). The authors based the model on the hypothesis that fluid flow over the biofilm creates horizontal and vertical pressures, leading to fiber vibration causing biofilm consolidation, or higher density packing.

2.5.5 Biofilm layer

Beside the denitrification by suspended bacteria, biofilm which includes bacteria in attach form also contributes to the denitrification. However when the biofilm becomes too thick it reduces performance of a membrane by decreasing hydrogen transfer through the membrane. It also decreases activity of biofilm. Ergas and Reuss (2001) found that after four months of operation of hollow fiber membrane bioreactor, denitrification rates decreased significantly due to the build up of a thick layer of biofilm on the surface of the membrane. Several operational strategies have been used to maintain biofilm thickness at an optimum level including the use of cross-flow membrane configurations (Ahmed and Semmens, 1996) and periodic shearing of biomass from the membranes using high liquid velocities combined with scouring with gas bubbles (Pankhania et al. 1994). CO_2 is normally used for this purpose, it also acts as buffer agent to reduce increase of pH produced by the denitrification. In depth study on biofilm layer in hydrogenotrophic denitrification is under investigation.

2.5.6 Dissolved oxygen and oxidation reduction potential

Dissolved oxygen reacts with hydrogen in water is as follows:

$$2H_2 + O_2 = 2H_2O. \qquad \text{Eq. 2.17}$$

From the Eq. 2.17, 1 g of dissolved oxygen in water requires 0.125 g of hydrogen gas theoretically. Based on this equation Grommen et al. (2006) calculated hydrogen consumed in denitrification and found that 40% of the hydrogen gas was used to remove DO in order to create anoxic conditions for the denitrification of influent containing 8 mg/L of dissolved oxygen and 18.5mg NO_3^--N/L . Moreover, too high concentration of DO can lead to accumulation of the denitrification intermediate: NO_2^- and N_2O (Rittmann and McCarty, 2001). Hence DO of the influent of denitrification tank must be minimized to enhance the denitrification. This results in a reduction of both the hydrogen gas consumption and the minimum hydraulic retention time of the system. The influent DO is reduced by minimizing contact with the atmosphere or sparging with nitrogen gas to make it anoxic before entering the denitrification tank (Grommen et al., 2006).

Oxidation reduction potential (ORP) is the electrical potential associated with the oxidation or reduction of a substance, such as an element or molecule. In a study on kinetics of hydrogen-dependent denitrification (Rezania et al., 2005), it was reported that under anaerobic conditions, for example, at ORP below – 250 mV, hydrogen can be consumed by methanogenic, sulfate reducing, and homoacetogenic bacteria. Under anoxic conditions, at higher ORP, for example, above – 50 mV, the presence of nitrate limits the activity of methanogens and sulfate-reducing bacteria. ORP significance was measured in the research on hydrogenotrophic denitrification (Mo et al., 2005). The system was operated

continuously 174 days in four stages with nitrate loadings of 24, 48, 96 and 192 mg NO_3^--N/L.d. In the fourth stage higher values of ORP were observed due to incomplete denitrification and the residual nitrate in the reactor. The study concluded that on-line ORP served as a good indicators of denitrification and general performance of the system.

2.5.7 Phosphorous requirement

Phosphate is a necessary nutrient for the synthetic of bacteria. In activated sludge process for BOD removal, the empirical ratio BOD:N:P is 100:5:1. This ratio for hydrogenotrophic is different and it was found that 0.49 mg of phosphate was required for removal of 75 mg of nitrate (Germonpre et al. 1992 cited from Chang et al., 1999). In a study of hydrogenotrophic denitrification with influent nitrate of 22 – 25 mg NO_3^--N/L, Chang et al. (1999) tested denitrification in two separate reactors. In the reactor without adding phosphate, nitrate removal rate increased gradually and decreased rapidly after 6 days, meanwhile in the other reactor enriched with phosphate denitrification increased rapidly. This study found that phosphate had a significant effect on the accumulation of nitrite, but its effect on nitrate removal was small at a concentration greater than 0.5 mg-P/L. It implies that for denitrification a suitable ratio N:P is at least 50:1.

2.6 Kinetics of hydrogenotrophic denitrification

The understanding of kinetics involved in hydrogenotrophic denitrification is required to provide insight into observations of the denitrification process and also helps to establish optimum reactor design and operating conditions. Although studies on hydrogenotrophic denitrification have been conducted to in various systems, a few detailed studies on kinetics on this process were carried out. Recently, it has been focused in two researches as follows:

A study on kinetics of hydrogen-dependent denitrification under varying pH and temperature conditions was implemented by Rezania et al. (2005). In this study, a zero-order kinetic model was proposed for nitrate reduction and kinetic coefficients were obtained at two temperatures (25^0C and 12^0C) at pH ranging from 7 to 9.5. Based on Monod's kinetics model and low half velocity constants of hydrogen and nitrate from previous studies, this research assumed that kinetics of nitrate reduction was independent of nitrate and hydrogen. The rate of nitrate consumption was only dependent on biomass concentration and maximum specific nitrate utilization rate. The research showed that the zero-kinetic model for hydrogenotrophic reduction of nitrate was highly correlated with the experimental results. At biomass concentration of 500 mg/L and pH from 7.5 to 9.5, maximum specific nitrate utilization rates were found between 0.38 and 0.74 mg NO_3^--N/mg VSS.d at 25^oC, and between 0.2 and 0.28 at 12^oC.

Recently, Vasiliadou et al. (2006) investigated the kinetics by implementing five test runs with the same initial biomass but different NO_3^--N concentrations. Carbon source and hydrogen concentrations were chosen to be in excess to ensure that they were not rate limiting. The kinetic model was developed considering denitrification as a two-step process occurring by the consecutive reduction of nitrates to nitrites and then to nitrogen gas without accumulation of intermediate gaseous products. The kinetic equation is presented in Eq. 2.19.

$$\frac{dN_1}{dt} = -\frac{1}{Y} X \frac{kN_1}{K_s + N_1 + k_d N_2 + \frac{N_1^2}{K_i}} \qquad \text{Eq. 2.18}$$

Where: $\frac{dN_1}{dt}$ is the rate of nitrate consumption, mg NO_3^--N/L.d

k : maximum specific nitrate utilization rate, mg NO_3^--N/mg VSS.d
X : biomass concentration, mg/L
N_1 : concentration of nitrate, mg/L
N_2 : concentration of nitrite, mg/L
Y : growth yield coefficient, mg biomass/ mg NO_3^--N
K_s : half velocity constant for nitrate, mg/L
k_d : a constant in growth rate expression, mg NO_3^--N/mg NO_2^--N
K_i : the nitrate inhibition constant, mg/L

The maximum specific nitrate utilization rate (k) was 0.0485 mg NO_3^--N/mg VSS.h or 1.164 mg NO_3^--N/mg VSS.d at 29 – 31^{o}C and pH between 6.4 and 7. Model validation was tested by running experiment with three different initial biomass and nitrate concentrations and the experiment data was in very good agreement with the kinetic model.

Although kinetics of hydrogenotrophic denitrification is different in the two studies, they both showed that the rate of nitrate consumption is dependent on biomass concentration and maximum specific nitrate utilization rate. The value of latter parameter varies depending on operating conditions such as temperature, pH. Table 2.7 shows maximum specific nitrate utilization rate from studies on denitrification.

Table 2.6 Maximum specific nitrate utilization rates (adapted from Rezania et al., 2005)

Electron donor	Biomass concentration mg/L	Temperature, ^{o}C	pH	Nitrate reduction rate, mg NO_3^--N/mg VSS.d	Reference
Hydrogen	500	25	7.5 – 9.5	0.38 – 0.74	Rezania et al., 2005
Hydrogen	500	12	7.5 – 9.5	0.21 – 0.28	Rezania et al., 2005
Hydrogen	32.89	29 - 31	6.4 - 7	1.164	Vasiliadou et al., 2006
Methanol	1200-3100	25	6.8	0.43 – 0.61	Foglar and Briski, 2003
Thiosulphate	-	25 - 33	6.5 – 7.5	7.2 – 9.6	Oh et al., 2000
Sulfur	1190 – 6610	25	7 – 8	0.14 – 0.19	Koenig and Liu, 2004

Chapter 3

Methodology

3.1 Experimental process

In this study, Denitrification – Aeration sequence system (D-A) with hollow fiber membrane to diffuse hydrogen gas was used to treat synthetic aquaculture wastewater in hydrogenotrophic denitrification process. Because the performance of such system was proved better than the Aeration Denitrification sequence system (A-D) in a study on hydrogenotrophic denitrification (Hung, 2006), this system was chosen.

The research was carried out under laboratory – scale experiment and in the ambient conditions. The experiment started with sludge acclimatization followed by three runs with three salinities to determine its performance and efficiency. Operating parameters were measured to find optimum conditions. The experimental procedure is described in Fig. 3.1.

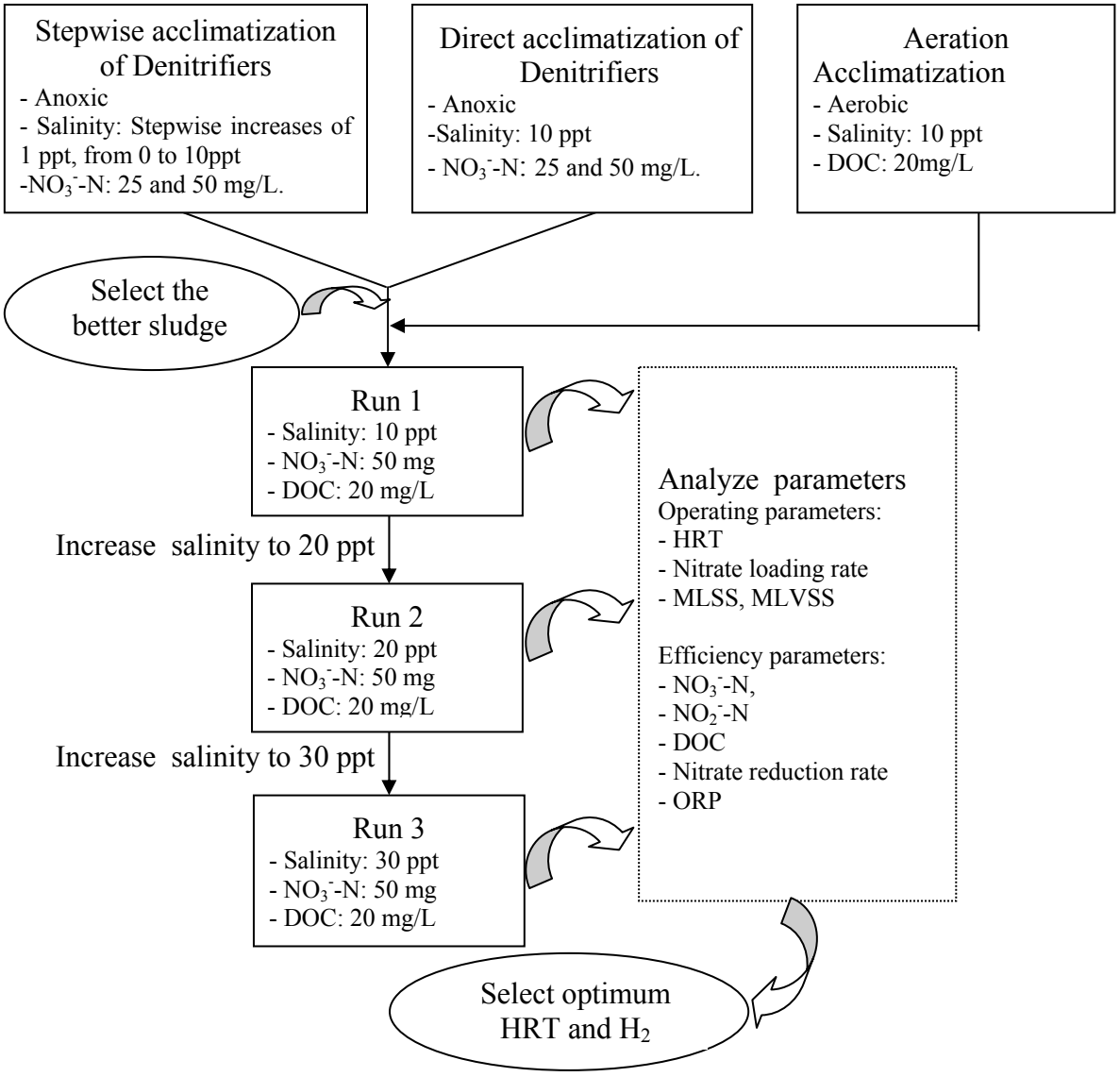

Figure 3.1 Experimental procedure

3.2 Feed wastewater and sludge acclimatization

3.2.1 Feed wastewater

This study used synthetic wastewater containing 50 mg NO_3^--N/L and 20 mg DOC/L. Nutrient salts and inorganic carbon ($NaHCO_3$) were added to make the feed wastewater favorable for growth of microorganisms. Except that salinities were varied every run, other chemicals were fixed during the experiment. The composition of synthetic wastewater is summarized in Table 3.1.

Table 3.1 Feed wastewater

Medium	Chemicals	Concentration
Medium A	DOC (glucose)	20 mg/L
Medium B	$NaNO_3$	303.6 (50 mg/L NO_3^--N)
Medium C	KH_2PO_4	80 mg/L
Medium D	$NaHCO_3$	200 mg/L
Medium E	Salinity (NaCl)	10, 20, and 30 ppt
Medium F: Trace element (1mL/L) (Chang et al., 1999)	$MgSO_4.7H_2O$	10 g/L
	$ZnSO_4.7H_2O$	2.2 g/L
	$CaCl_2.4H_2O$	2.5 g/L
	$CoCl_2.6H_2O$	0.5 g/L
	$(NH_4)_6Mo_7O_{24}.4H_2O$	0.5 g/L
	$FeSO_4.7H_2O$	5 g/L
	$CuSO_4.5H_2O$	0.2 g/L

3.2.2 Sludge acclimatization

Sludge acclimatization in this study was a crucial step for bacteria to adapt to changes of salinity and nitrate concentrations, so that they can grow and perform their roles of denitrification or DOC removal. NaCl and NO_3^--N were varied depending on sludge acclimatization methods. Phosphate buffer was to control the pH of the solution approximately 7 and enough nutrient (trace element) was added. Return sludge from wastewater treatment plant of Thamasat University was used for microorganism seeding.

Denitrifier sludge acclimatization with hydrogen gas

The sludge was cultured in batch reactors with working volume of 4 L, in which hydrogen gas was supplied through a hollow fiber membrane module. The membrane configuration is presented in table 3.2. Hydrogen gas pressure of 0.6 atm was be maintained in the membrane. These reactors were operated with a cycle of 24 hours including 22.5 hours for hydrogen diffusion, 1.5 hours for settling, decantation and feeding. Suspended biomass in term of MLVSS was measured to determine microorganism growth rate. NO_3^--N in the supernatant was analyzed to identify denitrification efficiency. The procedure of acclimatization of denitrifier sludge and DOC removal sludge is illustrated in Fig. 3.4.

Table 3.2 Characteristics of membrane

Item	Configuration
Membrane type	Hollow fiber
Membrane material	Polyethylene (PE)
Pore size	0.1μm
Surface area	$0.42m^2$

In order to acclimatize sludge to the synthetic wastewater with salinity of 10 ppt and 50 mg NO_3^--N/L and to test if rapid or gradual shift of salinity was preferable for bacteria, two acclimatization methods in this step were carried out in two separate reactors as follows:

- Stepwise acclimatization in the first reactor: The initial wastewater containing salinity nearly 0 ppt and 25 mg NO_3^--N/L was supplied to reactor. When the removal efficiency reaches at least 85%, 50 mg NO_3^--N/L was applied. After this step, stepwise increases of 1ppt salinity was applied. The salinity increase was implemented if only the denitrification efficiency reached at least 85%. These steps continued until the removal efficiency was stable with wastewater containing 10 ppt salinity.

- Direct acclimatization in the second reactor: similar to the first method but initial wastewater contained salinity of 10 ppt and 25 mg NO_3^--N/L. Only stepwise increase of 25 mg NO_3^--N/L was applied in this acclimatization. The acclimatization unit is illustrated in Fig. 3.2.

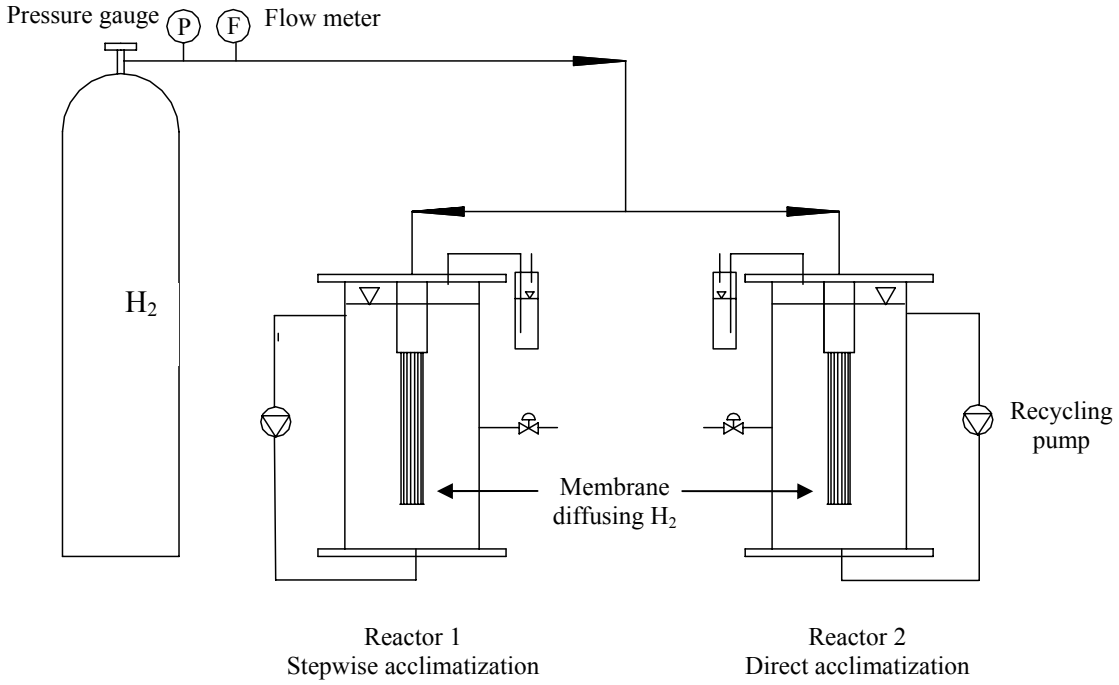

Figure 3.2 Diagram of denitrifier sludge acclimatization

Conditions for denitrifier sludge acclimatization are described in the Table 3.3.

Table 3.3 Operating conditions for denitrifier sludge acclimatization

Parameter	Value
pH	7
Temperature (^oC)	ambient
Salinity (ppt)	0 – 10
NO_3^--N (mg/L)	25 and 50
MLVSS (mg/L)	1550

Aerobic sludge acclimatization

Activated sludge was acclimatized in four-litter batch reactor supplied with air through a ceramic diffuser. Diagram of reactor is described in Fig. 3.3. A cycle of 24 hours included 22.5 hours for hydrogen diffusion, 1.5 hours settling, decantation and feeding. Biomass in term of MLVSS was measured to determine microorganism growth rate. DOC in the supernatant was analyzed to identify DOC removal efficiency. The wastewater containing salinity of 10 ppt and 20 mg DOC/L was supplied to reactor. Conditions for aeration sludge acclimatization are described in the Table 3.4.

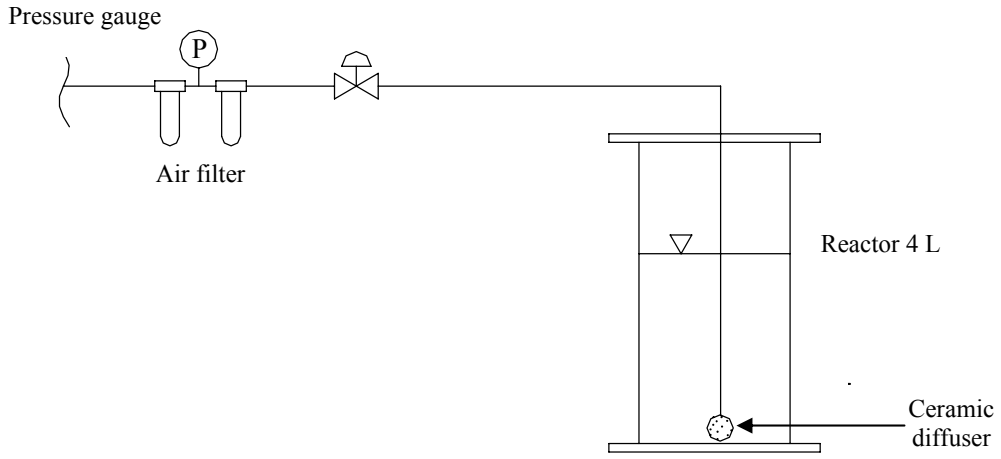

Figure 3.3 Diagram of aeration sludge acclimatization

Table 3.4 Operating conditions for aerobic sludge acclimatization

Parameter	Value
pH	7
Temperature, ^{o}C	ambient
DO, mg/L	6
Salinity, ppt	10
DOC, mg/L	20
MLSS, mg/L	6000

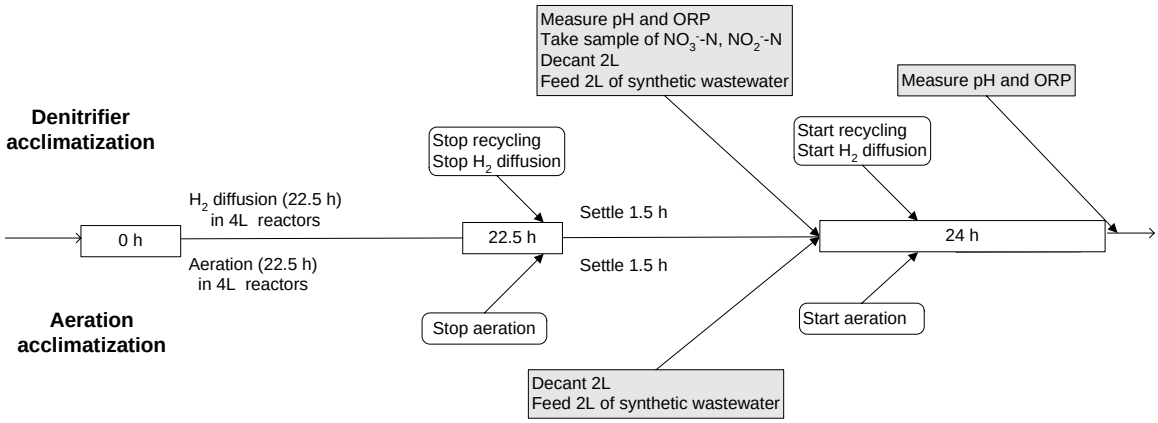

Figure 3.4. Sludge acclimatization procedure

3.3 Experimental setup and runs

3.3.1 Hollow fiber membrane bioreactor (MBR)

The reactor system including two tanks, a denitrification tank followed by an aeration tank (D – A system), was designed to remove NO_3^--N in the first tank and DOC in the second one. Experimental setup diagram is presented in Fig. 3.5. Function of each component and operation of the system are described as follows:

Denitrification reactor with working volume of 4.5 L created anoxic condition for nitrate removal by hydrogen oxidizing bacteria. The main part of the reactor was submerged hydrophobic hollow fiber membrane module which diffused hydrogen gas into the water for the denitrification and served as media for microorganism to attach. Hydrogen gas was supplied from a cylinder with high pressure of 140 atm so its pressure and flow rate was reduced before coming to the membrane. Internal water was recycled in order to create well mixing and to control biofilm layer on the membrane. Synthetic wastewater from feed tank entered the reactor and underwent denitrification process. CO_2 supplied from the bottom of the reactor was to supply carbon source and pH buffer. The effluent from the reactor flowed to a sedimentation tank where sludge was recycled and washed out properly to maintain suitable MLVSS in the denitrification tank. Diagram of the system is described in Fig. 3.5.

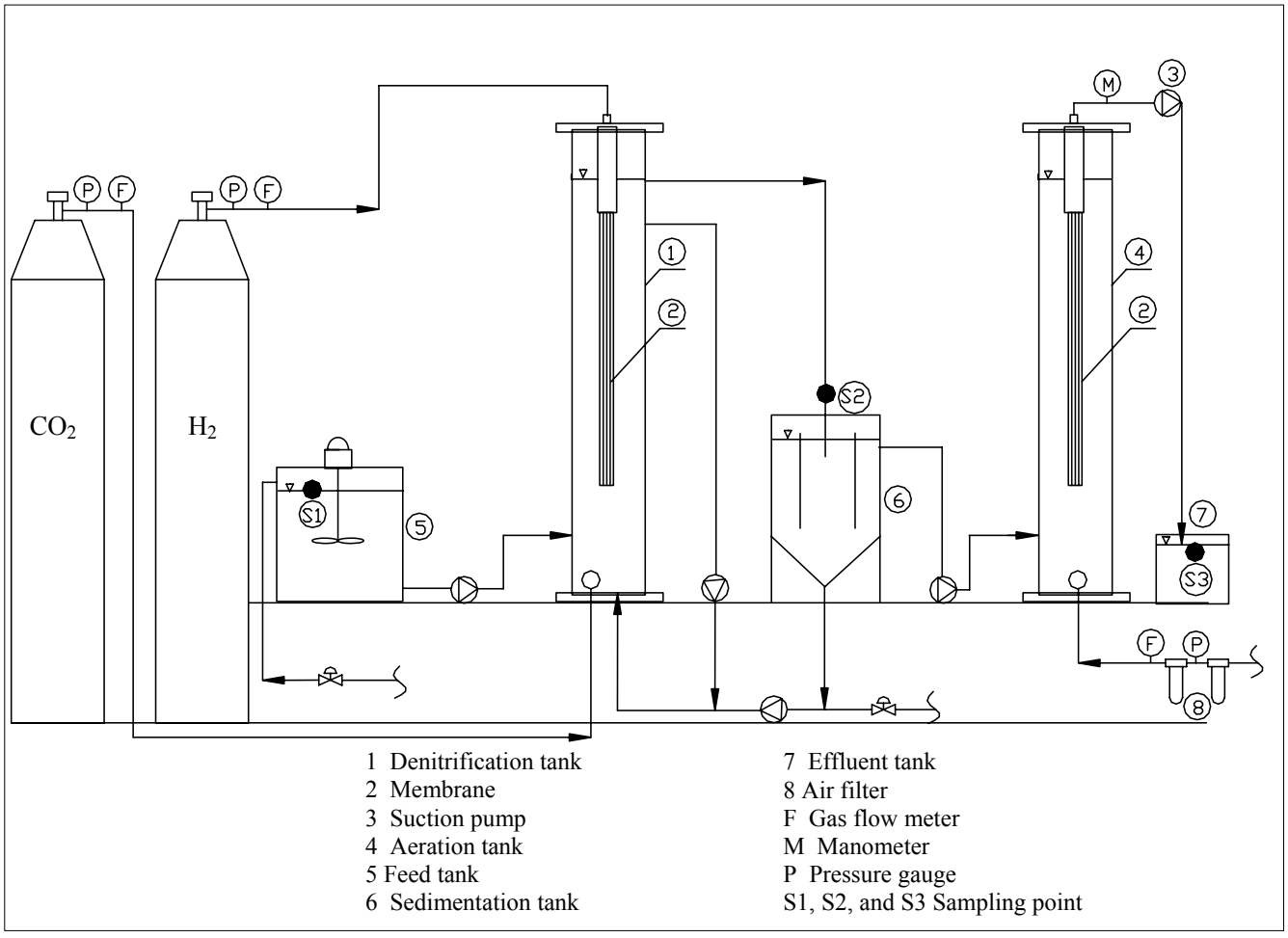

Figure 3.5 Experimental setup

Aeration reactor with working volume of 4.5 L created aerobic condition by airflow. Flow rate of air supplied into the aeration reactor was controlled properly to maintain dissolved

oxygen approximately 6 mg/L as well as to play a role of mixing the wastewater. The reactor received wastewater from the sedimentation tank, removed DOC and transferred wastewater to an effluent tank by a suction through the membrane. A suction time of 12 minutes and a resting time (relaxation) of 3 minutes for releasing of the negative pressure within the membrane unit were applied for the aeration membrane. This operation was essential for stable operation of membrane bioreactor. MLVSS in the reactor was adjusted to the lowest level, enough to remove DOC efficiently. The retention time in the reactor was the same as in denitrification tank.

3.3.2 Experimental runs

After acclimatization step, the better-acclimatized denitrifier sludge of two methods as described in section 3.2.2 will be put into the denitrification reactor. The experiment including three runs: run 1, run 2, and run 3 with synthetic wastewater containing 10, 20, and 30 ppt salinity respectively. If the sludge in stepwise acclimatization was used for three experimental runs, the same stepwise increase of 1 ppt salinity was carried out to increase salinity in wastewater for run 2 and run 3. Whereas, if the sludge in direct acclimatization was used, a stepwise increase of 5 ppt salinity was applied. Recycling rate was controlled at 2 L/min to maintain nearly complete mixing in the denitrification reactor. MLVSS was controlled at approximately 2,500 mg/L in the reactor.

The research sequentially operated three runs with three levels of salinity. Run 1 started with hydraulic retention time (HRT) of 6h or nitrate nitrogen loading rate (NLR) 200 $g/m^3.d$. In this run, HRT and hydrogen pressure were adjusted to find the optimum HRT and hydrogen pressure.

Run 2 started with the acclimatization of sludge from salinity of 10 ppt to 20 ppt. The initial HRT of run 2 was started at 9 hrs and adjusted to find the optimum one. Similarly, run 3 with initial HRT of 12 was conducted. Finally optimum HRT and hydrogen pressure of three runs were identified. At optimum HRT, the system achieved the highest nitrate reduction rate, at least 90% removal efficiency and outlet nitrite lower than 0.6 mg/L (safety level for fish). This process is presented in Fig. 3.6.

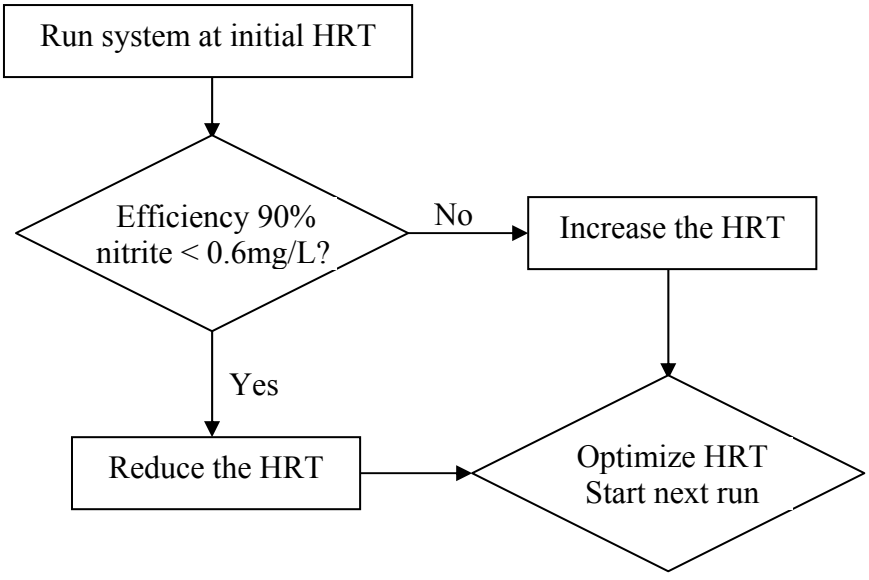

Figure 3.6 Process of finding optimum HRT

Parameters measured to determine efficiency of the system are described in section 3.4. The procedure of the experimental runs as well as their estimated duration are presented in Fig. 3.7.

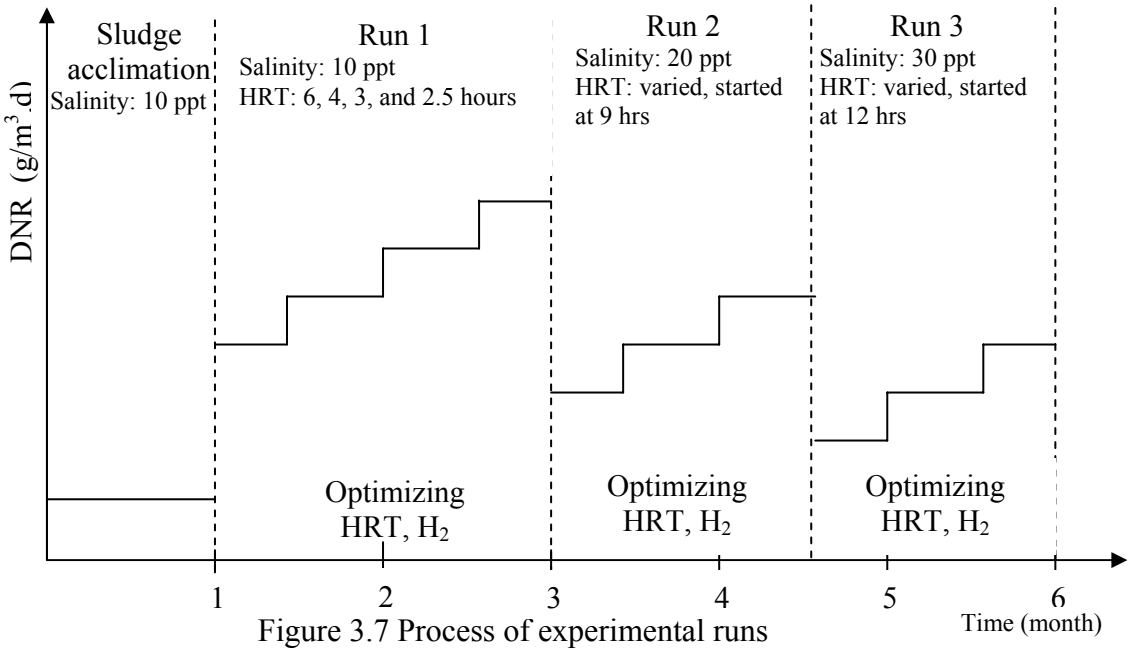

Figure 3.7 Process of experimental runs

3.4 Study parameters and analytical methods

3.4.1 Study parameters

To analyze the efficiency of the hydrogenotrophic denitrification, following equations were developed to calculate operating and kinetic parameters

Denitrification rate

$$DNR = \frac{Q * (TN_{in} - TN_{ef})}{V} = \frac{(TN_{in} - TN_{ef})}{HRT} \quad \text{Eq. 3.1}$$

Where DNR: denitrification rate, $g/m^3.day$

TN_{in} concentration of total nitrogen in influent of the reactor, g/m^3

TN_{ef} : concentration of total nitrogen in effluent of the reactor, g/m^3
Q: wastewater flow rate, m^3/d
V: volume of the reactor, m^3
HRT hydraulic retention time, d

Efficiency of nitrogen removal

$$E_N = \frac{(TN_{in} - TN_{ef})}{TN_{in}} * 100 \quad \text{Eq. 3.2}$$

Where E_N is efficiency of nitrogen removal, %

Hydrogen utilization efficiency

$$E_H = \frac{H_{ut}}{H_{in}} * 100 \quad \text{Eq. 3.4}$$

Where E_H : hydrogen utilization efficiency, %

H_{in} : the amount of hydrogen supplied per period of time, g/d

H_{ut} is the amount of hydrogen utilized per period of time, g/d

Biomass yield

$$Y = \frac{Biomass_{gen}}{TN_{rem}} = \frac{(Biomass_{aft} + Biomass_{was} - Biomass_{ini})}{TN_{rem}} \quad \text{Eq. 3.6}$$

Where Y : biomass yield, g VSS/ g N

$Biomass_{gen}$: biomass generation after a period of time, g

$Biomass_{aft}$: biomass in the reactor after a period of time, g

$Biomass_{ini}$: initial biomass in the reactor, g

TN_{rem} : amount of nitrate nitrogen removal after a period of time, g

Nitrate reduction rate to biomass concentration

$$K_N = \frac{Q\,(TN_{in} - TN_{ef})}{VX} \quad \text{Eq. 3.7}$$

Where K_N is nitrate reduction rate to biomass concentration, g NO_3^--N/g VSS.d

Q : flowrate, m^3/d

TN_{in} : concentration of total nitrogen in the influent, g/m^3

TN_{ef} : concentration of total nitrogen in the effluent, g/m^3

V : volume of the reactor, m^3

X : biomass concentration in the reactor, g/m^3

3.4.2 Analytical methods

Other parameters to be measured in this study included pH, DO, ORP, temperature, MLSS/MLVSS, DOC, DO, alkalinity, NO_2^--N, NO_3^--N, and PO_4^{3-}-P. Methods for analysis of these parameters are presented in table 3.5.

Table 3.5 Parameters and analytical methods

Parameter	Analytical method	Analytical Equipment	Range	Interference	Frequency	Sampling point
pH	-	pH meter	1 – 14	-	Daily	S1, S2, S3
DO	-	DO meter		H_2S, N_2, etc.	Daily	S1, S2
Temperature	-	Thermometer	-	-	Daily	S1
ORP	-	pH meter	-	-	Daily	S1, S2
MLSS/ MLVSS	Filtration/evaporation/ weighting	-	-	-	Every 3 days	S2
DOC	High-temperature combustion method	TOC analyzer (TOC – V_{CSN}, Shimadzu)	0-25000 mg/L	-	Daily	S1, S2, S3
NO_2^--N	Colorimetric method	Spectrophotometer	0 - 25 µg/L	Sb^{3+}, Au^{3+}, Bi^{3+} Fe^{3+}, Pb^{2+}, Hg^{2+}, Ag^{2+}, $PtCl_6^{2-}$, VO_3^{2-}	Daily	S2, S3
NO_3^--N	Cadmium reduction method	Spectrophotometer	0.01–1 mg/L	Oil and grease, SS, Fe, Cu, Cl_2.	Daily	S1, S2, S3

pH, DO, temperature, and ORP are measured by portable equipment
Measurement of other parameters follows Standard Methods (APHA et al., 1999)

3.5 Membrane cleaning and membrane resistance measurement

3.5.1 Membrane cleaning

Membrane cleaning is to reduce the increase of transmembrane pressure and to bring it back to a level close to the initial level. It helps to recover the working efficiency of the membrane. Sine the membrane was used for treating saline wastewater, it was cleaned with both hypochloride and hydrochloric acid. Membrane was removed out of the reactor and underwent a procedure of membrane cleaning as follows (Samarakoon, 2005):

- Remove cake layer on the membrane by flushing tap water
- Immerse the membrane for 2 – 6 hours in a solution containing sodium hypochloride (effective chloride about 3000 mg/L) and 4% aqueous sodium hydroxide
- Clean membrane thoroughly with tape water and put it in hydrochloric 2% for 6 hours.
-
- Take the membrane out of the solution and rinse with tap water to remove chemicals before installing it in the reactor
- Measure membrane resistance (R_m). The resistance should be 85% of the initial value.

3.5.2 Membrane resistance

Membrane resistance will be measured based on the resistance-in-series model (Choo and Lee, 1996) according Equation 3.8 and 3.9

$$J = \frac{\Delta P}{\mu R_t} \qquad \text{Eq. 3.8}$$

Where J : permeate flux, $m^3/m^2.s$
ΔP : transmembrane pressure, Pa
μ : viscosity of the permeate, Pa. s
R_t : total resistance for filtration

$$Rt = R_m + R_c + R_f$$

Where R_m : intrinsic membrane resistance
Rc : cake layer resistance
R_f : fouling resistance due to irreversible and pore plugging.

Membrane resistance will be measured by filtrating with filtered water at different filtration fluxes and recording the corresponding TMP. The membrane resistance is derived from the slope of the linear curve of ΔP versus J as described by the following equation

$$\Delta P = Rt.\ \mu.\ J + \Delta Po \qquad \text{Eq. 3.9}$$

Where ΔPo : the initial pressure to overcome the membrane set-up system resistance.
R_t : measured right after finishing run.
$R_m + R_f$ are obtained by measuring the resistance of the membrane after washing with tap water, R_m is measured after chemical cleaning
R_c : derived from equation 3.8

Chapter 4

Results and Discussion

This chapter presents the results from laboratory scale experiments for treatment of saline aquaculture wastewater using hollow fiber membrane bioreactor. It included sludge acclimatization and a series of three experimental runs with synthetic aquaculture wastewater of three salinities of 10, 20, and 30 ppt respectively. Each run started with acclimatization for the bacteria to adapt to increase of salinity. In these runs, hydraulic retention time and hydrogen pressure were adjusted to achieve the optimum values.

The results were analyzed and compared with previous studies on heterotrophic and hydrogenotrophic denitrification, especially with the hydrogen-dependent denitrification of fresh aquaculture wastewater with similar system.

4.1 Sludge acclimatization

In order to enrich enough microorganism for the experiment of hydrogenotrophic denitrification, activated sludge from Thamasat University wastewater treatment plant was acclimatized in two reactors supplied with hydrogen gas. The acclimatization was conducted with two method, stepwise acclimatization (no salinity added at the beginning and gradually increasing salinity to 10 ppt) and direct acclimatization with salinity of 10 ppt at the beginning. Conditions for both methods were the same with MLVSS of 1550 mg/L and hydrogen pressure of 0.6 bars. The result is represented in Figure 4.1 and 4.2 (see more detailed in Appendix A).

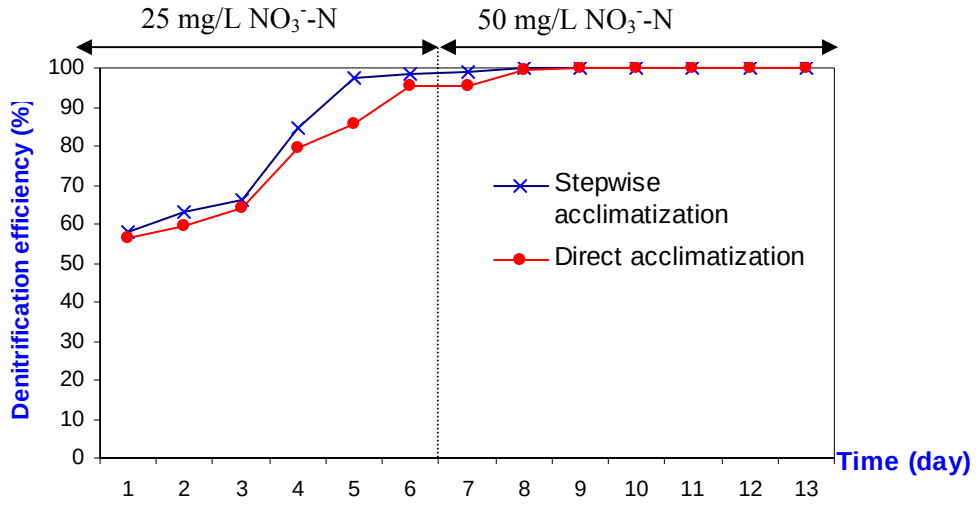

Figure 4.1 Denitrification efficiency of bacteria acclimatization process

Hydrogenotrophic denitrifiers in the two setups (with and without adding salinity) took 6 days to be acclimatized to the nitrate inlet of 25 mg/L. After this period, the denitrifiers quickly adapted to the increase of nitrate inlet concentration from 25 mg/L to 50 mg/L. Denitrification efficiency and rate finally reached to 100% and 25g/m^3.day after 8 days (Figure 4.1 and 4.2). In a similar study, due to limited hydrogen diffusion of silicon tube, the efficiency reached to around 90% with inlet NO_3^--N of 50 mg/L after two months (Hung, 2006). These results showed that the use of hollow fiber membrane in hydrogen diffusion for the sludge acclimatization was more efficient than that of silicon tube.

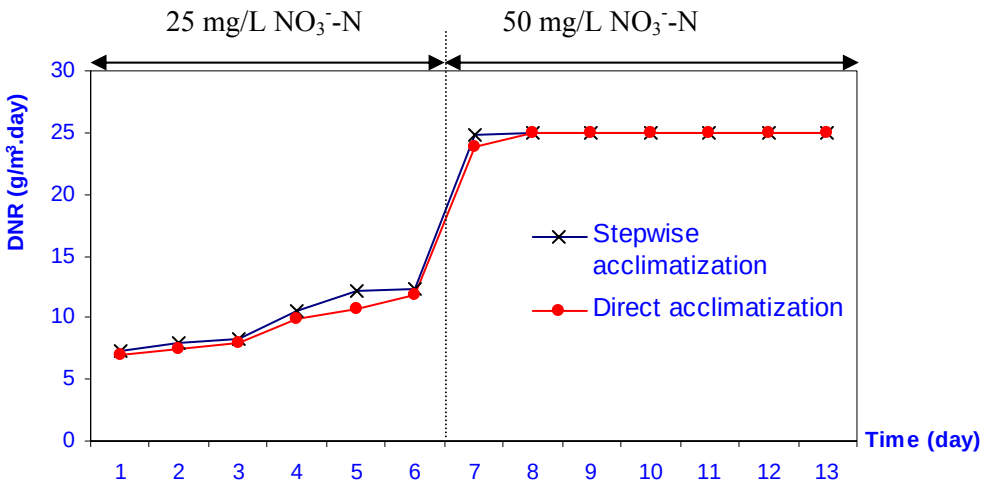

Figure 4.2 Denitrification rate of bacteria acclimatization process

In this study, denitrification rate and efficiency of direct acclimatization with salinity of 10 ppt was similar the acclimatization without adding salinity. Accordingly, compared to direct acclimatization, stepwise increases of salinity took longer time for bacteria to adapt to environment with salinity of 10 ppt. The result was in accordance with the study on acclimatization of heterotrophic denitrifiers by Park et al. (2005).

4.2 Nitrogen removal of the denitrification tank

4.2.1 Denitrification of experimental run 1

The membrane used in acclimatization stage for one month was continuously used in run 1. The result from day 1 to day 49 at HRT of 6 h is presented in Figure 4.3. In this period membrane fouling was observed and suitable recycling rate was found for the system.

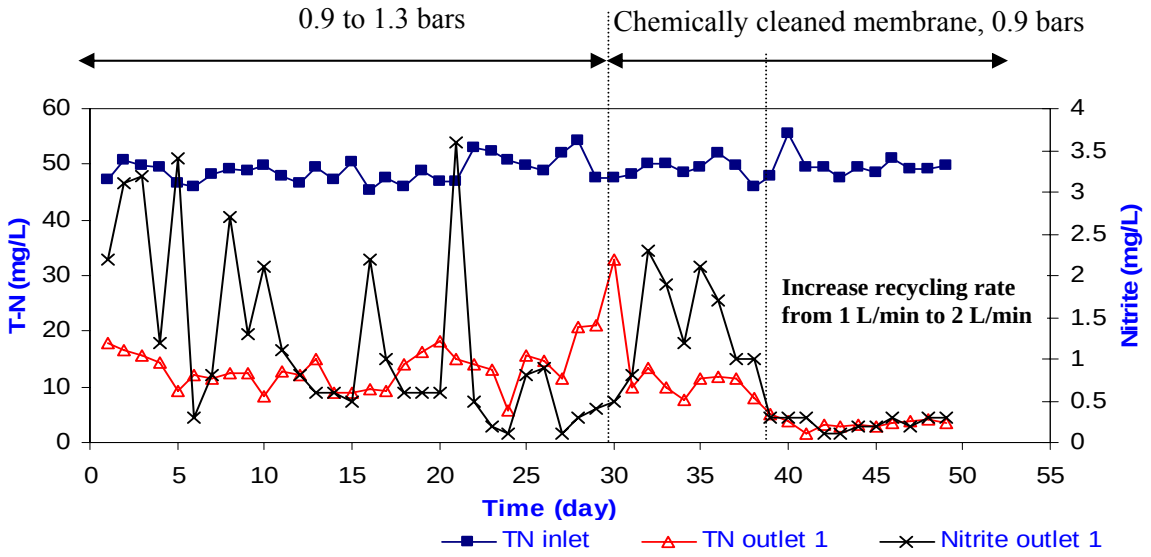

Figure 4.3 Effect of hydrogen pressure and mixing condition on denitrification

During the denitrification process of the system at HRT of 6 h and salinity of 10 ppt, the nitrogen removal depended on dissolved hydrogen in wastewater which was closely correlated to hydrogen pressure and the diffusion capacity of the membrane. From day 1 to day 17, the removal efficiency did not increase considerably with total nitrogen (TN)

effluent from denitrification tank (outlet 1) was higher than 10 mg/L. From day 18 to day 23 this value was even higher, from 13.1 to 18.1 mg/L although the hydrogen pressure increased to 1.3 bars (Appendix B1 and Figure 4.3). This was due to membrane fouling mainly by salt and the salt cake in crystal form was observed on the membrane. Negative impact of precipitation of mineral solids on performance of hydrogen diffusing membrane was reported by previous studies (Egras and Reuss, 2001 and Rezania et al, 2005).

In the day 24, after the salt cake was physically removed, the removal efficiency increased to 88.4%. However, it was only the short rised since in subsequent days the removal efficiency reduced to less than 70% with TN effluent from denitrification tank (outlet 1) higher than 10 mg/L. On the day 29, it reached to 21 mg/L. Physical cleaning of the membrane surface was found to be insufficient for fully restoring hydrogen diffusion (Rezania et al., 2005). Therefore, chemical cleaning was necessary to recover the diffusion capacity of the membrane. The denitrifcation efficiency after day 30 increased when membrane was chemically cleaned to remove salt and other deposits. Three days after operating with cleaned membrane, the efficiency of denitrification tank always reached to more than 75% (Appendix B1).

In addition, recycling rate contributes to the performance of the system because it plays the role of mixing in denitrification reactor and enhance the release of hydrogen gas from the membrane. Besides, the higher velocity of the recycling rate reduced the biofilm thickness on the membrane. Ergas and Reuss (2001) reported that high recirculation velocity was used to control biofilm thickness. When recycling rate was adjusted from 1 L/min to 2 L/min from day 39, the efficiency increased to more than 90% with nitrate and nitrite concentration lower than 5 mg/L and 0.3 mg/L respectively. Whereas, nitrite in the outlet was generally higher than 0.5 mg/L and sometimes more than 2 mg/L at recycling rate of 1 L/min (Figure 4.3).

Due to the high efficiency at recycling rate of 2 L/min, this recycling rate was applied from the day 39 onward for three runs of the experiment. The result of run 1 from this day is presented in Figure 4.4 and 4.5.

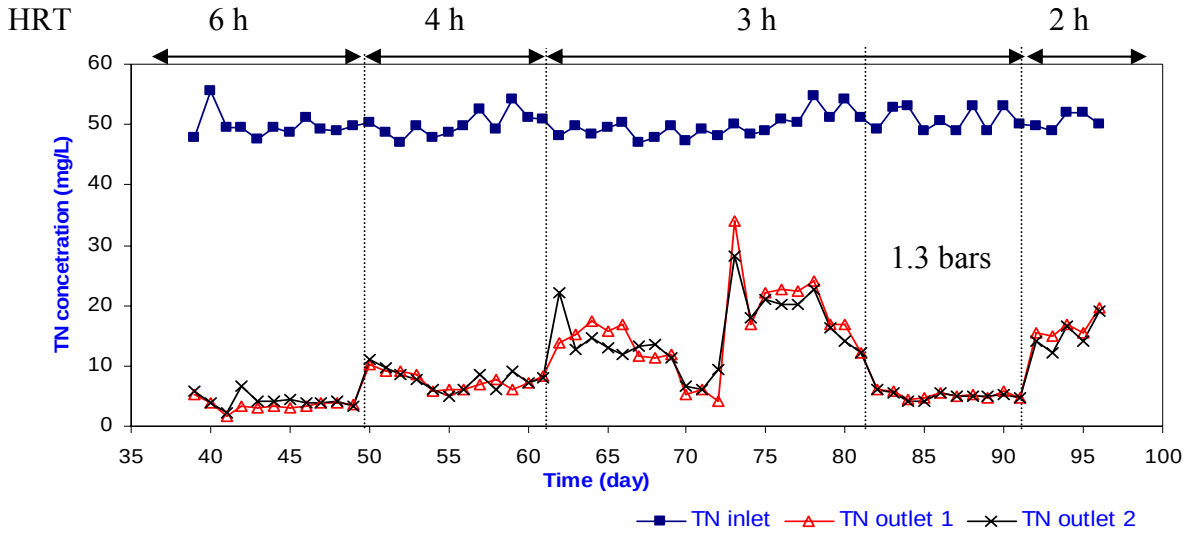

Figure 4.4 Total nitrogen (TN) concentrations in run 1

Based on Figure 4.4, TN from denitrification tank (outlet 1) increased after reduction of HRT which made bacteria under shock loading of nitrogen inlet. This increase was also found in the similar system when nitrogen loading increased (Hung, 2006).

The dependence of denitrification efficiency on the hydrogen diffusion in wastewater was clearly observed. At HRT of 3 h, the increase of hydrogen pressure from 1.2 bars to 1.3 bars on day 70 – 72 enhanced the nitrogen removal. Efficiency of these days increased from 75% to approximate 90% (Figure 4.5). However, due to the membrane leaking and the operation restarted on day 73 at hydrogen pressure of 1 bar which made the removal reduced due to lower hydrogen supplied. When the pressure increased to 1.2 and then 1.3 bars, the removal efficiency recovered and reached to 89.8% from day 82 to day 91 with denitrification rate of 365.7 g/m^3.day (Table 4.1). At HRT of 2 h, the efficiency reduced to 67.4% although the hydrogen pressure was increased to 1.4 bars. Total nitrogen outlet in the effluent was around 16.5 mg/L and nitrite in the effluent was found 1.9 mg/L on the average. This nitrite level was not efficiently removed in the aeration tank to meet standard of 0.6 mg/L for aquaculture fish. Therefore, HRT of 3 h was selected as the optimum in terms of denitrification rate, efficiency and water quality (see more detailed in Appendix B1). Hydrogen pressure at this HRT was 1.3 bars.

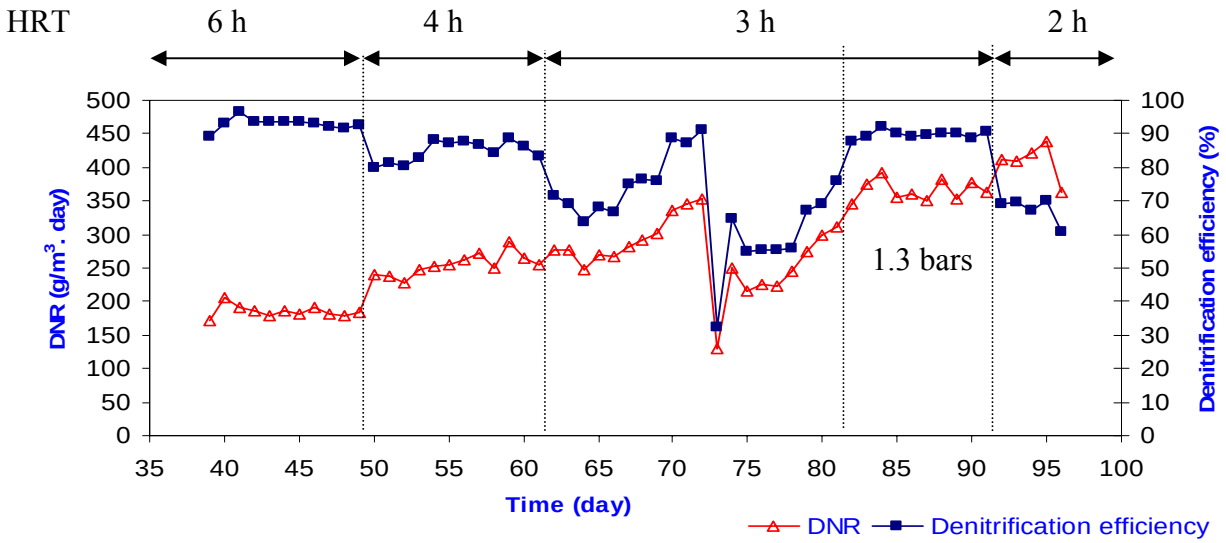

Figure 4.5 Denitrification rate and efficiency of denitrification tank in run 1

The table 4.1 is summary of data analysis for the system at HRT of 2 h and 3 h in comparison with the system treating fresh wastewater at HRT of 3 h (Hung, 2006)

Table 4.1 Denitrification of fresh and saline wastewater at HRT of 2 h and 3 h

HRT	Denitrification rate (g/m^3. day)		Denitrification efficiency (%)		N_2O^--N (mg/L)		Reference
	D tank	Total system	D tank	Total system	Outlet 1	Outlet 2	
3 h	365.7	366.8	89.8	90.0	0.54	0.10	Current study
2 h	327.2	339.3	67.4	69.9	1.90	0.74	Current study
3 h	363.7	365.0	91.4	91.5	0.10	0	Hung (2006)

Based table 4.1, denitrification of fresh wastewater and saline wastewater at HRT of 3 h was similar except that nitrite outlets in treating saline wastewater were slightly higher than those in fresh wastewater case. It implies that salinity of 10 ppt slightly affect the

denitrification rate and efficiency. That was found in accordance with the result of acclimatization process, in which the denitrification of direct acclimatization with saline wastewater of 10 ppt salinity was similar to the denitrification of stepwise acclimatization with fresh wastewater (Section 4.1).

4.2.2 Denitrification of experimental run 2

Run 2 started from day 97 with HRT of 9 h, gradually reduced to 4 h and salinity from 15 ppt was increased to 20 ppt from day 103. Figure 4.6 and 4.7 show the result of run 2.

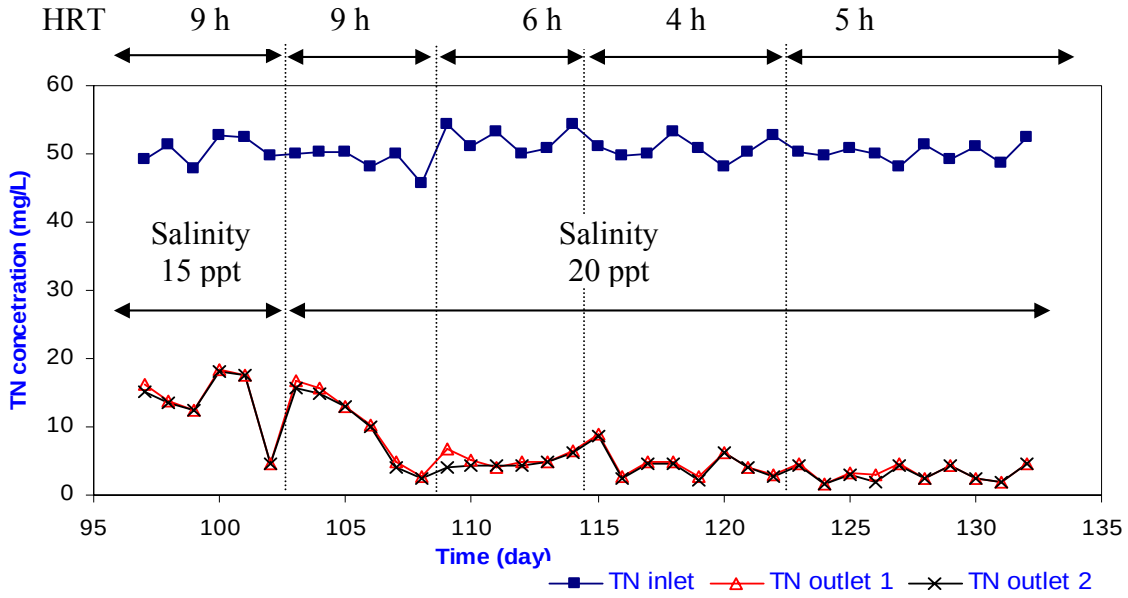

Figure 4.6 Total nitrogen (TN) concentrations in run 2

Figure 4.6 and 4.7 show that bacteria have a good ability to adapt in the increased salinity. When salinity increased from 15 ppt to 20 ppt at HRT of 9 h, removal efficiency reduced but it recovered and reached to more than 90% after 6 days with total nitrogen outlet after denitrification tank (outlet 1) lower than 5 mg/L.

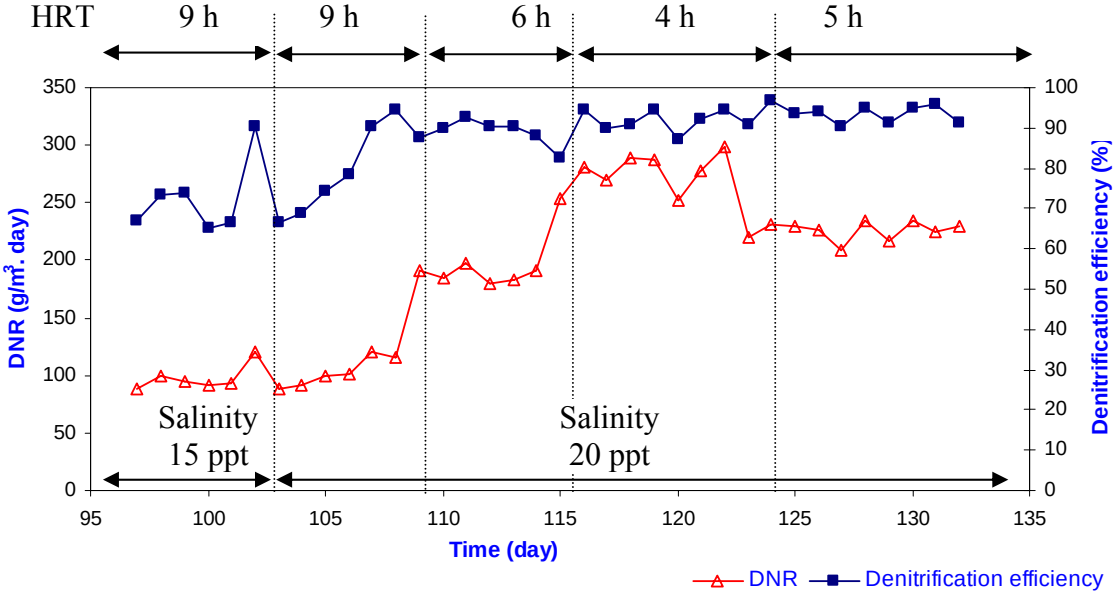

Figure 4.7 Denitrification rate and efficiency of denitrification tank in run 2

Similar to run 1, the denitrification efficiency in run 2 was slightly decreased when HRT was reduced from 9 h to 6 h and from 6 h to 4 h, which increased nitrogen loading for the system (Figure 4.7 and Appendix B2).

Based on Figure 4.7, the denitrification rate increased with the increase of nitrogen loading or reduction of HRT. Nitrogen removal efficiency after 18 days reached to more than 90% at HRT of 4 and 5 h with denitrifiation rate of 276.1 g/m^3 and 225.2 g/m^3.day respectively (Table 4.2). Although the denitrification rate at HRT of 4 h was found higher that at HRT of 5 h, nitrite in the effluent from denitrification tank (outlet 1) and from aeration tank (outlet 2) were 1.4 mg/L and 0.95 mg/L higher than the safety level for fish. Consequently, HRT of 5 h was the optimum for the system in treating saline wastewater with salinity of 20 ppt. Hydrogen pressure at this HRT was 1.2 bars.

Nitrate produced in the denitrifcation process is reduced to the Nitrite and from Nitrite to Nitrogen as following reaction

$$NO_3^- + H_2 \longrightarrow NO_2^- + H_2O \text{ and } NO_2^- + H_2 \longrightarrow N_2 + H_2O$$

The first priority is reduction of nitrate to nitrite and after that from nitrite to nitrogen and nitrite reductase is more sensitive than nitrate reductase (Chang et al., 1999). That explains the high nitrite concentration at HRT of 4 h since nitrite reductase did not have enough HRT to efficiently convert nitrite to nitrogen gas. Grommen et al (2006) also reported that reducing the HRT from 12 to 9 h led to an accumulation of nitrite in the effluent of the denitrifcation reactor, indicating that the reduction of nitrite was the rate-limiting step of denitrification process.

Table 4.2 Denitrification at HRT of 4 h and 5 h in run 2

HRT	Denitrification rate (g/m^3. day)		Denitrification efficiency (%)		N_2O^--N concentration (mg/L)	
	D tank	Total system	Total system	D tank	Outlet 1	Outlet 2
5 h	225.2	226.2	93.5	93.9	0.33	0.19
4 h	276.1	276.2	90.7	90.8	1.40	0.95

4.2.3 Denitrification of experimental run 3

Run 3 started from day 133 with HRT of 12 h and salinity of 25 ppt. Salinity was increased to 30 ppt on day 138 and HRT was reduced gradually from 9 h to 5 h. Figure 4.8 and 4.9 show the result of run 3 (See more detailed in Appendix B3).

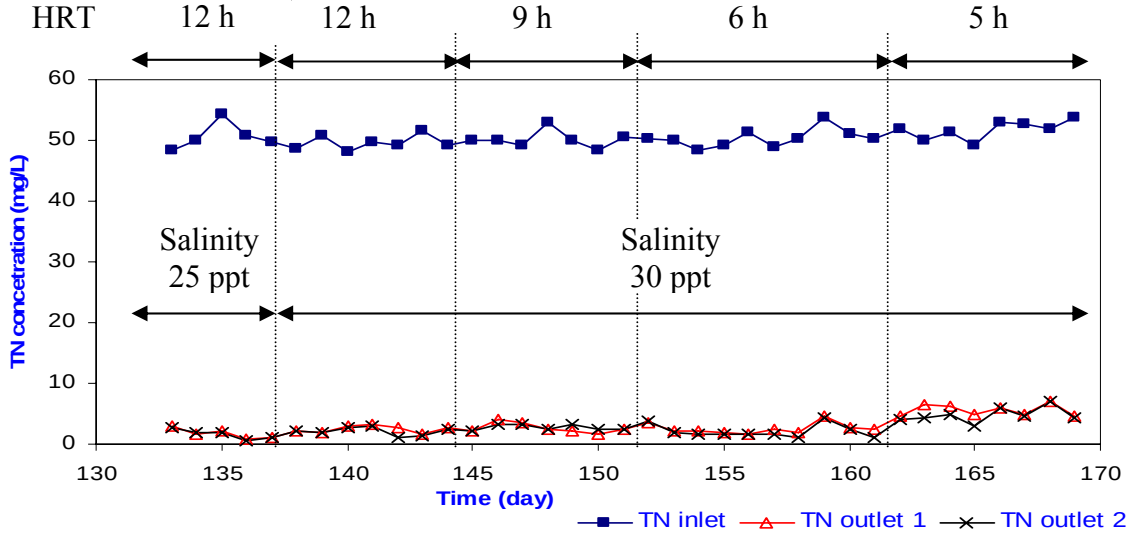

Figure 4.8 Total nitrogen (TN) concentration in run 3

From Figure 4.8, the denitrification was stable from day 133 to day 162 with total nitrogen effluent from denitrification tank (outlet 1) was lower than 5 mg/L. TN concentration increased little when HRT decreased to 5 h. Different from run 1 and run 2, Figure 4.8 and 4.9 show that when salinity or nitrogen loading increased, the phenomenon of lower denitification efficiency was not found at the beginning of HRT of 12 h, 9 h and 6 h. These longer HRTs allowed bacteria to perform denitrification in a new environment with higher salinity or nitrogen loading.

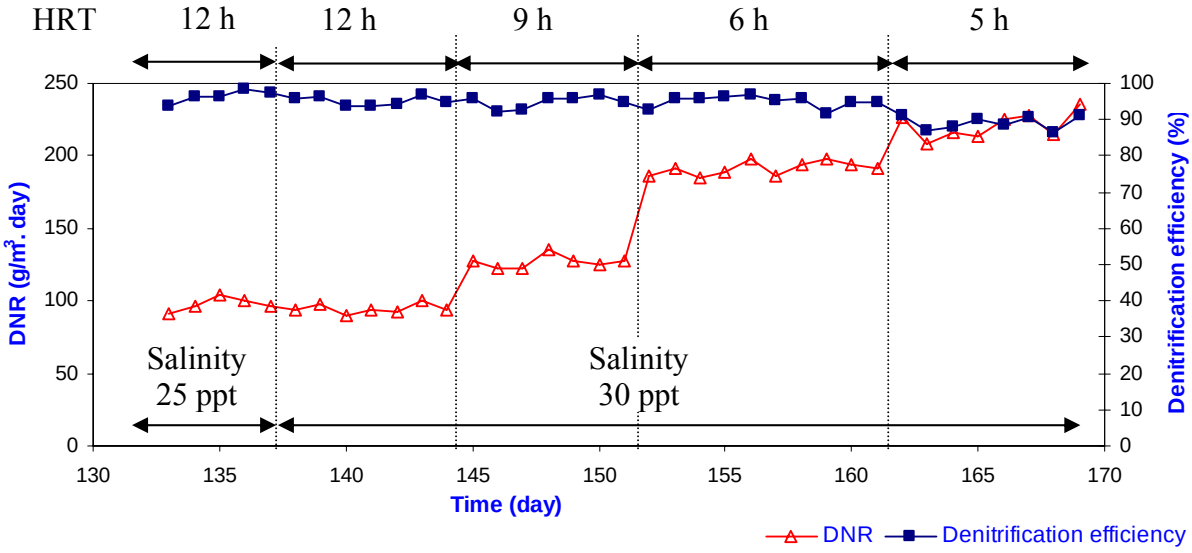

Figure 4.9 Denitrification rate and efficiency of denitrification tank in run 3

Similar to the result of run 2, nitrite concentration in the effluent from denitrification tank (outlet 1) and from aeration tank (outlet 2) in run 3 at HRT of 5 h did not meet safety level of 0.6 mg/L although the denitrification efficiency was high and nitrogen outlet was less than 5 mg/L (Figure 4.8 and 4.9). At HRT of 5 h, the denitrification rate of denitrification tank was 221.1 g/m^3.day higher than 191.3 g/m^3.day at HRT of 6 h. But nitrite outlet 1 and 2 at HRT of 5 h were 1.23 and 1.03 mg/L higher than safety level of 0.6 mg/L (Table 4.3). Therefore, nitrite outlet 1 and outlet 2 decided optimum HRT of 6 h for run 3. Hydrogen pressure at this HRT was 1.1 bars.

Table 4.3 Denitrification at HRT of 5 h and 6 h of run 3

HRT	Denitrification rate (g/m^3. day)		Nitrogen removal efficiency (%)		N_2O^--N concentration (mg/L)	
	D tank	Total system	Total system	D tank	Outlet 1	Outlet 2
6 h	191.3	193.2	95	95.9	0.29	0.21
5 h	221.1	225.2	89.1	90.8	1.23	1.03

4.2.4 Oxydation reduction potential (ORP) in denitrification tank

Fluctuation of ORP was observed throughout the experiment from run 1 to run 3 (Figure 4.10). Positive ORP values from 60 to 72 mV were observed in the first three days of operation after cleaning membrane (day 30 to day 32, Appendix B1). In these days, the removal efficiency was lower than 80%. It was because during the cleaning of membrane sludge did not have enough hydrogen. This resulted in unstable operational condition at the initial stage of operation when membrane was reinstalled. These high values were due to incomplete denitrication and the presence of residual nitrate in the reactor (Mo et al.,

2005). The authors also reported that positive values of ORP were observed in the initial two days of operation in their study.

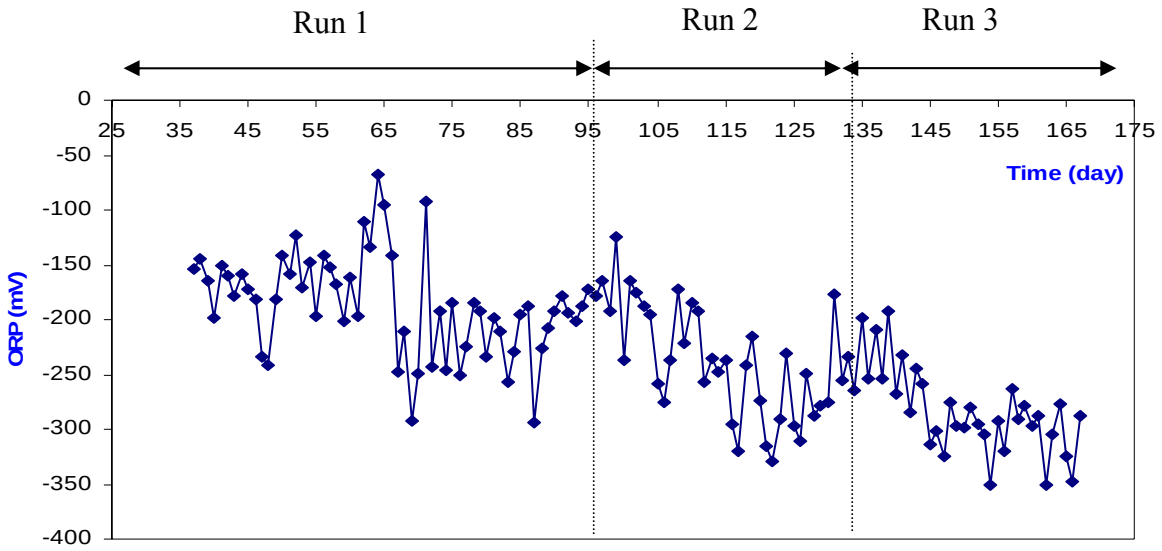

Figure 4.10 ORP in the denitrification tank

Because the system did not run for long time at each HRT, ORP was not found to be a good indicator for the denitrification performance. However, positive ORP can be used as indicator of low efficiency when the system was unstable, for example in the case of first day after cleaning membrane in run 1 as mentioned above. Table 4.4 shows that ORP values in this study was different from the results of Mo et al (2005). According to Fuerhacker et al. (2000), ORP was a very complex parameter and depended on both the wastewater quality and the performance of the sludge.

Table 4.4 ORP in denitrification tank at optimum HRTs

Run	TN loading ($g/m^3.d$)	ORP (mV)	Reference
Run 1	400	-224	
Run 2	240	-286	Current study
Run 3	200	-297	
Stage 1	24	-120	
Stage 2	48	-180	Mo et al. (2005)
Stage 3	96	-230	

4.3 Nitrogen removal of total system

4.3.1 Nitrite accumulation

The nitrite effluents of three runs are presented in Figure 4.11. From this figure, nitrite peaks were observed in beginning days of new HRTs when the nitrogen loading increased suddenly and denitrification efficiency decreased. In run 1, after 9 days operated with recirculating rate of 1 L/min, nitrite effluent from denitrification tank (outlet 1) was stable and lower than 0.5 mg/L when the recirculating increased to 2L/min. Nitrite outlet 1 increased slightly when the HRT reduced to 3 h. However at the HRT of 2 h, nitrite outlet 1 increased to around 1.9 mg/L due to low retention time for the conversion from nitrite into nitrogen gas.

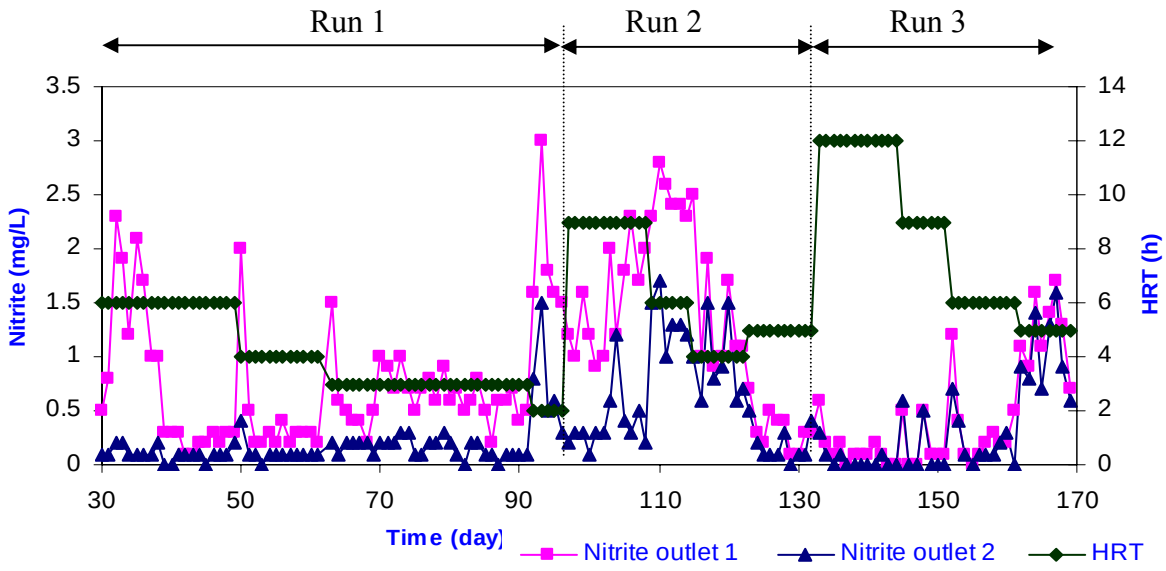

Figure 4.11 Nitrite concentrations in three runs

In run 2, nitrite concentration in outlet 1 was high, often more than 1.5 mg/L at HRT of 9 h and 6 h with hydrogen pressure of 0.9 and 1 bar respectively. It reduced to 1.4 and 0.33 mg/L at HRT of 4 h and 5 h when the hydrogen pressure increased to 1.3 and 1.2 bars (Appendix B2). It implies that hydrogen supplied at HRT of 9 and 6 h was limited for the denitrification. As discussed in section 4.2.2, the accumulation of nitrite might occur if there was not sufficient hydrogen available for the biomass. Nitrite in run 3 was stable at HRTs from 12 to 6 h. However, it increased to around 1.23 mg/L at HRT of 5 h although the hydrogen pressure was increased to 1.3 bars (Appendix B3).

Nitrite concentrations in the effluent from denitrification (outlet 1) and from aeration tank (outlet 2) of systems for treating fresh and saline aquaculture wastewater are presented in Table 4.5 to compare nitrite accumulation between two systems.

Table 4.5 Nitrite concentration in hydrogenotrophic denitrification

Wastewater	HRT	Efficiency of D tank (%)	Nitrite (mg/L)		Nitrite removal efficiency of A tank (%)
			Outlet 1	Outlet 2	
Saline (10 ppt)	3	89.8	0.54	0.10	81
Saline (20 ppt)	5	93.5	0.33	0.19	42
Saline (30 ppt)	6	95.0	0.29	0.21	28
Fresh (Hung, 2006)	3	91.4	0.10	0	100
Fresh (Hung, 2006)	2.5	85.8	1.24	0.04	97

In the system for treating fresh wastewater at HRT of 3 h, nitrite effluent from denitrification tank (outlet 1) was 0.1 mg/L (Hung, 2006). Meanwhile, it remained at higher concentration in the system for saline wastewater although the nitrogen removal efficiency were equal or better (Table 4.5).

Concentration of nitrite in the Table 4.5 showed that nitrite removal efficiency of aeration tank in treating fresh wastewater was higher. In aerobic condition, nitrite was mainly converted to nitrate by *Nitrobacter* under nitrification process as follows:

$$NO_2^- + 0.5\ O_2 \longrightarrow NO_3^-$$

In fresh wastewater case, the nitrite removal after aeration tank achieved was up to 100% that reduced completely the nitrite in the outlet 2 at HRT of 3 h and 97% at HRT of 2.5 h. This efficiency in treating saline wastewater was low, it was 81, 42 and 28% in run 1, 2 and 3 respectively. It implies that the conversion of nitrite into nitrate was inhibited under higher salinity condition. This was also found in the study on organic and nitrogen removal of fish market wastewater at salinity of 5, 10 and 20 ppt with TN of around 62.7 mg/L using SBR. It was reported that salinity showed negligible effect on organics removal, while it affected the nitrification and denitrification efficiency to a larger extent. Increased the salt concentrations decreased the nitrification efficiency (Rene et al, 2007).

4.3.2 Nitrogen removal of total system

Nitrogen was removed simultaneously in both reactors. The denitrification tank under anoxic condition took the main role in nitrogen removal, which converted nitrate and nitrite into nitrogen gas. Little amount of nitrogen was removed in the aeration tank (Table 4.6). The amount of nitrogen lost under aerobic condition was used for the assimilation of bacteria.

Throughout the experiment, total nitrogen from aeration tank (outlet 2) sometime was higher than total nitrogen from denitrification tank (outlet 1). It was due to the biomass degraded in the aeration tank which contributed to the nitrogen of outlet 2. This phenomenon was observed in initial days of operation after cleaned membrane was installed or when there was operational problem in sedimentation tank. In these cases, more biomass washed out from sedimentation tank flowed into the aeration tank where anaerobic bacteria were degraded by endogenous decay under aerobic condition. Table 4.6 summaries results of denitrification at optimum HRTs in comparison with the system treating fresh wastewater.

Table 4.6 Denitrifcation rate and nitrogen removal efficiency at optimum HRTs

Wastewater	Denitrification rate (g/m^3. day)		Denitrification efficiency (%)		Reference
	D tank	Total system	D tank	Total system	
Saline (10 ppt)	365.7	366.8	89.8	90.0	Current study
Saline (20 ppt)	225.2	226.2	93.5	93.9	
Saline (30 ppt)	191.3	193.2	95.0	95.9	
Fresh (HRT = 3h)	363.7	365.0	91.4	91.5	Hung (2006)

The denitrification result of run 1 with salinity of 10 ppt was close to the result obtained from fresh wastewater. However, longer retention times were required for the bacteria to perform denitrification efficiently when the salinity increased to 20 ppt and 30 ppt. Optimum HRT increased to 5 h and 6 h in run 2 and run 3 respectively for the system to achieve high efficiency and meet standards of treated wastewater.

4.4 Removal of organic carbons and involvement of heterotrophic denitrification

Beside nitrogen removal, the aeration tank was designed for organic matter removal. Dissolved organic carbon (DOC) measured in effluents of denitrification and aeration tank is presented in Figure 4.12 and Table 4.7.

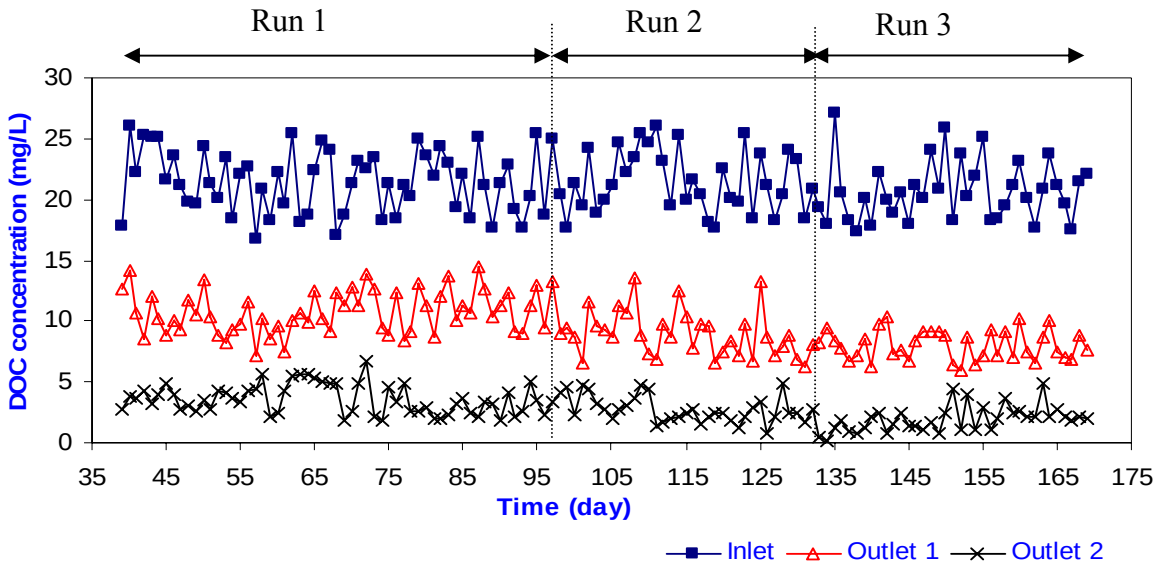

Figure 4.12 Dissolved organic carbon (DOC) in the denitrification process

From Figure 4.12, the removal of dissolved organic carbon (DOC) in denitrification tank and aeration tank fluctuated slightly and it did not depend on HRTs. In the current study, methane in the head-space gas was too low from 0.24 to 1.88%, which ensured that organic matter removed by anaerobic process was low and neglected. Therefore, DOC removal in denitrification tank was due to involvement of heterotrophic denitrifiers utilizing organic matters as electron donor. Organic matters consumed in the denitrification tank was also found in the system for treating fresh aquaculture wastewater. At HRT of 3 h, COD influent of 49 mg/L was reduced to 33 mg/L in the effluent of denitrification (Hung, 2006). In the current study, glucose as DOC in the inlet was consumed in the heterotrophic denitrification reaction as follows (Christensen and Harremoës, 1972)

$$5C_6H_{12}O_6 + 24\ NO_3^- \longrightarrow 30\ CO_2 + 18\ H_2O + 24\ OH^- + 12\ N_2$$

According to the above equation, 1 mg DOC was consumed to remove 0.93 mg NO_3^- - N theoretically. In practice, the amount of DOC used is higher since a part of DOC is consumed in the assimilation of bacteria. Table 4.7 presents concentration of organic matters.

Table 4.7 DOC concentration of in treating fresh and saline wastewaters

Wastewater	DOC of whole run (mg/L)			DOC/COD of optimum HRT (mg/L)			Reference
	Inlet	Outlet 1	Outlet 2	Inlet	Outlet 1	Outlet 2	
Saline (10 ppt)	21.5	10.7	3.5	21.5	11.9	2.8	Current study
Saline (20 ppt)	21.6	8.9	2.4	21.4	8.4	2.5	
Saline (30 ppt)	22.0	8.5	2.2	21.2	7.9	2.3	
Fresh (HRT = 3h)	-	-	-	49*	33*	6.5*	Hung (2006)

** COD concentration*

The organic carbon used in two studies was glucose, which is easily degraded by biological process. In the current study, organic carbon removal in the denitrification tank was 45, 61 and 63% in run 1, 2 and run 3 respectively. The removal efficiency in the case of fresh wastewater was 33% (Extrapolated from Hung, 2006). It can be concluded that in

saline wastewater, heterotrophic denitrification was higher than in fresh wastewater. Nitrate removal mediated by both heterotrophic and autotrophic denitrification was also observed in denitrification system of marine recirculating system for culture of gilthead seabream (Cytryn et al., 2003 cited by van Jijn et al., 2005). In this system, autotrophic denitrifiers used sulfate as electron donor for the denitrication.

On the contrast, the increase of DOC from 12 mg/L to 32 mg/L in the effluent of hydrogenotrophic denitrification was observed by Ergas and Reuss (2001). It was reported that the source of organic carbon added in the effluent was most likely to be soluble microbial products, such as proteins and polysaccharides, leaking from the microbial cell. Lee and Rittmann (2000) also found an increase of DOC from 1.39 to 2.3 mg/L in their work.

Since organic matters as DOC from the denitrification tank (outlet 1) was low, biomass in the aeration tank from 500 – 1000 mg/L was sufficient to remove organic matters.

4.5 Water quality after treatment

Water quality after treatment of three runs is summarized in Table 4.8

Table 4.8 Quality of treated wastewater at different runs

Parameter	Inlet	Outlet			Safety level
		Run 1	Run 2	Run 3	
pH	6.9 - 7.2	7.6-8.1	7.6-7.9	7.6-8.0	6.5-8.3 Blancheton, 2000
DOC (mg/L)	21.2-21.5	2.8	2.5	2.3	-
NO_3^--N (mg/L)	48-54	5.0	2.9	1.9	<50 Lucas and Southgate, 2003
NO_2^--N (mg/L)	0-1.1	0.1	0.2	0.2	<0.6 Lucas and Southgate, 2003
SS (mg/L)	-	0	0	0	<15-200 Jewell and Cummings, 1990
CO_2 (mg/L)	0	0	0	0	<40 Blancheton, 2000

Based on Table 4.8, all parameters in the effluent meet the requirement for aquaculture fish. They are even lower than safety level many times so the system water can be applied in reality to treat the aquaculture wastewater which requires good quality for aqua livings in recirculating systems. The treatment helps to avoid accumulation of nitrogen compounds which can create algae blooming in the aquaculture pond and to reduce wastewater discharge into the environment.

4.6 Kinetic of the denitrification

4.6.1 Biomass yield

The stoichiometric reaction of hydrogenotrophic denitrification is as follows

$$H_2 + 0.35\ NO_3^- + 0.35\ H^+ + 0.052CO_2 \rightarrow 0.17N_2 + 1.1\ H_2O + 0.010\ C_5H_7NO_2$$

From the above equation, the cell yield is 0.24 g cells/g NO_3^--N theoretically. According to calculation in this study (refer to Appendix C), this value was 0.42, 0.44 and 0.48 g cells/g NO_3^--N in run 1, run 2 and run 3 respectively (Table 4.9).

Table 4.9 Biomass yields of three runs

Wastewater	HRT	Biomass yield (g cells/g NO_3^-N)	Reference
Saline (10 ppt)	3	0.42	
Saline (20 ppt)	5	0.44	Current study
Saline (30 ppt)	6	0.48	
Fresh	3	0.34	Hung (2006)

These values are higher than theory but considerably lower than the 0.6 to 0.9 g cells/g NO_3^--N of heterotrophic denitrification (Ergas and Reuss, 2001). As discussed in Section 4.4, proportion of organic matters degraded in denitrification tank was higher than in the system for fresh wastewater. That explains the higher biomass yield since the yield of heterotrophic denitrifiers is higher than that of hydrogenotrophic denitrifiers

4.6.2 Nitrate reduction rate

The nitrate reduction rate to biomass concentration is calculated for run 1, 2 and 3 are 0.15, 0.12, and 0.13 mg N/mg VSS.d (Appendix C2). These are much lower than 0.38 – 0.74 mg N/mg VSS.d at temperature of 25^{o}C and 0.21 – 0.28 mg N/mg VSS.d at 12^{o}C in hydrogenotrophic denitrification (Rezania et al, 2005). The main reason is high biomass in the denitrification tank. However, this system was efficient in treating fresh and saline wastewater at high biomass concentration, biomass in the denitrification tank was not reduced to test the nitrate reduction rate at lower biomass concentration.

4.7 Membrane fouling

4.7.1 Membrane fouling in denitrification tank

Visually, almost all the sludge attached on the membrane in denitrification tank after 2 – 3 days of installing membrane in denitrification tank. To examine the efficiency of membrane in denitrification, the nitrogen removal rate per surface area of membrane is calculated and presented in Table 4.10 (more detailed in Appendix D2).

Table 4.10 shows that except the result from study by Rezania et al. (2005), nitrogen removal rate per membrane areas was comparable to other studies although the pore size of the membrane is bigger than those of other membranes.

Table 4.10 Nitrate removal rate per membrane surface area

Membrane surface area (m^2)	Pore size (μm)	Nitrogen removal rate (g N/m^2. day)	Reference
0.42	0.1	2.05 - 3.92	Current study
0.42	0.1	3.88	Hung (2006)
0.093	0.04	1.76 – 2.87	Mo et al. (2005)
0.093	0.04	8.2 – 14.2	Rezania et al. (2005)
0.37	0.05	2.2	Ergas and Reuss (2001)

Membrane fouling always occurs after a period of operation and it is one of the main problems reducing efficiency of the reactor. As discussed in section 4.2.1, membrane fouling occurred around one and a half month after operating in acclimatization stage and run 1. The salt in crystal form cake and thick biofilm was observed on the membrane (Appendix D1). Membrane resistance (Rm) increased to 8.98195 x $10^{11}m^{-1}$ after 2 months of operation. After membrane was chemically cleaned to recover diffusion capacity,Rm reduced to 5.00752 x $10^{11}m^{-1}$, resulting in the increase of removal rate.

Efficiency of nitrate removal was affected by biofilm layer and precipitation of mineral salt on the membrane. Two main reasons related to the fouling included flow rate of recirculating and CO_2 gas supplied. The recirculating rate in acclimatization was 1 L/min, and phosphate buffer solution was used to control pH instead of CO_2. Therefore, the membrane from sludge acclimatization stage was fouling when it was continuously used in run 1 without cleaning. The recycling rate was increased to 2 L/min later in three runs to enhance mixing condition and release of hydrogen gas from the membrane thus increasing nitrogen removal rate. In addition, CO_2 supplied in three runs to both control pH and scour the membrane which helped reducing membrane fouling.

Sodium hypochloride was found not able to remove salt deposit and the hypochloric acid was necessary to clean the deposit.

The membrane resistance (Rm) in three runs was measured after one month of operation each run is calculated and presented in Table 4.11 (more detailed in Appendix D2)

Table 4.11 Rm of the membrane after one month of operation

Run	Initial Rm (m^{-1})	Rm after one month (m^{-1})	Percentage increase of TMP (%)	Rm after chemical cleaning (m^{-1})	Recovery percentage (%)
1	5.11579 x 10^{11}	6.71729 x 10^{11}	31	5.35489 x 10^{11}	96
2	6.12632 x 10^{11}	7.56992 x 10^{11}	24	6.41955 x 10^{11}	95
3	6.41955 x 10^{11}	7.43459 x 10^{11}	16	6.87519 x 10^{11}	93

During one month of operation in every run, percentage of membrane resistance (Rm) increase was from 16 to 31% lower than that of fresh wastewater case (Table 4.11). During one month, no reduction of nitrogen removal caused by membrane fouling was found. In this study, biofilm attached loosely on the membrane and it was easily physically removed by shaking membrane in water (Appendix D1). The sludge on membrane was observed mainly in cake form, biofilm in gel form was little. In the system of treating fresh wastewater, Rm increased rather faster, from 5.26917 x $10^{11}m^{-1}$ to 7.62857 x $10^{11}m^{-1}$ only after 15 days. Visual observation indicated that the nitrogen removal rate decreased due to thick biofilm attached on the hollow fiber (Hung, 2006). This increase was rather high, 45% of the initial Rm. It can be concluded that the biofilm was the main reason to increase Rm in fresh wastewater case. The effect of salinity on biofilm formation on diffusion membrane is still new subject to researchers.

4.7.2 Membrane fouling in aeration tank

Membrane resistance as the indicator for membrane fouling was recorded during operation of the system. The result is presented in Figure 4.13 and Appendix B.

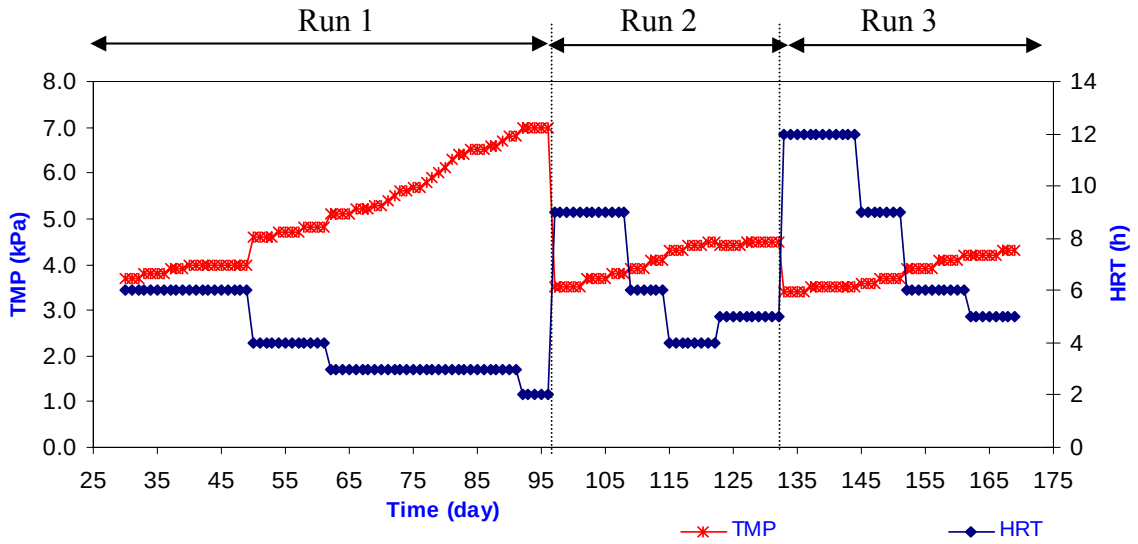

Figure 4.13 Variation of TMP with HRTs of three runs

TPM increased from 3.7 to 7 kPa in run 1, from 3.5 to 4.5 kPa in run 2 and from 3.4 to 4.3 kPa in run 3. The increase in TMP was small since the permeate flux through membrane was low. Maximum flux at HRT of 2 h was 5.36 L/m^2.h, comparably lower than the membrane capacity with 10.4 L/m^2.h. The small increase of TMP was also due to low biomass in the aeration tank (500 – 1000 mg/L). Within HRTs, TMP slightly increased. Meanwhile, in fresh wastewater case, except the increase of 0.1 kPa at HRT of 3 h, TMP was constant in other HRTs (Hung, 2006). That means possibility of membrane fouling in treating wastewater with higher salinity was higher than in fresh wastewater case. Salt precipitation was the main cause of the membrane fouling.

4.8 Estimation of hydrogen utilization and cost analysis

Stoichiometric reaction of hydrogenontrophic denitrification is

$$H_2 + 0.35\ NO_3^- + 0.35\ H^+ + 0.052CO_2 \rightarrow 0.17N_2 + 1.1\ H_2O + 0.010\ C_5H_7NO_2$$

According to the above equation, 1g of NO_3^--N converted to N_2 consumes 0.357 g of hydrogen gas (Ho et al., 2001).

The hydrogen utilization of run 1, 2 and 3 were 0.426, 0.565 and 0.583 g H_2/g N respectively. Compared to the theoretical value of 0.357 g H_2/g N, the hydrogen utilization efficiency were 84, 63 and 61% (Appendix E2). These values are higher than 54% in the study with fresh wastewater (Hung, 2006) and 40% in the study with drinking water (Ergas and Reuss (2001). As discussed in section 4.4, higher proportion of heterotrophic denitrification was found in the current study. That reduced hydrogen required for hydrogenotrophic denitrification. Due to the loss of hydrogen gas released into the head-space and the remaining hydrogen dissolved in the effluent, the actual hydrogen utilization rate was lower than theoretical value.

In order to evaluate the applicability of the system in practice, cost analysis is required to calculate the cost for one nitrate removed and the cost for one m^3 of wastewater. The result was compared with the cost in the case of heterotrophic denitrification with methanol as electron donor. Table 4.12 shows the calculation result.

Table 4.12 Cost analysis for treatment of aquaculture wastewater

Type of denitrification	Cost for 1 g removed NO_3^--N (Baht/g)		Cost for 1 m^3 wastewater (Baht/m^3)		Reference
	Theory	Reality	Theory	Reality	
Heterotrophic (methanol as electron donor)	0.49	0.52 – 0.96*	22.1	23.4 – 44.8*	-
Autotrophic (hydrogen as electron donor) – fresh wastewater	0.44	0.78	19.8	34.8	Hung (2006)
Autotrophic (hydrogen as electron donor) – saline wastewater	0.43	0.52 – 0.71	19.7–20.6	23.6 – 33.7	Current study

** The calculation is based on the methanol consumption: 2.08 – 3.98 g methanol/g NO_3^--N (Boley et al., 2000).*

From Table 4.12, the actual cost of current study is comparably lower than the cost of system for fresh wastewater. It is also lower than the cost in heterotrophic denitrification case using methanol as electron donor. The methanol is considered as low-cost electron donor compared to other organic matters such as acetic acid and ethanol. Moreover, organic remained in the effluent in heterotrophic denitrification needs further treatment to remove it, thus increasing the cost further.

4.9 Results of this study in comparison with previous studies

The result of three runs to treat saline aquaculture wastewater in this study is presented in Table 4.13 in comparison with previous study regarding denitrification rate.

Except results from studies of Ergas and Reuss (2001) and Rezania et al. (2005), the result of run 1 at HRT of 3 h is better than other studies. The higher nitrate inlet of the two above studies was the reason for too high denitrification rates. The nitrate inlet was 150 and 300 mg/L in studies of Ergas and Reuss (2001) and Rezania (2005) respectively. Whereas this value in this study was 50 mg/L. Although at high salinity concentrations, results of run 2 and run 3 at HRT of 5 and 6 h are also comparable to other studies.

Table 4.13 Comparison of current study with previous studies

Reactor type	Influent, NO_3^-- N mg/L	HRT	Denitrification rate, gNO_3^--N/m^3/d	Efficiency %	Reference
Hollow fiber membrane	10	42 min	228.3*	66.6*	Lee and Rittmann. (2000)
	12.5	42 min	370.6*	86.5*	
Hollow fiber membrane	48	12 h	96	100	Mo et al. (2005)
Hollow fiber membrane	145	4.1 h	770	100	Ergas and Reuss (2001)
Hollow fiber membrane	300	22h	800	-	Rezania et al.(2005)
Microporous membrane	40	`-	-	92	Mansell and Schroeder. (2002)
Polyurethane Carrier Reactor	50	353min	200	80-100	Dries et al. (1988)
Trickling filter	20	-	18.5	-	Grommen et al. (2006)
Fixed film	80	-	250	-	Gros et al. (1988)
Fluidized-bed sand reactor	25	4.5 h	130	-	Kurt et al. (1987)
Packed bed of granulated activated carbon	21-27	1 h	250	85	Kiss et al. (2001)
Hollow fiber membrane	50	3 h	363.7	91.4	Hung (2006)
Saturation tank	25	3 h	110	100	Rezania et al. (2007)
Hollow fiber membrane	**50**	**3 h**	**366.8**	**90.0**	**Current study**
		5 h	**226.2**	**93.9**	
		6 h	**193.2**	**95.9**	

Chapter 5

Conclusions and Recommendation

In this study, laboratory scale experiment to remove nitrate in saline aquaculture wastewater by hydrogenotrophic denitrification using hollow fiber membrane bioreactor was conducted. The experiment started with acclimatization of hydrogen-dependent denitrifiers in activated sludge to salinity of 10 ppt. Followed by the acclimatization stage was a series of three experimental runs with salinity concentrations in wastewater of 10, 20, and 30 ppt for run 1, 2 and 3 respectively. During the experiment, optimum hydraulic retention time, denitrification efficiency and organic carbon removal for each run were investigated. Other parameters including biomass production, membrane fouling and quality of treated wastewater were also examined to evaluate the performance of the system and its applicability in practice for recirculating aquaculture ponds. The conclusions drawn from these results are presented as follows:

5.1 Conclusions

1. Direct acclimatization method which acclimatized hydrogenotrophic denitrifiers directly to salinity of 10 ppt was the ideal method compared to the acclimatization with gradual increase of salinity. The use of hollow fiber membrane in hydrogen diffusion for the sludge acclimatization was found more efficient than that of silicon tube.

2. At higher salinity in wastewater, bacteria required longer time to efficiently perform the denitrification, resulting in increase of optimum hydraulic retention time from 3 h in run 1 to 5 h and 6 h in run 2 and run 3. At these HRTs, the nitrogen removal efficiency reached to more than 90% with denitrification rate of total system was 366.8, 226.2 and 193.2 g/m^3.day respectively.

3. In denitrification stage, nitrite reductase which convert nitrite to nitrogen gas was sensitive to the environment. Nitrite concentration in the effluent remained higher if there was not enough hydrogen supplied or retention time in the denitrification tank.

4. Dissolved organic carbon (DOC) was reduced in the denitrification process due to the involvement of hetrotrophic denitrification, resulting in high biomass yield. The yield was from 0.42 to 0.48 g cells/g NO_3^--N higher than theoretical value of 0.24 cells/g NO_3^--N.

5. Water quality of treated wastewater in terms of nitrate, nitrite, DOC, SS was very good at optimum HRTs. All these values were lower than safety level several times, so treated wastewater can be recycled back to the recirculating system in practice.

6. Compared to the theory, hydrogen utilization efficiency was from 61 to 84%. Cost of hydrogen as electron donor for removal of 1 gram nitrate of wastewater was from 0.52 to 0.71 Baht/g and for 1 m^3 was from 23.6 to 33.7 Baht/m^3

7. Recirculation flow rate for good mixing and CO_2 supplied to control pH contributed to the reduction of membrane fouling in denitrification tank. During one month of operation for every experimental run, no salt cake and reduction of

denitrification performance caused by membrane fouling was found. In the aeration tank, due to low biomass concentration and low permeate flux, the membrane in aeration tank was not fouled.

5.1 Recommendation

1. In this study, dissolved hydrogen in wastewater was not measured, in future study it must be determined to find the relation between dissolved hydrogen and efficiency of nitrogen removal at different salinities in wastewater.

2. Study of diversity and abundance of microbial communities is required to investigate the denitrification performance in relation with microbial aspect.

3. The performance of this system in treating aquaculture wastewater with different concentrations of nitrate and organic matter should be studied in future since these concentrations vary depending on types of aquaculture fish.

4. To understanding in detailed about kinetics of hydrogenotrophic denitrification, batch study is necessary to develop kinetic model for cellular growth, nitrite and nitrate utilization.

5. In the future study, the system should be operated at optimum hydraulic retention time (HRT) for a long period to observe oxidation reduction potential (ORP) change which is considered as a good indicator of the denitrification efficiency. Operation of the system with lower biomass concentration in denitrification tank should be conducted to investigate the denitrification efficiency at low biomass.

6. Membrane fouling of hydrogen diffusing membrane at high salinity is still new aspect. Mechanism and model of the fouling should be developed since it is the important parameter in operation.

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Appendix A

Result of sludge acclimatization with hydrogen condition

Table A1 result of sludge acclimatization with hydrogen condition

Date	Inlet		Outlet				DNR ($g/m^3.d$)	Efficiency (%)
	pH	NO_3^--N (mg/L)	pH	NO_3^--N (mg/L)	NO_2^--N (mg/L)	T-N (mg/L)		
Results of stepwise acclimatization (fresh wastewater)								
13/9/06	7.1	25	8.4	10.5	0	10.5	7.3	58
14	7.2	25	7.8	9.2	0	9.2	7.9	63.2
15	7.1	25	8.1	8.5	0	8.5	8.3	66
16	7.1	25	8.4	3.8	0	3.8	10.6	84.8
17	7.2	25	8.7	0.7	0	0.7	12.2	97.2
18	7.1	25	8.7	0.4	0	0.4	12.3	98.4
19	7.0	50	8.7	0.5	0	0.5	24.8	99
20	7.0	50	9.1	0	0	0	25	100
21	7.0	50	9.4	0	0	0	25	100
22	6.7	50	9.4	0	0	0	25	100
23	6.7	50	8.3	0	0	0	25	100
24	6.8	50	7.0	0	0	0	25	100
25	6.8	50	7.0	0	0	0	25	100
Results of direct acclimatization (salinity of 10 ppt)								
13/9/06	7.1	25	8.2	10.9	0	10.9	7.1	56.4
14	7.1	25	7.8	10.1	0	10.1	7.5	59.6
15	7.1	25	7.8	9.0	0	9	8.0	64
16	7.0	25	8.2	5.1	0	5.1	10.0	79.6
17	7.0	25	8.5	3.6	0	3.6	10.7	85.6
18	7.0	25	8.5	1.2	0	1.2	11.9	95.2
19	6.8	50	8.5	1.1	1.2	2.3	23.9	95.6
20	6.8	50	8.6	0.2	0	0.2	24.9	99.6
21	6.5	50	9.1	0	0	0	25	100
22	6.5	50	9.2	0	0	0	25	100
23	6.5	50	7.1	0	0	0	25	100
24	6.7	50	6.8	0	0	0	25	100
25	6.7	50	6.8	0	0	0	25	100

Appendix B

Appendix B1. Result analysis of run 1 with salinity of 10 ppt

Day	Date	pH			NO_2^--N (mg/L)			T-N (mg/L)			Denitrification rate (g/m^3.day)		Denitrification efficiency (%)		DOC concentration (mg/L)			ORP (mV)	TMP (kPa)	H_2 pressure (bar)
		Inlet	Outlet 1	Outlet 2	Inlet	Outlet 1	Outlet 2	Inlet	Outlet 1	Outlet 2	D tank	Total system	D tank	Total system	Inlet	Outlet 1	Outlet 2			
HRT = 6 h, hydrogen diffusion membrane after one month used in sludge acclimatization																				
1	14/10/2006	6.9	7.1	7.1	0.4	2.2	0.3	47.2	17.9	15.8	117.2	125.6	62.1	66.5	9.3	7.1	4.8	-157		0.9
2	15	7.1	7.2	7.2	0.3	3.1	0.4	50.9	16.7	20.6	136.8	121.2	67.2	59.5	12.0	4.8	5.4	-79		0.9
3	16	6.8	7.1	7.2	0.5	3.2	0.3	49.9	15.7	15.8	136.8	136.4	68.5	68.3	9.9	6.6	3.6	-63		0.9
4	17	6.7	7.1	7.3	0.8	1.2	0.5	49.6	14.5	11.7	140.4	151.6	70.8	76.4	6.5	7.2	4.0	-44		0.9
5	18	6.7	7.2	7.5	0.2	3.4	0.7	46.7	9.1	11.5	150.4	140.8	80.5	75.4	14.3	7.9	3.7	-126		1
6	19	6.8	7.3	7.6	0	0.3	0	46.1	12.1	12.6	136.0	134.0	73.8	72.7	13.9	7.8	3.3	-96		1
7	20	6.8	7.2	7.5	0.4	0.8	0.1	48.2	11.6	13.3	146.4	139.6	75.9	72.4	11.5	8.9	3.0	-71		1
8	21	6.7	7.1	7.6	0.5	2.7	0.1	49	12.6	13.6	145.6	141.6	74.3	72.2	22.6	9.1	3.7	-140		1
9	22	6.7	7.2	7.5	0.5	1.3	0.2	48.9	12.3	12.5	146.4	145.6	74.8	74.4	12.1	7.5	5.1	-103		1
10	23	6.8	7.5	7.6	0.3	2.1	0.3	49.9	8.4	10.1	166.0	159.2	83.2	79.8	27.7	11.9	3.5	-113		1.1
11	24	6.7	7.1	7.4	0.5	1.1	0.8	48	12.7	13	141.2	140.0	73.5	72.9	19.3	10.2	3.3	-126		1.1
12	25	6.9	7.2	7.4	0.4	0.8	0.3	46.5	12.1	12.3	137.6	136.8	74.0	73.5	27.1	12.1	3.4	-157		1.1
13	26	6.7	7.1	7.3	0.2	0.6	0.1	49.6	15.1	15.3	138.0	137.2	69.6	69.2	24.2	8.7	3.7	-169		1.1
14	27	6.7	6.9	7.5	0.4	0.6	0.3	47.3	9	9.2	153.2	152.4	81.0	80.5	19.9	10.9	3.4	-121		1.2
15	28	6.8	7.9	7.6	0.3	0.5	0.5	50.5	9	9.2	166.0	165.2	82.2	81.8	20.1	9.8	4.2	-97		1.2
16	29	6.7	7.1	7.2	0.7	2.2	0.6	45.3	9.5	11.2	143.2	136.4	79.0	75.3	23.3	10.8	3.7	-90		1.2
17	30	6.5	7.0	7.2	0.8	1	0	47.5	9.2	12	153.2	142.0	80.6	74.7	22.8	11.2	3.5	-108		1.2
18	31	6.7	7.2	7.2	1.4	0.6	0.1	45.8	14.2	13.3	126.4	130.0	69.0	71.0	20.1	7.3	4.2	-218		1.2
19	01/11/2006	6.5	7.1	7.4	1.1	0.6	0.1	48.7	16.3	17.4	129.6	125.2	66.5	64.3	25.8	7.8	3.7	-158		1.2
20	02	6.7	6.9	7.2	1.6	0.6	0.2	46.8	18.1	21.4	114.8	101.6	61.3	54.3	25.3	9.9	4.2	-107		1.2
21	03	6.7	6.9	7.1	1.2	3.6	0.5	47	15.1	18.2	127.6	115.2	67.9	61.3	16.5	5.7	3.6	-115		1.3
22	04	6.8	7.2	7.2	0.4	0.5	0.1	53	14.1	16.2	155.6	147.2	73.4	69.4	17.9	13.2	3.1	-229		1.3

Day	Date	pH			NO_2^--N (mg/L)			T-N (mg/L)			Denitrification rate (g/m^3.day)		Denitrification efficiency (%)		DOC concentration (mg/L)			ORP (mV)	TMP (kPa)	H_2 pressure (bar)
		Inlet	Outlet 1	Outlet 2	Inlet	Outlet 1	Outlet 2	Inlet	Outlet 1	Outlet 2	D tank	Total system	D tank	Total system	Inlet	Outlet 1	Outlet 2			
23	05/11/2006	6.8	7.3	7.4	0.6	0.2	0	52.3	13.1	16.7	156.8	142.4	75.0	68.1	20.3	9.9	2.8	-260		1.3
24	06	7.0	7.9	8.0	3.4	0.1	0.3	50.7	5.9	9.1	179.2	166.4	88.4	82.1	16.7	20.6	1.5	-212		1.3
25	07	6.9	7.3	7.8	2.9	0.8	0.1	49.8	15.6	15.7	136.8	136.4	68.7	68.5	18.4	13.0	6.6	-123		1.3
26	08	6.9	7.5	7.9	0.9	0.9	0.1	48.8	14.7	15.3	136.4	134.0	69.9	68.6	23.5	5.6	2.6	-242		1.3
27	09	6.9	7.4	7.8	1.3	0.1	0.3	51.9	11.5	17.6	161.6	137.2	77.8	66.1	17.2	16.8	7.8	-239		1.3
28	10	7.2	7.7	7.8	1.1	0.3	0.1	54.2	20.8	14.6	133.6	158.4	61.6	73.1	21.3	7.1	2.6	-89		1.3
29	11	7.1	7.7	7.7	1	0.4	0.6	47.5	21	19.7	106.0	111.2	55.8	58.5	19.3	6.4	6.0	-69		1.3
HRT = 6 h, membrane was already chemically cleaned																				
30	15/11/2006	7.0	7.3	7.4	0.4	0.5	0.1	47.4	32.8	43.9	58.4	14.0	30.8	7.4	23.7	10.2	4.4	72	3.7	0.9
31	16	7.1	7.5	7.5	0.4	0.8	0.1	48.1	9.8	12.6	153.2	142.0	79.6	73.8	19.5	10.8	3.6	60	3.7	0.9
32	17	7.2	7.6	7.8	1.1	2.3	0.2	50	13.4	16.2	146.4	135.2	73.2	67.6	21.9	8.3	2.5	70	3.7	0.9
33	18	6.9	7.5	7.8	1	1.9	0.2	50.1	10	10	160.4	160.4	80.0	80.0	23.2	10.1	3.0	-234	3.8	0.9
34	19	7.1	7.6	7.8	1.4	1.2	0.1	48.4	7.7	10.8	162.8	150.4	84.1	77.7	18.7	9.2	2.7	-250	3.8	0.9
35	20	7.0	7.4	7.7	0.9	2.1	0.1	49.4	11.4	11.5	152.0	151.6	76.9	76.7	25.1	11.4	3.2	-90	3.8	0.9
36	21	6.9	7.1	7.5	1.6	1.7	0.1	51.9	11.7	11.8	160.8	160.4	77.5	77.3	22.6	12.3	2.1	-117	3.8	0.9
37	22	6.9	7.3	7.7	1.2	1	0.1	49.8	11.4	10.6	153.6	156.8	77.1	78.7	19.5	8.2	4.2	-121	3.9	0.9
38	23	7.1	7.5	8.1	1	1	0.2	45.9	8.1	9.9	151.2	144.0	82.4	78.4	24.0	11.0	3.5	-259	3.9	0.9
39	24	6.9	7.5	7.8	0.2	0.3	0	47.8	5.2	5.9	170.4	167.6	89.1	87.7	17.8	12.7	2.7	-154	3.9	0.9
40	25	6.9	7.8	8.1	1	0.3	0	55.5	3.9	4	206.4	206.0	93.0	92.8	26.1	14.2	3.8	-145	4.0	0.9
41	26	6.9	7.7	8.1	1.2	0.3	0.1	49.6	1.6	2.1	192.0	190.0	96.8	95.8	22.3	10.6	3.6	-165	4.0	0.9
42	27	6.9	7.4	7.7	0.3	0.1	0.1	49.6	3.2	6.6	185.6	172.0	93.5	86.7	25.3	8.6	4.2	-199	4.0	0.9
43	28	7.3	7.8	8.1	0.3	0.1	0.1	47.6	3	4.1	178.4	174.0	93.7	91.4	25.2	12.1	3.2	-150	4.0	0.9
44	29	7.2	7.7	8.0	0.2	0.2	0.1	49.5	3.2	4.1	185.2	181.6	93.5	91.7	25.1	10.2	3.9	-160	4.0	0.9
45	30	7.0	7.7	8.1	0.1	0.2	0	48.6	3	4.4	182.4	176.8	93.8	90.9	21.6	8.9	4.8	-178	4.0	0.9
46	01/12/2006	7.1	7.6	7.9	0.2	0.3	0.1	51.1	3.4	3.9	190.8	188.8	93.3	92.4	23.6	10.1	3.9	-159	4.0	0.9
47	02	7.0	7.6	8.0	0.2	0.2	0.1	49.1	3.9	4	180.8	180.4	92.1	91.9	21.1	9.3	2.8	-173	4.0	0.9
48	03	7.0	7.6	7.9	0.3	0.3	0.1	49	4	4.1	180.0	179.6	91.8	91.6	19.8	11.7	3.1	-181	4.0	0.9
49	04	7.0	7.5	7.7	0.2	0.3	0.2	49.8	3.6	3.3	184.8	186.0	92.8	93.4	19.7	10.5	2.6	-234	4.0	0.9

Day	Date	pH			NO_2^--N (mg/L)			T-N (mg/L)			Denitrification rate (g/m^3.day)		Denitrification efficiency (%)		DOC concentration (mg/L)			ORP (mV)	TMP (kPa)	H_2 pressure (bar)
		Inlet	Outlet 1	Outlet 2	Inlet	Outlet 1	Outlet 2	Inlet	Outlet 1	Outlet 2	D tank	Total system	D tank	Total system	Inlet	Outlet 1	Outlet 2			
HRT = 4 h																				
50	05/12/06	7	7.0	7.4	0.2	2	0.4	50.3	10.1	11.1	241.2	235.2	79.9	77.9	24.4	13.4	3.5	-242	4.6	1
51	06	6.9	7.2	7.8	0.6	0.5	0.1	48.8	9.2	9.6	237.6	235.2	81.1	80.3	21.3	10.4	2.8	-181	4.6	1
52	07	6.9	7.2	7.7	0.8	0.2	0.1	46.9	9.1	8.5	226.8	230.4	80.6	81.9	20.1	8.9	4.3	-142	4.6	1
53	08	6.9	7.3	7.9	0.7	0.2	0	49.8	8.6	7.7	247.2	252.6	82.7	84.5	23.5	8.2	4.1	-159	4.6	1
54	09	6.8	7.0	7.6	0.9	0.3	0.1	47.8	5.7	6	252.6	250.8	88.1	87.4	18.5	9.3	3.7	-123	4.7	1
55	10	7.0	7.1	7.5	0.7	0.2	0.1	48.7	6.1	5.1	255.6	261.6	87.5	89.5	22.1	9.8	3.4	-171	4.7	1
56	11	6.9	7.3	7.6	0.4	0.4	0.1	49.8	6.1	6.1	262.2	262.2	87.8	87.8	22.7	11.5	4.2	-148	4.7	1
57	12	7.1	7.4	7.7	0.7	0.2	0.1	52.5	7	8.5	273.0	264.0	86.7	83.8	16.8	7.1	4.4	-197	4.7	1
58	13	7.1	7.4	7.6	0.4	0.3	0.1	49.2	7.7	6.1	249.0	258.6	84.3	87.6	20.8	10.2	5.7	-141	4.8	1
59	14	7.0	7.4	7.6	0.5	0.3	0.1	54.2	6.2	9	288.0	271.2	88.6	83.4	18.3	8.6	2.1	-153	4.8	1
60	15	7.0	7.4	7.6	0.5	0.3	0.1	51.1	7.1	7.3	264.0	262.8	86.1	85.7	22.2	9.6	2.5	-168	4.8	1
61	16	7.0	7.3	7.5	0.4	0.2	0.1	50.9	8.4	8	255.0	257.4	83.5	84.3	19.6	7.5	4.2	-201	4.8	1
HRT = 3 h																				
62	19/12/06	7.1	7.3	7.5	0.5	6.2	2.1	48.2	13.7	22.1	276.0	208.8	71.6	54.1	25.5	10.0	5.5	-162	5.1	1.1
63	20	7.0	7.4	7.7	0.4	1.5	0.2	49.8	15.3	12.8	276.0	296.0	69.3	74.3	18.1	10.6	5.6	-197	5.1	1.1
64	21	7.0	7.3	7.4	0.1	0.6	0.1	48.4	17.5	14.7	247.2	269.6	63.8	69.6	18.7	9.9	5.7	-110	5.1	1.1
65	22	7.0	7.2	7.4	0.1	0.5	0.2	49.5	15.8	13	269.6	292.0	68.1	73.7	22.4	12.5	5.4	-134	5.1	1.1
66	23	7.0	7.3	7.4	0.3	0.4	0.2	50.3	16.8	11.8	268.0	308.0	66.6	76.5	24.8	10.2	5.1	-67	5.2	1.2
67	24	7.1	7.2	7.4	0.2	0.4	0.2	46.9	11.7	13.3	281.6	268.8	75.1	71.6	24.1	9.2	4.8	-96	5.2	1.2
68	25	7.1	7.2	7.5	0.5	0.2	0.2	47.9	11.3	13.6	292.8	274.4	76.4	71.6	17.0	12.4	4.8	-142	5.2	1.2
69	26	7.1	7.3	7.4	0.6	0.5	0.1	49.8	12	11.3	302.4	308.0	75.9	77.3	18.8	11.3	1.8	-247	5.3	1.2
70	27	7.1	7.2	7.4	0.5	1	0.2	47.4	5.3	6.7	336.8	325.6	88.8	85.9	21.3	12.8	2.6	-210	5.3	1.3
71	28	7.1	7.3	7.5	0.9	0.9	0.2	49.3	6.2	6.1	344.8	345.6	87.4	87.6	23.1	11.3	4.8	-292	5.4	1.3
72	29	7.0	7.2	7.4	0.5	0.7	0.2	48.2	4.2	9.3	352.0	311.2	91.3	80.7	22.6	13.9	6.7	-249	5.5	1.3
73	31	7.0	7.2	7.8	0.6	1	0.3	50.1	33.9	28.2	129.6	175.2	32.3	43.7	23.4	12.6	2.1	-92	5.6	1
74	01/01/2007	7.1	7.3	7.4	0.8	0.7	0.3	48.3	17	18.1	250.4	241.6	64.8	62.5	18.3	9.4	1.9	-243	5.6	1
75	02	7.0	7.0	7.3	0.5	0.5	0.1	48.9	22	20.9	215.2	224.0	55.0	57.3	21.3	8.8	4.6	-193	5.7	1

Day	Date	pH			NO_2^--N (mg/L)			T-N (mg/L)			Denitrification rate (g/m^3.day)		Denitrification efficiency (%)		DOC concentration (mg/L)			ORP (mV)	TMP (kPa)	H_2 pressure (bar)
		Inlet	Outlet 1	Outlet 2	Inlet	Outlet 1	Outlet 2	Inlet	Outlet 1	Outlet 2	D tank	Total system	D tank	Total system	Inlet	Outlet 1	Outlet 2			
76	03/01/2007	6.9	7.3	7.6	0.2	0.7	0.1	51	22.8	20.1	225.6	247.2	55.3	60.6	18.5	12.4	3.4	-246	5.7	1.1
77	04	7.0	7.1	7.3	0.7	0.8	0.2	50.2	22.3	20.1	223.2	240.8	55.6	60.0	21.1	8.4	4.9	-185	5.8	1.1
78	05	7.1	7.3	7.4	1.1	0.6	0.2	54.8	24.1	22.8	245.6	256.0	56.0	58.4	20.2	9.2	2.6	-251	5.9	1.1
79	06	6.9	7.2	7.9	1	0.9	0.3	51.1	16.9	16.2	273.6	279.2	66.9	68.3	24.9	13.1	2.6	-224	6.0	1.2
80	07	7.0	7.3	7.6	1.2	0.6	0.2	54.1	16.8	14.2	298.4	319.2	68.9	73.8	23.6	11.2	2.9	-184	6.1	1.2
81	08	7.2	7.3	7.6	0.9	0.7	0.1	51.2	12.3	12.1	311.2	312.8	76.0	76.4	22.0	8.7	2.0	-192	6.3	1.2
HRT = 3 h, hydrogen pressure = 1.3 bars (optimum)																				
82	09/01/07	7.1	7.4	7.7	1.1	0.5	0	49.2	6.0	6.2	345.6	344.0	87.8	87.4	24.4	12.1	2.0	-234	6.4	1.3
83	10	7.1	7.4	7.6	0.8	0.6	0.2	52.7	5.8	5.5	375.2	377.6	89.0	89.6	23.0	13.7	2.3	-199	6.4	1.3
84	11	7.2	7.5	7.7	0.8	0.8	0.2	53.2	4.3	4.2	391.2	392.0	91.9	92.1	19.3	10.0	3.2	-210	6.5	1.3
85	12	7.1	7.3	7.7	0.9	0.5	0.1	49.0	4.7	4.2	354.4	358.4	90.4	91.4	22.1	11.2	3.7	-257	6.5	1.3
86	13	7.1	7.6	7.6	0.7	0.2	0.1	50.6	5.5	5.6	360.8	360.0	89.1	88.9	18.5	10.7	2.5	-229	6.5	1.3
87	14	7.0	7.7	7.8	0.9	0.6	0	49.0	5.1	5.0	351.2	352.0	89.6	89.8	25.2	14.4	2.1	-196	6.6	1.3
88	15	7.2	7.4	7.7	0.6	0.6	0.1	53.2	5.3	5.0	383.2	385.6	90.0	90.6	21.1	12.7	3.4	-188	6.6	1.3
89	16	7.1	7.4	7.8	0.6	0.7	0.1	48.9	4.7	4.9	353.6	352.0	90.4	90.0	17.6	10.3	3.2	-294	6.7	1.3
90	17	7.1	7.5	8.1	0.4	0.4	0.1	53.2	5.9	5.3	378.4	383.2	88.9	90.0	21.3	11.2	1.8	-226	6.8	1.3
91	18	7.2	7.4	7.8	0.4	0.5	0.1	50.1	4.7	4.7	363.2	363.2	90.6	90.6	22.9	12.3	4.1	-207	6.8	1.3
Average					**0.72**	**0.54**	**0.1**	**50.9**	**5.2**	**5.1**	**365.7**	**366.8**	**89.8**	**90.0**	**21.5**	**11.9**	**2.8**	**-224**		
HRT = 2 h																				
92	19/01/07	7.1	7.4	7.6	0.5	1.6	0.8	49.7	15.4	14.2	411.6	426.0	69.0	71.4	19.2	9.2	2.1	-193	7.0	1.4
93	20	7.0	7.2	7.5	0.4	3.0	1.5	49	14.8	12.2	410.4	441.6	69.8	75.1	17.6	9.0	2.6	-178	7.0	1.4
94	21	7.1	7.3	7.4	0.6	1.8	0.5	52.1	17	16.5	421.2	427.2	67.4	68.3	20.2	11.2	5.0	-194	7.0	1.4
95	22	7.0	7.3	7.6	0.7	1.6	0.6	51.9	15.4	14.1	438.0	453.6	70.3	72.8	25.4	13.0	3.5	-202	7.0	1.4
96	23	7.1	7.4	7.7	0.3	1.5	0.3	50	19.7	19	363.6	372.0	60.6	62.0	18.7	9.5	2.3	-188	7.0	1.4
Average					**0.5**	**1.9**	**0.74**	**50.5**	**16.5**	**15.2**	**409.0**	**424.1**	**67.4**	**69.92**	**20.2**	**10.4**	**3.1**	**-191**		

Appendix B2. Result analysis of run 2 with salinity of 20 ppt

Day	Date	pH			NO_2^--N (mg/L)			T-N (mg/L)			Denitrification rate ($g/m^3.day$)		Denitrification efficiency (%)		DOC concentration (mg/L)			ORP (mV)	TMP (kPa)	H_2 pressure (bar)
		Inlet	Outlet 1	Outlet 2	Inlet	Outlet 1	Outlet 2	Inlet	Outlet 1	Outlet 2	D tank	Total system	D tank	Total system	Inlet	Outlet 1	Outlet 2			
HRT = 9 h, salinity = 15 ppt																				
97	24/01/2007	7.1	7.2	7.4	0.6	1.2	0.2	49.3	16.3	15.1	88.0	91.2	66.9	69.4	24.9	13.2	3.3	-172	3.5	0.9
98	25	7.0	7.4	7.6	0.7	1	0.3	51.3	13.7	13.5	100.3	100.8	73.3	73.7	20.4	9.0	4.1	-178	3.5	0.9
99	26	7.0	7.4	7.5	0.6	1.6	0.3	47.8	12.4	12.4	94.4	94.4	74.1	74.1	17.7	9.4	4.5	-164	3.5	0.9
100	27	7.2	7.4	7.4	0.4	1.2	0.1	52.7	18.3	18	91.7	92.5	65.3	65.8	21.3	8.7	2.3	-193	3.5	0.9
101	28	7.1	7.3	7.6	1	1	0	52.5	17.7	17.6	92.8	93.1	66.3	66.5	19.5	6.5	4.7	-124	3.5	0.9
102	29	7.0	7.2	7.5	0.2	1	0.3	49.8	4.7	4.6	120.3	120.5	90.6	90.8	24.2	11.6	4.4	-237	3.7	0.9
HRT = 9 h, salinity = 20 ppt																				
103	30/01/2007	7.0	7.3	7.6	0.1	2	0.6	50.1	16.7	15.8	89.1	91.5	66.7	68.5	18.9	9.6	3.2	-165	3.7	1
104	31	7.0	7.3	7.4	0.4	1.2	1.2	50.2	15.7	14.9	92.0	94.1	68.7	70.3	19.9	9.3	2.7	-176	3.7	1
105	01/02/2007	7.0	7.6	7.5	0.1	1.8	0.4	50.2	13	12.9	99.2	99.5	74.1	74.3	21.1	8.7	2.0	-187	3.7	1
106	02	7.0	7.3	7.6	0.4	2.3	0.3	48.2	10.3	9.9	101.1	102.1	78.6	79.5	24.6	11.3	2.8	-195	3.8	1
107	03	7.1	7.4	7.6	0.2	1.7	0.5	50.1	4.9	4	120.5	122.9	90.2	92.0	22.2	10.7	3.1	-259	3.8	1
108	04	7.1	7.4	7.8	0.1	2	0.2	45.8	2.6	2.5	115.2	115.5	94.3	94.5	23.5	13.5	3.6	-276	3.8	1
HRT = 6 h																				
109	05/02/2007	7.0	7.4	7.6	0.3	2.3	1.5	54.4	6.7	4.1	190.8	201.2	87.7	92.5	25.4	8.8	4.7	-237	3.9	1.1
110	06	7.0	7.4	7.6	0.1	2.8	1.7	51.2	5.2	4.3	184.0	187.6	89.8	91.6	24.6	7.3	4.4	-172	3.9	1.1
111	07	7.0	7.3	7.9	0.1	2.6	1	53.3	4	4.2	197.2	196.4	92.5	92.1	26.0	6.8	1.3	-222	3.9	1.1
112	08	6.9	7.6	7.8	0.1	2.4	1.3	49.9	4.9	4.4	180.0	182.0	90.2	91.2	23.1	9.7	1.7	-184	4.1	1.1
113	09	6.9	7.3	7.6	0.3	2.4	1.3	50.9	5	4.9	183.6	184.0	90.2	90.4	19.5	8.7	2.0	-193	4.1	1.1
114	10	7.0	7.3	7.8	0.1	2.3	1.2	54.3	6.5	6.2	191.2	192.4	88.0	88.6	25.3	12.5	2.2	-257	4.1	1.1
HRT = 4 h																				
115	11/02/2007	7.1	7.3	7.9	0.1	2.5	1	51.1	8.9	8.6	253.2	255.0	82.6	83.2	19.9	10.4	2.5	-235	4.3	1.2
116	12	7.0	7.4	7.8	0.2	1	0.6	49.6	2.8	2.5	280.8	282.6	94.4	95.0	21.7	7.8	2.8	-248	4.3	1.2
117	13	7.0	7.3	7.8	0.1	1.9	1.5	50	5	4.6	270.0	272.4	90.0	90.8	20.4	9.8	1.5	-237	4.3	1.3
118	14	7.0	7.4	7.7	0.1	0.9	0.8	53.2	5	4.5	289.2	292.2	90.6	91.5	18.1	9.6	2.2	-296	4.4	1.3

Day	Date	pH			NO_2^--N (mg/L)			T-N (mg/L)			Denitrification rate (g/m^3.day)		Denitrification efficiency (%)		DOC concentration (mg/L)			ORP (mV)	TMP (kPa)	H_2 pressure (bar)
		Inlet	Outlet 1	Outlet 2	Inlet	Outlet 1	Outlet 2	Inlet	Outlet 1	Outlet 2	D tank	Total system	D tank	Total system	Inlet	Outlet 1	Outlet 2			
119	15/02/2007	7.1	7.3	7.7	0.1	1	0.9	50.7	2.8	2.2	287.4	291.0	94.5	95.7	17.6	6.5	2.5	-320	4.4	1.3
120	16	7.0	7.3	7.9	0.1	1.7	1.5	48.1	6.1	6.2	252.0	251.4	87.3	87.1	22.5	7.4	2.4	-241	4.4	1.3
121	17	7.0	7.3	7.9	0.1	1.1	0.6	50.2	4	4.1	277.2	276.6	92.0	91.8	20.1	8.3	1.8	-215	4.5	1.3
122	18	7.1	7.5	7.9	0.1	1.1	0.7	52.7	2.9	4.6	298.8	288.6	94.5	91.3	19.8	7.1	1.2	-274	4.5	1.3
Average					**0.11**	**1.4**	**0.95**	**50.7**	**4.7**	**4.7**	**276.1**	**276.2**	**90.7**	**90.8**	**20.0**	**8.4**	**2.1**	**-258**		
HRT = 5 h, hydrogen pressure 1.2 bars (optimum)																				
123	19/02/2007	7.0	7.5	7.8	0.1	0.7	0.5	50.2	4.5	4.2	219.4	220.8	91.0	91.6	25.4	9.8	2.2	-315	4.4	1.2
124	20	7.1	7.4	7.8	0.1	0.3	0.2	49.7	1.5	1.5	231.4	231.4	97.0	97.0	18.4	6.7	2.9	-329	4.4	1.2
125	21	7.0	7.4	7.8	0.1	0.2	0.1	50.9	3.2	3.1	229.0	229.4	93.7	93.9	23.8	13.2	3.3	-290	4.4	1.2
126	22	7.0	7.4	7.7	0.1	0.5	0.1	50.1	3	1.9	226.1	231.4	94.0	96.2	21.1	8.7	0.8	-231	4.4	1.2
127	23	7.0	7.5	7.9	0	0.4	0.1	48.1	4.7	4.3	208.3	210.2	90.2	91.1	18.3	7.2	2.1	-297	4.5	1.2
128	24	7.0	7.4	7.8	0.1	0.4	0.3	51.4	2.5	2.4	234.7	235.2	95.1	95.3	20.4	7.9	4.9	-311	4.5	1.2
129	25	7.1	7.4	7.6	0.1	0.1	0	49.2	4.2	4.3	216.0	215.5	91.5	91.3	24.1	8.9	2.5	-249	4.5	1.2
130	26	7.1	7.4	7.9	0.2	0.1	0.1	51.2	2.5	2.4	233.8	234.2	95.1	95.3	23.3	6.9	2.4	-287	4.5	1.2
131	27	7.0	7.4	7.9	0.1	0.3	0.1	48.7	2	1.8	224.2	225.1	95.9	96.3	18.5	6.3	1.6	-279	4.5	1.2
132	28	7.0	7.3	7.7	0.1	0.3	0.4	52.4	4.6	4.7	229.4	229.0	91.2	91.0	20.9	8.1	2.7	-275	4.5	1.2
Average					**0.1**	**0.33**	**0.19**	**50.2**	**3.3**	**3.1**	**225.2**	**226.2**	**93.5**	**93.9**	**21.4**	**8.4**	**2.5**	**-286**		

Appendix B3. Result analysis of run 3 with salinity of 30 ppt

Day	Date	pH			NO_2^--N (mg/L)			T-N (mg/L)			Denitrification rate (g/m^3.day)		Denitrification efficiency (%)		DOC concentration (mg/L)			ORP (mV)	TMP (kPa)	H_2 pressure (bar)
		Inlet	Outlet 1	Outlet 2	Inlet	Outlet 1	Outlet 2	Inlet	Outlet 1	Outlet 2	D tank	Total system	D tank	Total system	Inlet	Outlet 1	Outlet 2			
HRT = 12 h, salinity = 25 ppt																				
133	02/03/07	7.0	7.4	7.9	0.1	0.6	0.3	48.3	3	2.8	90.6	91.0	93.8	94.2	19.3	8.2	0.4	-177	3.4	0.9
134	03	7.0	7.3	7.8	0	0.2	0.1	50	1.7	2	96.6	96.0	96.6	96.0	17.9	9.5	0.2	-255	3.4	0.9
135	04	7.1	7.4	7.9	0.1	0.1	0	54.2	2.1	1.9	104.2	104.6	96.1	96.5	27.1	8.4	1.2	-234	3.4	0.9
136	05	7.0	7.3	7.9	0.1	0.2	0.1	50.7	0.7	0.6	100.0	100.2	98.6	98.8	20.5	7.7	1.8	-264	3.4	0.9
137	06	7.0	7.3	7.8	0.1	0	0	49.7	1.2	1	97.0	97.4	97.6	98.0	18.3	6.7	0.9	-199	3.5	0.9
HRT = 12 h, salinity = 30 ppt																				
138	07/03/07	6.9	7.3	8.0	0	0.1	0	48.7	2.1	2.2	93.2	93.0	95.7	95.5	17.3	7.1	0.8	-254	3.5	0.9
139	08	7.0	7.4	8.0	0.1	0.1	0	50.9	1.9	1.8	98.0	98.2	96.3	96.5	20.1	8.6	1.2	-209	3.5	0.9
140	09	7.0	7.5	8.1	0.1	0.1	0	48.1	2.9	2.7	90.4	90.8	94.0	94.4	17.8	6.2	2.2	-254	3.5	0.9
141	10	7.0	7.4	8.0	0.1	0.2	0	49.8	3.2	3	93.2	93.6	93.6	94.0	22.3	9.7	2.5	-193	3.5	0.9
142	117	7.1	7.6	7.9	0	0.1	0.1	49.2	2.8	1	92.8	96.4	94.3	98.0	19.9	10.3	0.7	-268	3.5	0.9
143	12	6.9	7.3	7.6	0.1	0	0	51.7	1.5	1.3	100.4	100.8	97.1	97.5	18.9	7.3	1.5	-232	3.5	0.9
144	13	7.0	7.5	7.9	0.1	0	0	49.2	2.6	2.5	93.2	93.4	94.7	94.9	20.6	7.6	2.4	-285	3.5	0.9
HRT = 9 h																				
145	14/03/07	7.0	7.7	8.0	0	0.5	0.6	50	2.1	2.2	127.7	127.5	95.8	95.6	18.0	6.7	1.3	-244	3.6	1
146	15	7.0	7.5	7.8	0.1	0	0	50.1	4	3.2	122.9	125.1	92.0	93.6	21.2	8.3	1.4	-258	3.6	1
147	16	7.0	7.4	7.6	0.1	0	0	49.2	3.5	3.3	121.9	122.4	92.9	93.3	20.1	9.2	1.0	-314	3.6	1
148	17	6.9	7.5	7.9	0.8	0.5	0.5	52.9	2.3	2.5	134.9	134.4	95.7	95.3	24.1	9.2	1.7	-302	3.7	1
149	18	7.2	7.7	7.7	0.2	0.1	0	50.1	2.1	3.2	128.0	125.1	95.8	93.6	20.8	9.2	0.7	-324	3.7	1
150	19	7.0	7.3	7.9	0.2	0.1	0	48.3	1.6	2.4	124.5	122.4	96.7	95.0	25.9	8.9	2.4	-276	3.7	1
151	20	7.1	7.6	8.1	0.2	0.1	0	50.5	2.5	2.5	128.0	128.0	95.0	95.0	18.3	6.4	4.4	-297	3.7	1
HRT = 6 h, hydrogen pressure of 1.1 bars (optimum)																				
152	21/03/07	6.9	7.3	7.6	0.3	0.9	0.7	50.2	3.6	3.7	186.4	186.0	92.8	92.6	23.8	5.9	1.1	-298	3.9	1.1
153	22	7.1	7.8	7.9	0.2	0.4	0.4	50.1	2.2	2	191.6	192.4	95.6	96.0	20.2	8.7	4.0	-280	3.9	1.1
154	23	6.9	7.5	7.9	0.5	0.1	0.1	48.3	2.1	1.6	184.8	186.8	95.7	96.7	22.0	6.4	1.0	-295	3.9	1.1

Day	Date	pH			NO_2^--N (mg/L)			T-N (mg/L)			Denitrification rate (g/m^3.day)		Denitrification efficiency (%)		DOC concentration (mg/L)			ORP (mV)	TMP (kPa)	H_2 pressure (bar)
		Inlet	Outlet 1	Outlet 2	Inlet	Outlet 1	Outlet 2	Inlet	Outlet 1	Outlet 2	D tank	Total system	D tank	Total system	Inlet	Outlet 1	Outlet 2			
155	24/2/2007	7.0	7.4	7.8	0.2	0	0	49.2	1.9	1.6	189.2	190.4	96.1	96.7	25.2	7.2	2.9	-305	3.9	1.1
156	25	7.1	7.4	8.0	0.3	0.1	0.1	51.3	1.7	1.6	198.4	198.8	96.7	96.9	18.3	9.3	1.0	-350	3.9	1.1
157	26	7.1	7.4	8.0	0.3	0.2	0.1	49	2.3	1.5	186.8	190.0	95.3	96.9	18.4	7.2	1.9	-292	4.1	1.1
158	27	6.9	7.5	7.8	0.4	0.3	0.1	50.4	2	1	193.6	197.6	96.0	98.0	19.5	9.1	3.6	-320	4.1	1.1
159	28	6.9	7.5	7.6	0.5	0.2	0.2	53.9	4.5	4.3	197.6	198.4	91.7	92.0	21.2	7.0	2.5	-263	4.1	1.1
160	29	7.0	7.5	7.9	0.9	0.3	0.3	51.1	2.7	2.3	193.6	195.2	94.7	95.5	23.1	10.2	2.6	-290	4.1	1.1
161	30	7.0	7.3	7.6	0.7	0.5	0.1	50.2	2.5	1.1	190.8	196.4	95.0	97.8	20.1	7.5	2.2	-278	4.2	1.1
Average					**0.43**	**0.29**	**0.21**	**50.4**	**2.6**	**2.1**	**191.3**	**193.2**	**95**	**95.9**	**21.2**	**7.9**	**2.3**	**-297**		
HRT = 5 h																				
162	31/3/2007	7.0	7.6	8.0	0.7	1.1	0.9	51.9	4.7	4	226.6	229.9	90.9	92.3	17.6	6.6	2.1	-297	4.2	1.2
163	01/4/2007	7.0	7.4	7.6	0.5	0.9	0.8	50	6.5	4.3	208.8	219.4	87.0	91.4	20.9	8.7	4.8	-288	4.2	1.2
164	02	7.1	7.3	7.6	0.6	1.6	1.4	51.3	6.2	4.9	216.5	222.7	87.9	90.4	23.7	10.1	2.2	-350	4.2	1.2
165	03	6.9	7.5	7.9	0.3	1.1	0.7	49.3	4.9	3	213.1	222.2	90.1	93.9	21.1	7.4	2.8	-305	4.2	1.3
166	04	7.0	7.3	8.1	0.5	1.4	1.3	52.9	6	6	225.1	225.1	88.7	88.7	19.7	7.0	2.1	-277	4.2	1.3
167	05	7.0	7.5	8.2	1.1	1.7	1.6	52.6	5	4.5	228.5	230.9	90.5	91.4	17.5	6.8	1.9	-325	4.3	1.3
168	06	6.9	7.6	7.7	0.7	1.3	0.9	51.8	7	7	215.0	215.0	86.5	86.5	21.4	8.9	2.1	-347	4.3	1.3
169	07	7.2	7.3	7.9	0.9	0.7	0.6	53.7	4.7	4.4	235.2	236.6	91.2	91.8	22.1	7.6	2.0	-288	4.3	1.3
Average					**0.66**	**1.23**	**1.03**	**51.7**	**5.6**	**4.8**	**221.1**	**225.2**	**89.1**	**90.8**	**20.5**	**7.9**	**2.5**	**-310**		

Appendix C

Kinetic of the Denitrification

Appendix C.1 Biomass yield calculation

Nitrogen removal and biomass washed out in three run are presented in table C.1

Table C.1 Calculation of nitrogen removed and biomass washed out

Date	TN inlet (mg/L)	TN outlet (mg/L)	TN removed (mg/d)	Biomass washed out (mg VSS/L)	Biomass washed out (mg VSS/d)
Run 1, HRT = 3 h					
09/01/2007	49.2	6	1555.2	11.5	414
10	52.7	5.8	1688.4	11.5	414
11	53.2	4.3	1760.4	13	468
12	49	4.7	1594.8	22.5	810
13	50.6	5.5	1623.6	14.5	522
14	49	5.1	1580.4	13.5	486
15	53.2	5.3	1724.4	12.5	450
16	48.9	4.7	1591.2	13	468
17	53.2	5.9	1702.8	16.5	594
18	50.1	4.7	1634.4	17.5	630
Total			**16455.6**		**5256**
Run 2, HRT = 5 h					
19/02/2007	50.2	4.5	987.1	18.5	399.6
20	49.7	1.5	1041.1	17	367.2
21	50.9	3.2	1030.3	19	410.4
22	50.1	3	1017.4	15.5	334.8
23	48.1	4.7	937.4	7.5	162
24	51.4	2.5	1056.2	13.5	291.6
25	49.2	4.2	972.0	12.5	270
26	51.2	2.5	1051.9	17.5	378
27	48.7	2	1008.7	13	280.8
28	52.4	4.6	1032.5	13.5	291.6
Total			**10134.6**		**3186**
Run 3, HRT = 6 h					
21/03/2007	50.2	3.6	838.8	20	360
22	50.1	2.2	862.2	16.5	297
23	48.3	2.1	831.6	10	180
24	49.2	1.9	851.4	22.5	405
25	51.3	1.7	892.8	18	324
26	49	2.3	840.6	11.5	207
27	50.4	2	871.2	12.5	225
28	53.9	4.5	889.2	25.5	459
29	51.1	2.7	871.2	29	522
30	50.2	2.5	858.6	21	378
Total			**8607.6**		**3357**

Biomass in the reactor is calculated in the following equation and the result is presented in table C.2

The biomass in the reactor (mg) = MLVSS (mg/L) x volume of reactor (L)

Table C.2 Calculation of total biomass in denitrification tank

Run	**$MLVSS_{ini}$ (mg/L)**	**$Biomass_{ini}$ (mg)**	**$MLVSS_{aft}$ (mg/L)**	**$Biomass_{aft}$ (mg)**
Run 1	2060	9270	2440	10980
Run 2	1540	6930	1820	8190
Run 3	1260	5670	1440	6480

Biomass yield is calculated based on the following equation and the result is presented in Table C3.

$$Y = \frac{Biomass_{gen}}{TN_{rem}} = \frac{(Biomass_{aft} + Biomass_{was} - Biomass_{ini})}{TN_{rem}}$$

Where Y : biomass yield, mg VSS/ mg N

$Biomass_{gen}$: biomass generation after a period of time, mg

$Biomass_{aft}$: biomass in the reactor after a period of time, mg

$Biomass_{was}$: biomass washed from the denitrification tank, mg

$Biomass_{ini}$: initial biomass in the reactor, mg

TN_{rem} : amount of nitrate nitrogen removal after a period of time, mg

The result of biomass yield is showed in Table B3

Table C.3 Calculation of biomass yield

Run	**$Biomass_{ini}$ (mg)**	**$Biomass_{aft}$ (mg)**	**$Biomass_{was}$ (mg)**	**TN_{rem} (mg)**	**Biomass yield (mg VSS/ mg N)**
	A	**B**	**C**	**D**	**(B + C – A)/D**
Run 1	9270	10980	5256	16455.6	0.42
Run 2	6930	8190	3186	10134.6	0.44
Run 3	5670	6480	3357	8607.6	0.48

Appendix C.2 Nitrate reduction rate to biomass concentration

Nitrate reduction rate to biomass concentration is calculated in the following equation and the result is presented in C.4.

$$K_N = \frac{Q\,(TN_{in} - TN_{ef})}{V\,X}$$

Where K_N is nitrate reduction rate to biomass concentration, g NO_3^--N/g VSS.d

Q : flowrate, m^3/d

TN_{in} : concentration of total nitrogen in the influent, g/m^3

TN_{out} : concentration of total nitrogen in the outlet, g/m^3

V : volume of the reactor, m^3

X : biomass concentration in the reactor, g/m^3

Since Q/V = 1/HRT, the equation is presented as

$$K_N = \frac{24\,(TN_{in} - TN_{ef})}{HRT * X}$$

Where HRT is hydraulic retention time (h)

Table C.4 Calculation of nitrate reduction rate to biomass concentration

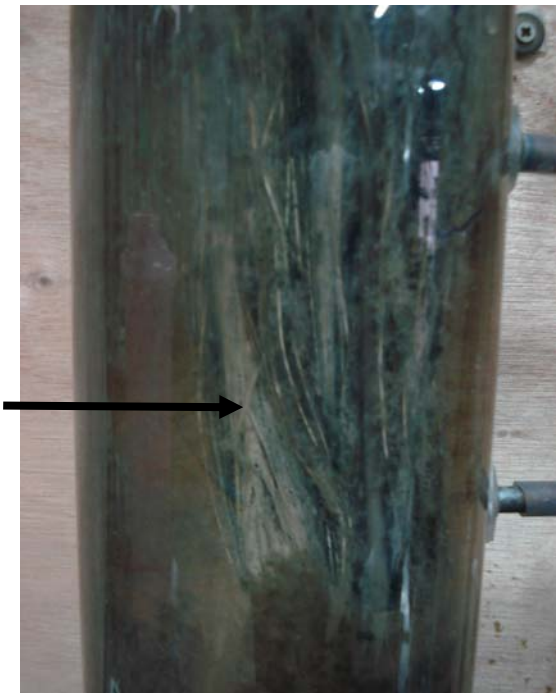
Run	HRT (h)	TN_{in} (mg/L)	TN_{out} (mg/L)	X (mg/L)	K_N
1	3	50.9	5.2	2440	0.15
2	5	50.2	3.3	1820	0.12
3	6	50.4	2.6	1440	0.13

Appendix D

Membrane resistance measurement

Appendix D1. Photos of membrane fouling

Cleaned membrane



Biofilm and salt cake on the membrane

Membrane after one month of diffusion

Membrane after physical cleaning

Appendix D2. Nitrogen flux to the membrane

$$\text{Nitrate removal rate} = \frac{24\ V\ (TN_{in} - TN_{out})}{HRT * A * 1000}$$

TN_{in} : concentration of total nitrogen in the influent, mg/L
TN_{out} : concentration of total nitrogen in the outlet, mg/L
V : volume of the reactor, L
HRT: hydraulic retention time, h
Nitrogen removal rate (g N/m^2. day)

According to the above equation, Table D.1 shows the result of nitrogen flux to the membrane

Table D.1 Nitrogen flux to the membrane

Run	HRT (h)	TN_{in} (mg/L)	TN_{out} (mg/L)	Membrane area (m^2)	Nitrogen removal rate (g N/m^2. day)
1	3	50.9	5.2	0.42	3.92
2	5	50.2	3.3	0.42	2.41
3	6	50.4	2.6	0.42	2.05

Appendix D3. Membrane Resistance Measurement

1. Membrane resistance after two months operation

Table D2. Filtration flux and TMP after two months of operation

No	Flux (mL/min)	Filtration flux ($L/m^2.h$)	TMP (kPa)
Resistance after 2 months			
1	26	3.7	4.4
2	52	7.4	4.9
3	76	10.9	5.6
4	98	14.0	6.2
5	127	18.1	7.0
6	161	23.0	8.1
7	179	25.6	8.7
Resistance after chemical cleaning			
1	28.5	4.1	4.1
2	54.0	7.7	4.5
3	78.0	11.1	4.9
4	108.0	15.4	5.4
5	135.0	19.3	5.7
6	166.0	23.7	6.25
7	189.0	27.0	6.7

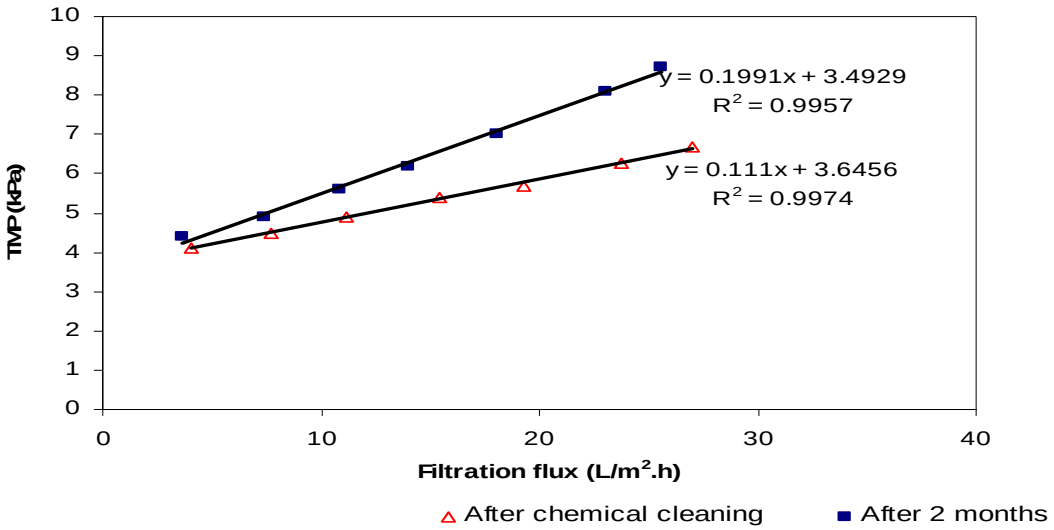


Figure D.1 Filtration flux versus TMP after two months of operation

Membrane surface area: $0.42 m^2$
Dynamic viscosity of water at 30^oC: 0.798×10^{-3} Pa.s

The membrane resistance was derived from the slope of the linear curve of Δ P versus J. With dynamic viscosity of pure water is 0.798 * 10-3 Pa.s (or N.s/m2), initial membrane resistance was calculated as follows:

$$Rm = \frac{0.111\ (kPa)\ x\ 1000\ (Pa/kPa) x 3600\ (s/h) x 1000\ (L/m^3)}{(L/m^2.h) x 0.798 x 10^{-3}\ (Pa.s)} = 5.00752\ x\ 10^{11}\ m^{-1}$$

Similarly membrane resistance after two months of diffusion $Rm = 8.98195\ x\ 10^{11}\ m^{-1}$

2. Membrane resistance in run 1

Table D3. Filtration flux and TMP after one month of operation in run 1

No	Flux (mL/min)	Filtration flux ($L/m^2.h$)	TMP (kPa)
Initial resistance			
1	27.0	3.9	3.6
2	54.0	7.7	4.0
3	82.0	11.7	4.4
4	116.0	16.6	5.0
5	137.5	19.6	5.3
6	167.5	23.9	5.8
7	192.0	27.4	6.3
Resistance after one months of diffusion			
1	28.0	4.0	3.7
2	54.5	7.8	4.3
3	77.0	11.0	4.8
4	107.0	15.3	5.4
5	136.0	19.4	6.1
6	165.5	23.6	6.7
7	187.5	26.8	7.05
Resistance after chemical cleaning			
1	29.0	4.1	3.6
2	44.5	6.4	4.0
3	81.0	11.6	4.4
4	107.0	15.3	4.9
5	138.5	19.8	5.4
6	164.0	23.4	5.9
7	192.0	27.4	6.45

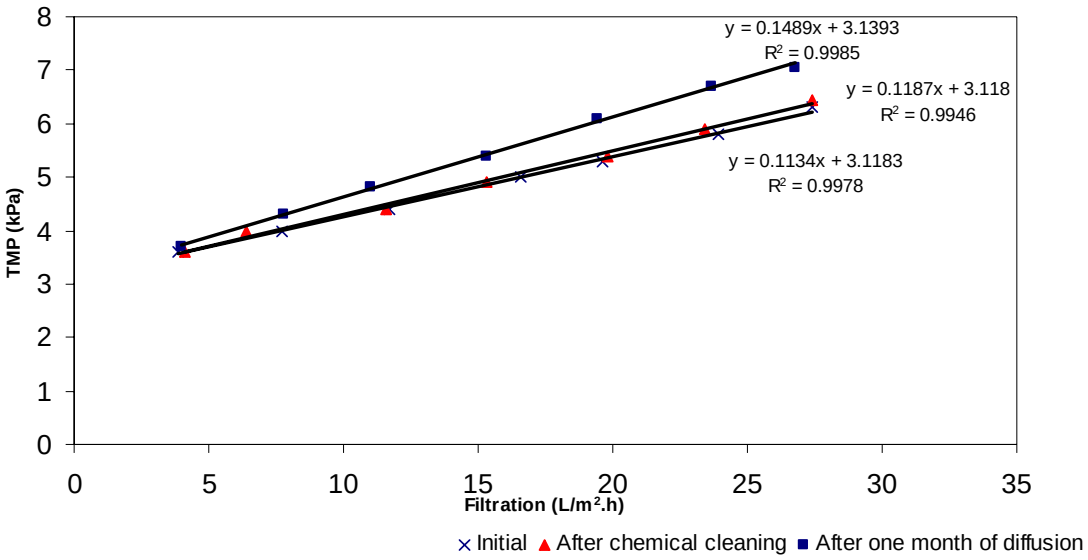


Figure D.2 Filtration flux versus TMP after one of operation in run 1

The calculation of membrane resistance (Rm) gives results as follows:

Initial Rm: 5.11579×10^{11} m^{-1}

Rm after one month: 6.71729 x 10^{11} m^{-1}
Percentage increase of Rm: 31%
Rm after chemical cleaning: 5.35489 x 10^{11} m^{-1}
Recovery percentage: 96%

3. Membrane resistance in run 2

Table D4. Filtration flux and TMP after one month of operation in run 2

No	Flux (mL/min)	Filtration flux ($L/m^2.h$)	TMP (kPa)
Initial resistance			
1	30	4.3	3.8
2	55	7.9	4.2
3	82	11.7	4.7
4	108	15.4	5.2
5	135	19.3	5.8
6	160	22.9	6.3
7	188	26.9	6.8
Resistance after one months of diffusion			
1	29	4.1	4.2
2	57	8.1	4.7
3	88	12.6	5.5
4	115	16.4	6.1
5	144	20.6	6.8
6	173	24.7	7.5
7	200	28.6	8.3
Resistance after chemical cleaning			
1	30	4.3	4.1
2	55	7.9	4.5
3	85	12.1	5.2
4	112	16.0	5.7
5	144	20.6	6.4
6	170	24.3	6.95
7	201	28.7	7.5

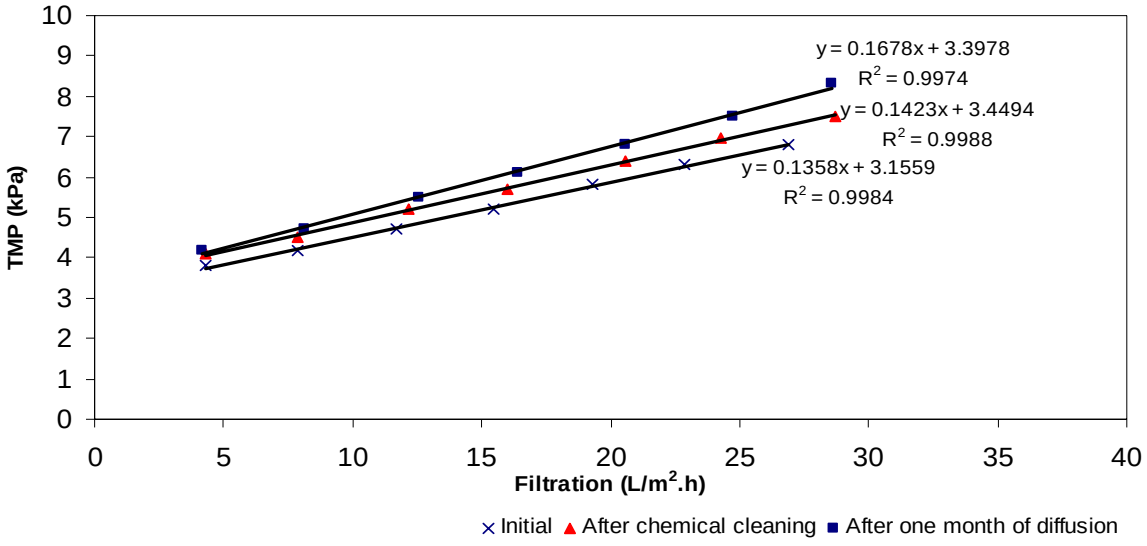


Figure D.3 Filtration flux versus TMP after one of operation in run 2

The calculation of membrane resistance (Rm) gives results as follows:

Initial Rm: $6.12632x\ 10^{11}\ m^{-1}$
Rm after one month: $7.56992 \times 10^{11}\ m^{-1}$
Percentage increase of Rm: 24%
Rm after chemical cleaning: $6.41955 \times 10^{11}\ m^{-1}$
Recovery percentage: 95%

4. Membrane resistance in run 3

Table D5. Filtration flux and TMP after one month of operation in run 3

No	Flux (mL/min)	Filtration flux ($L/m^2.h$)	TMP (kPa)
Initial resistance			
1	30	4.3	4.1
2	55	7.9	4.5
3	85	12.1	5.2
4	112	16.0	5.7
5	144	20.6	6.4
6	170	24.3	6.95
7	201	28.7	7.5
Resistance after one months of diffusion			
1	32.5	4.6	4.3
2	60	8.6	4.8
3	89	12.7	5.5
4	118	16.9	6.1
5	149	21.3	6.8
6	174	24.9	7.5
7	204	29.1	8.3
Resistance after chemical cleaning			
1	30	4.3	4.2
2	57	8.1	4.6
3	86	12.3	5.4
4	114	16.3	5.8
5	142	20.3	6.6
6	175	25.0	7.4
7	198	28.3	7.7

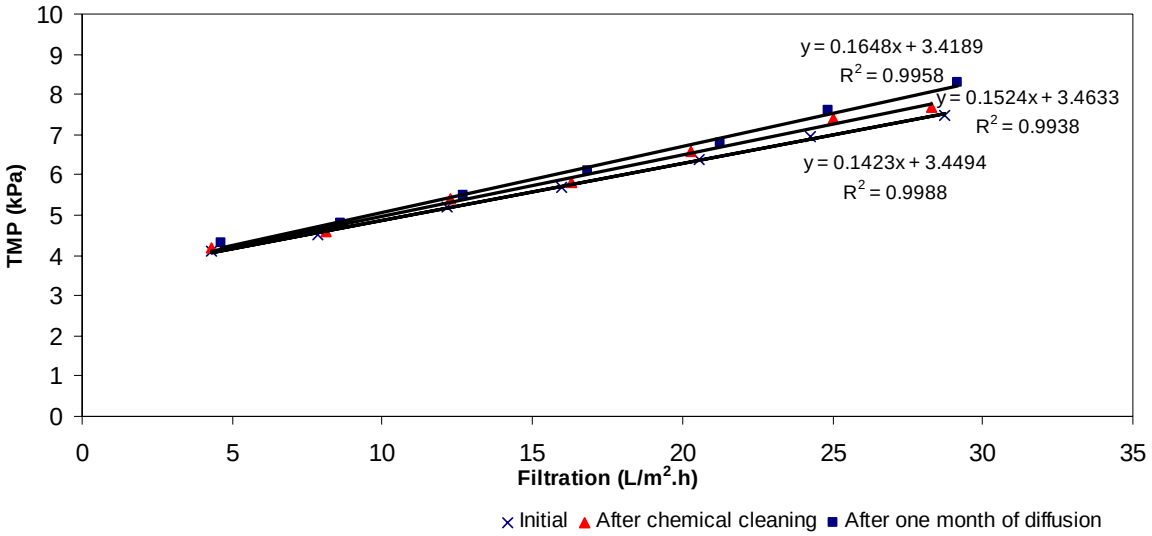


Figure D.4 Filtration flux versus TMP after one of operation in run 3

The calculation of membrane resistance (Rm) gives results as follows:

Initial Rm: 6.41955*x* 10^{11} m^{-1}
Rm after one month: 7.43459x 10^{11} m^{-1}
Percentage increase of Rm: 16%
Rm after chemical cleaning: 6.87519x 10^{11} m^{-1}
Recovery percentage: 93%

Appendix E
Hydrogen utilization and cost analysis

Appendix E.1 Measurement of hydrogen flow rate

Hydrogen flow rate was measured indirectly by applying the different pressures on the membrane (Figure E1). After a period of time when dissolved hydrogen was saturated in the tank, the volume hydrogen gas come out from the tank was collected and measured at the standard conditions (25^{o}C and 1 atm). The volume measured over the period of time was the hydrogen flow rate. The measurement at every pressure was measured 3 times. Table E1 shows the results of the measurement.

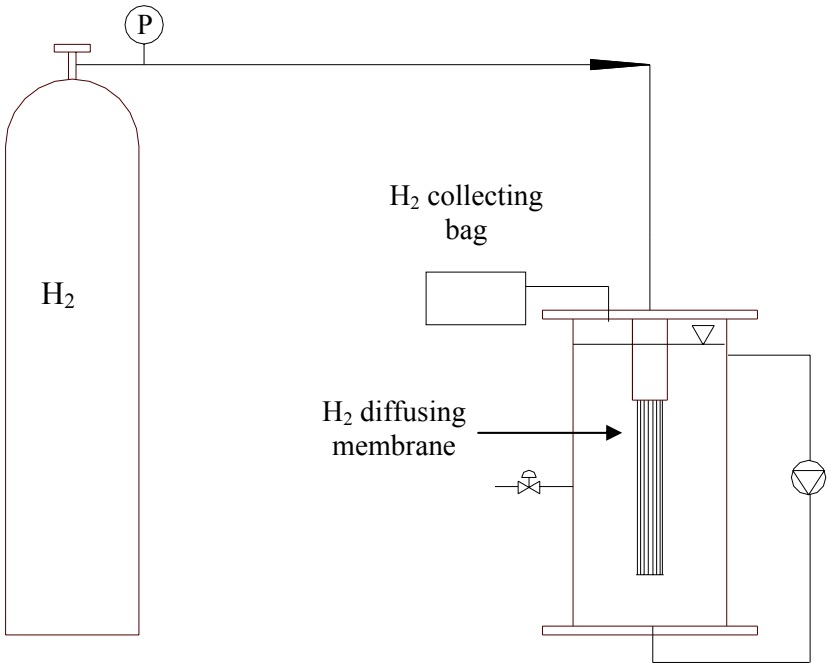


Figure E.1 Unit to measure hydrogen gas flow rate

Table E.1 Hydrogen flow rate at different pressures

Hydrogen pressure (bar)	Volume of gas (mL)	Time (min)	Flow rate (mL/min)
1.4	860	59	14.6
1.3	620	57	10.9
1.2	640	72	8.9
1.1	630	81	7.8
1	350	49	7.1
0.9	370	57	6.5
0.8	420	74	5.7
0.6	280	65	4.3

Appendix E.2 Estimation of hydrogen utilization rate and cost analysis

Hydrogen utilization rate

Stoichiometric reaction of hydrogenontrophic denitrification is

$$H_2 + 0.35\ NO_3^- + 0.35\ H^+ + 0.052CO_2 \rightarrow 0.17N_2 + 1.1\ H_2O + 0.010\ C_5H_7NO_2$$

According to the above equation, 1g of NO_3^--N converted to N_2 consumes 0.357 g of hydrogen gas (Ho et al., 2001).

Since the nitrite in the inlet and outlet in this study were too small, Nitrate removal was considered as total nitrogen removal and calculated as follows

$$Nitrate_{rem} = \frac{(TN_{in} - TN_{out}) * V}{HRT} \quad (1)$$

Where: $Nitrogen_{rem}$: nitrogen removal (mg/h)
TNin : nitrogen in the inlet, mg/L
TNout: nitrogen in the outlet, mg/L
V: volume of the reactor, L. Volume of reactor = 4.5L.
HRT: hydraulic retention time, h

From equation (1), Nitrate removal (mg/h) is calculated and presented in Table E2

Table E.2 Nitrogen removal of three runs

Run	HRT (h)	TN_{in} (mg/L)	TN_{out} (mg/L)	$Nitrate_{rem}$ (mg/h)
	A	B	C	4.5*(B-C)/A
1	3	50.9	5.2	68.55
2	5	50.2	3.3	42.21
3	6	50.4	2.6	35.85

$$\text{Amount of hydrogen measured (mg/h)} = \frac{\text{Hydrogen flow rate (mL/min)} * 60\ \text{(min/h)}}{22.4\ \text{(mL/mg)}} \quad (2)$$

$$\text{Hydrogen utilization (mg } H_2\text{/mg N)} = \frac{\text{Amount of hydrogen measured (mg/h)}}{Nitrate_{rem}\ \text{(mg/h)}} \quad (3)$$

Hydrogen pressure of run 1, 2 and run 3 were 1.3, 1.2 correlating to hydrogen flow rates of 10.9, 8.9 and 7.8 mL/min respectively. Based on equation (2) and (3), the calculation of hydrogen utilization and efficiency is presented in the Table E3

Table E.3 Hydrogen utilization and efficiency

Run	Hydrogen flow rate (mL/min)	Amount of hydrogen measured (mg/h)	$Nitrate_{rem}$ (mg/h)	Hydrogen utilization (mg H_2/mg N)	Theoretical hydrogen utilization (mg H_2/mg N)	Efficiency (%)
	A	B	C	B/C	D	D/(B/C)*100
1	10.9	29.20	68.55	0.426	0.357	84
2	8.9	23.84	42.21	0.565	0.357	63
3	7.8	20.89	35.85	0.583	0.357	61

Cost analysis

Volume of hydrogen gas in cylinder: $6m^3 = 6000L$
Amount of hydrogen (g)

$$\text{Amount of hydrogen (g)} = \frac{6000\ \text{L} * 2}{22.4\ (\text{L/g})} = 535.7\ \text{g}$$

Cost of 1 cylinder: 650 Baht

$$\text{Cost of 1 g hydrogen (Baht/g)} = \frac{650\ \text{Baht}}{535.7\ \text{g}} = \mathbf{1.21\ Baht/g}$$

Theoretical cost for removal of 1 gram of nitrate = 1.21 Baht/g x 0.357 = **0.43 Baht/g**

From the cost of hydrogen gas and hydrogen utilization, the cost for 1 gram nitrate removal and for 1 m^3 wastewater is calculated in Table E.4

Table E.4 Cost for nitrogen removal in wastewater

Run	Nitrogen removal (g/m^3)	Theoretical cost (Baht/g)	Theoretical cost ($Baht/m^3$)	Hydrogen utilization (g H_2/g N)	Actual cost (Bath/g N)	Actual cost ($Baht/m^3$)
	A	**B**	**AxB**	**C**	**1.21 C**	**AxC**
1	45.7	0.43	19.7	0.426	0.52	23.6
2	46.9	0.43	20.2	0.565	0.68	32.1
3	47.8	0.43	20.6	0.583	0.71	33.7

Calculation cost for methanol as electron donor
Reaction

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

6x14 5x32
45 85.7

If nitrate nitrogen in inlet wastewater is 50 mg/L, removal efficiency is around 90%, methanol required for treatment of $1m^3$ wastewater is 85.7 gram

Cost of 1 L methanol is 200 Baht (This price was provided by EEM Lab-AIT for analytical grade).

Specific density of methanol: 0.8, 1L of methanol is 800g

The cost of 1 gram methanol:

$$\frac{200}{800} = \mathbf{0.25\ Baht/g}$$

Theoretical cost for removal of 1 g of nitrate:

$$\frac{87.5}{45}\ \text{x}\ 0.25 = \mathbf{0.49\ Baht/g}$$

Theoretical cost for treatment of 1 m^3 of wastewater = 0.48 Baht/g x 45 g/m^3 = **22.1 Baht/m^3**

According Boley et al. (2000), the actual the consumption of methanol was 2.08 – 3.98 g methanol/g NO_3^--N. The actual cost of methanol for treating 1 gram nitrate is from (2.08 x 0.25) to (3.98 x 0.25) Baht/g or from **0.52 to 0.96 Baht/g**.

The actual cost of methanol for treating 1 m^3 of wastewater from (0.52 Bath/g x 45 g/m^3) to (0.96 Baht/g x 45 g/m^3) or from **23.4 to 44.8 Baht/m^3**.

Appendix F
Photo of experimental setup

Experimental setup of the system

HYDROGENOTROPHIC DENITRIFICATION OF SALINE AQUACULTURE WASTEWATER USING HOLLOW FIBER MEMBRANE BIOREACTOR

THESIS FINAL

Diep Dinh Phong

Examination Committee:

Prof. C. Visvanathan (Chairperson)

Dr. Nguyen Thi Kim Oanh

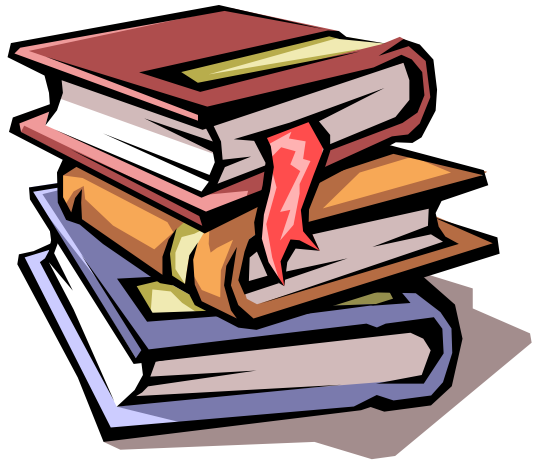
Dr. Oleg Shipin

Dr. Jega. V. Jegatheesan

AIT, 2 May 2007

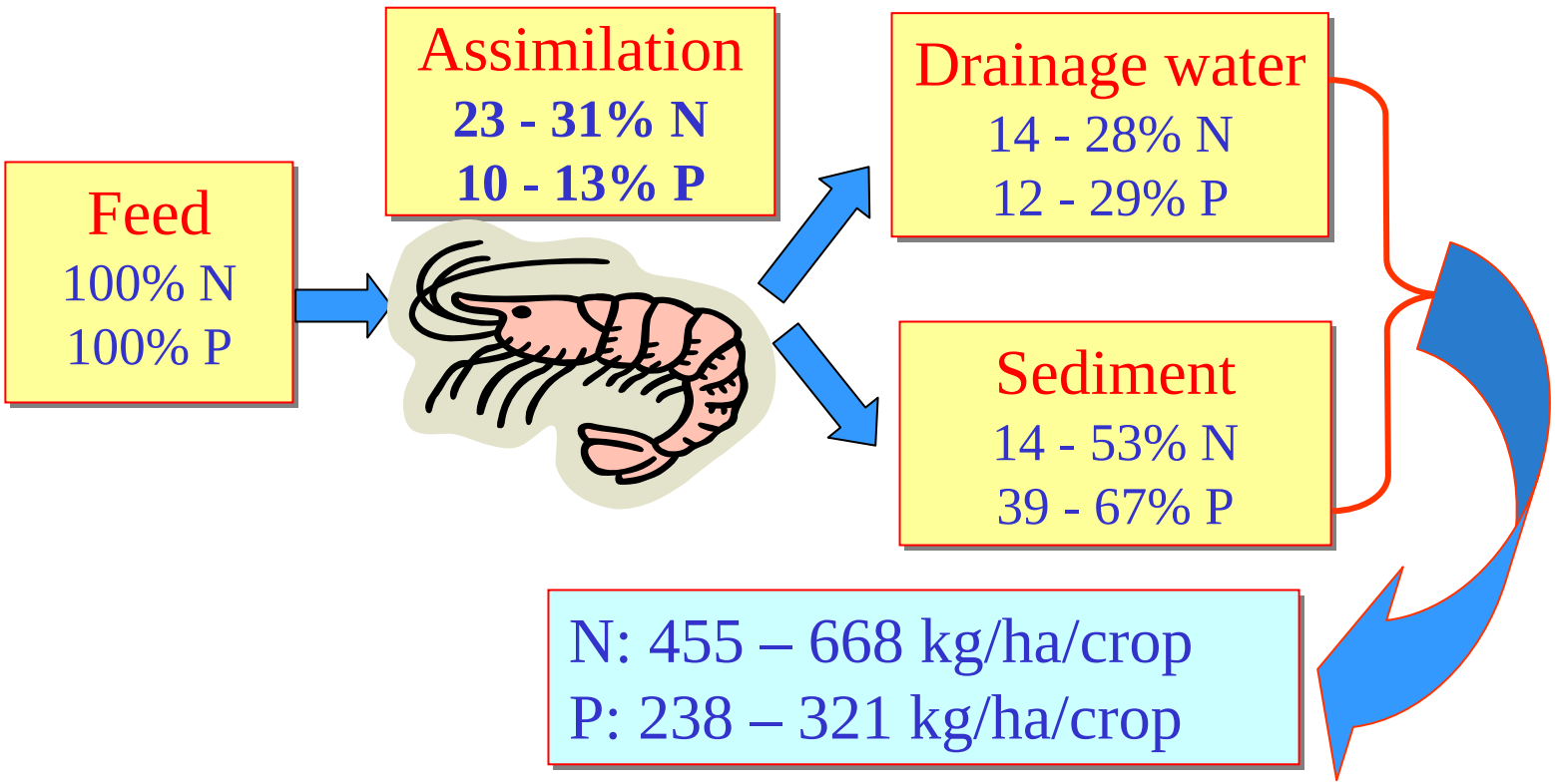
CONTENTS

- Introduction
- Objectives and scope of study
- Methodology
- Results and Discussion
- Conclusions and Recommendation



INTRODUCTION

- Discharge from aquaculture ponds



Source: Dierberg and Kiattisimkul, 1996; Thakur and Lin, 2003.

INTRODUCTION

- Discharge from aquaculture ponds

Clean water

Discharge
- Scarcity of water
- Eutrophication
- Effects on aquatic life
- High salinity affecting fresh receiving water

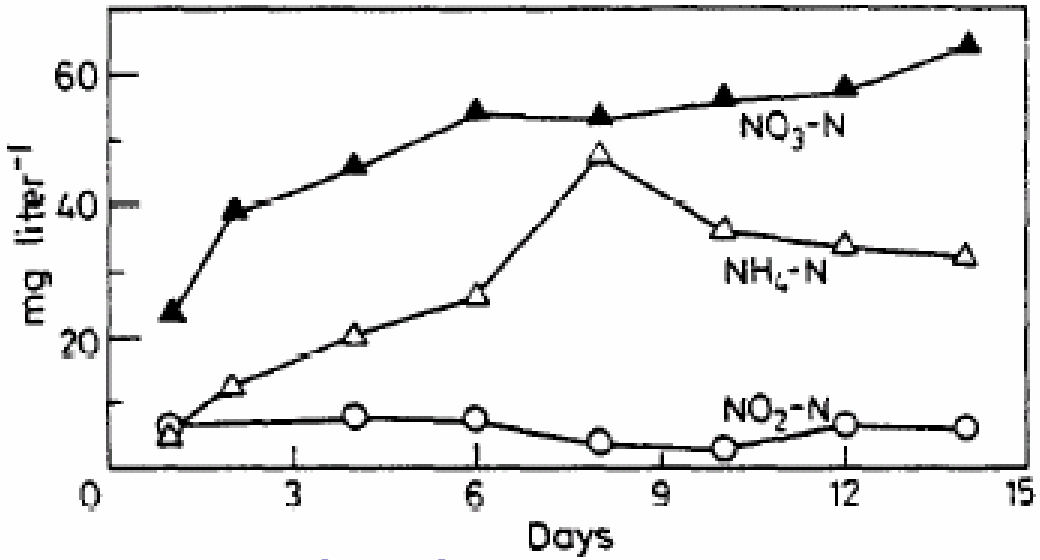
Treatment and Recirculating
- Reduce amount of clean water used
- Reduce pollution load to environment

INTRODUCTION

☞ Recirculating system (cont')

- No treatment or
- Improper treatment

Accumulation of pollutants especially Nitrate
- Eutrophication in the pond
- Effects on cultured fish



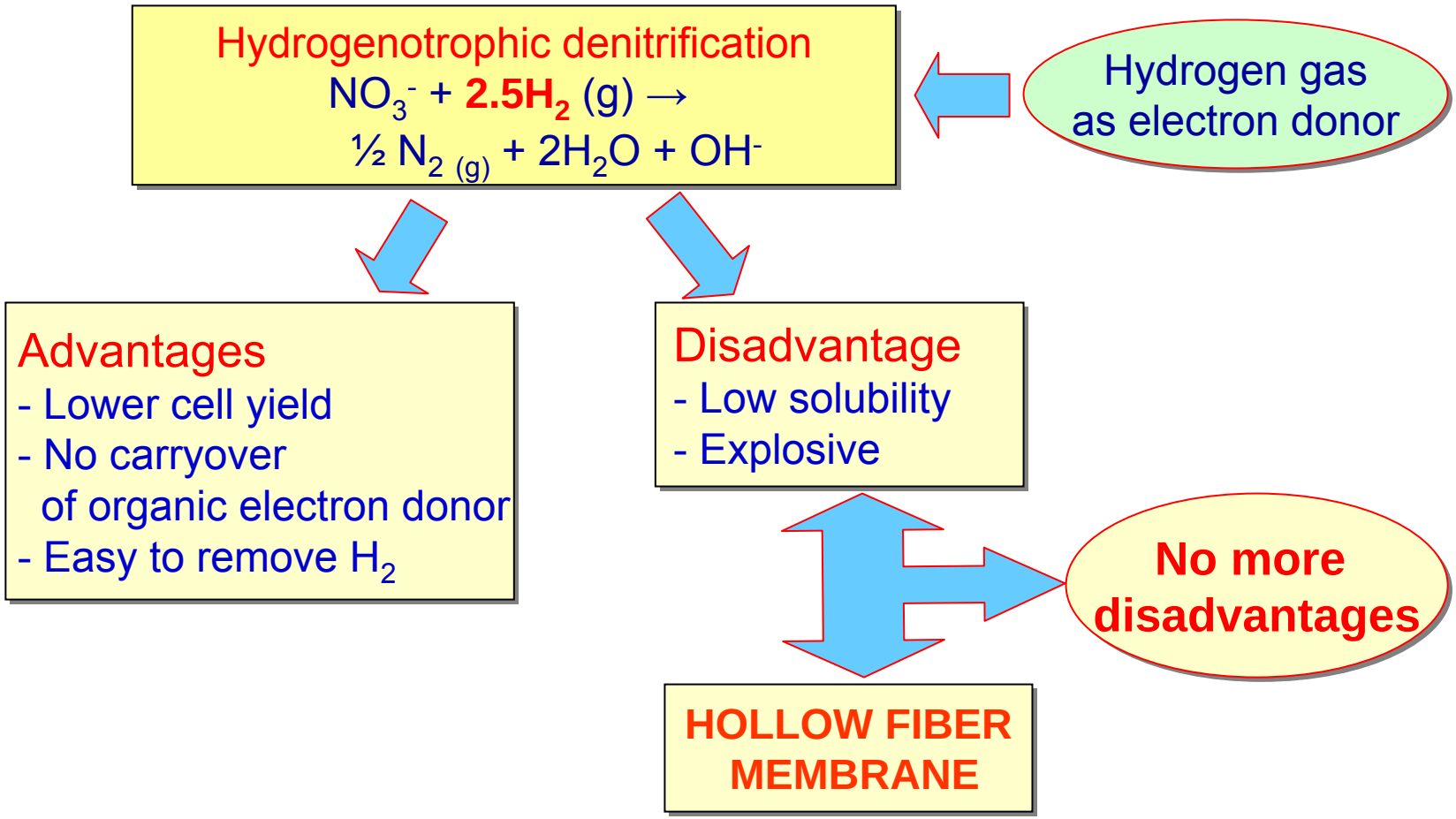
Source: Arbiv and van Rinjn, 1995.

Treatment is required
Nitrogen removal is important

Denitrification

INTRODUCTION

☞ Hydrogenotrophic denitrification



INTRODUCTION

Hydrogenotrophic denitrification using hollow fiber membrane

Drinking water
- Studied for long time
- Suitable: low organic carbon
- More safety

Aquaculture wastewater
- New approach
- Suitable: low organic carbon

Fresh wastewater

Saline wastewater

INTRODUCTION

Hydrogenotrophic denitrification using hollow fiber membrane

Fresh wastewater

- Denitrification – Aeration sequence is more efficient than Aeration – Denitrification sequence
- Denitrification rate: 367.3 mgNO_3^--N/L.d and efficiency: 91.4% at hydraulic retention time (HRT) = 3h.
- pH buffer: CO_2 is better than $H_2PO_4^-$ and HPO_4^-
- Hydrogen utilization efficiency: 54%

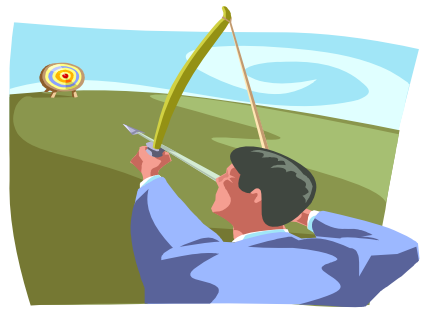
(Source: Hung, 2006)

Saline wastewater

This study

OBJECTIVES AND SCOPE OF STUDY

☞ Objectives



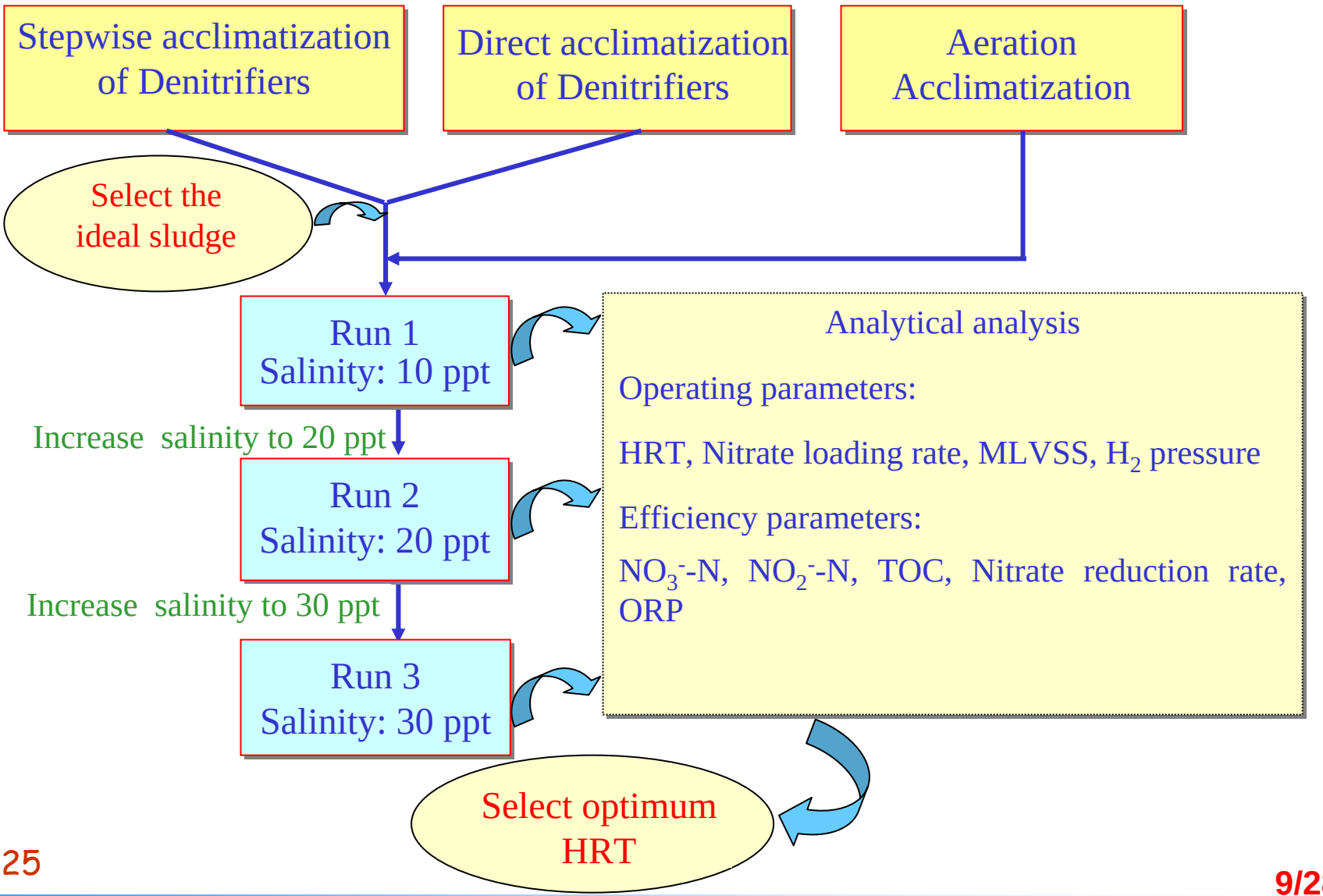
- Study the potential of hydrogenotrophic denitrification in treating saline aquaculture wastewater with three salinity of 10, 20, and 30 ppt;
- Determine parameters affecting the denitrification; and optimize the operating conditions such as hydraulic retention time (HRT).

☞ Scope of study

- Laboratory scale system of gas diffusing membrane bioreactor;
- Synthetic saline aquaculture wastewater; and
- Determination of operating conditions and parameters indicating performance and efficiency of the system.

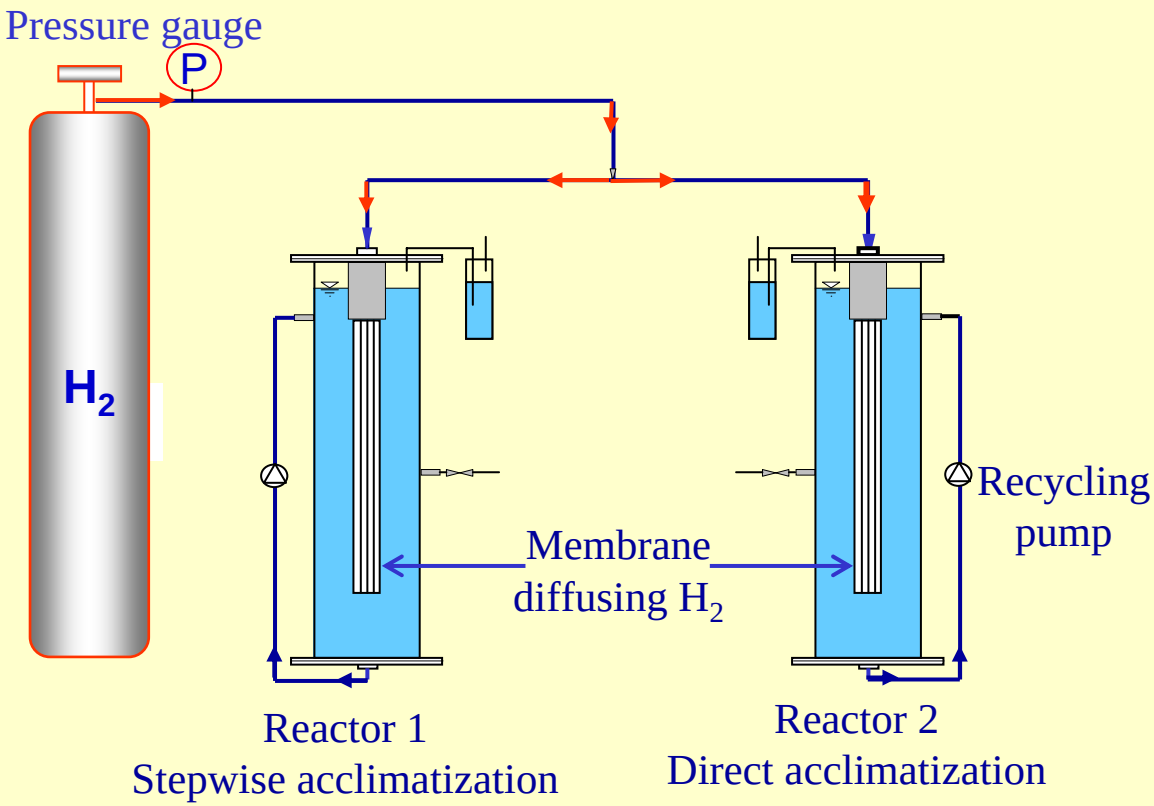
METHODOLOGY

Experimental procedure

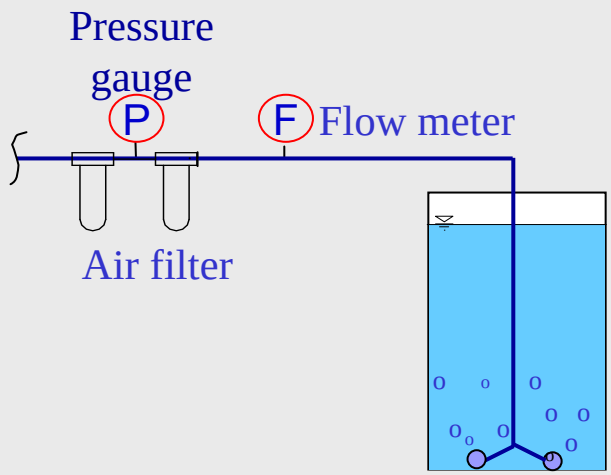


METHODOLOGY

☞ Diagram of sludge acclimatization



Sludge acclimatization of denitrifiers



Aeration sludge acclimatization

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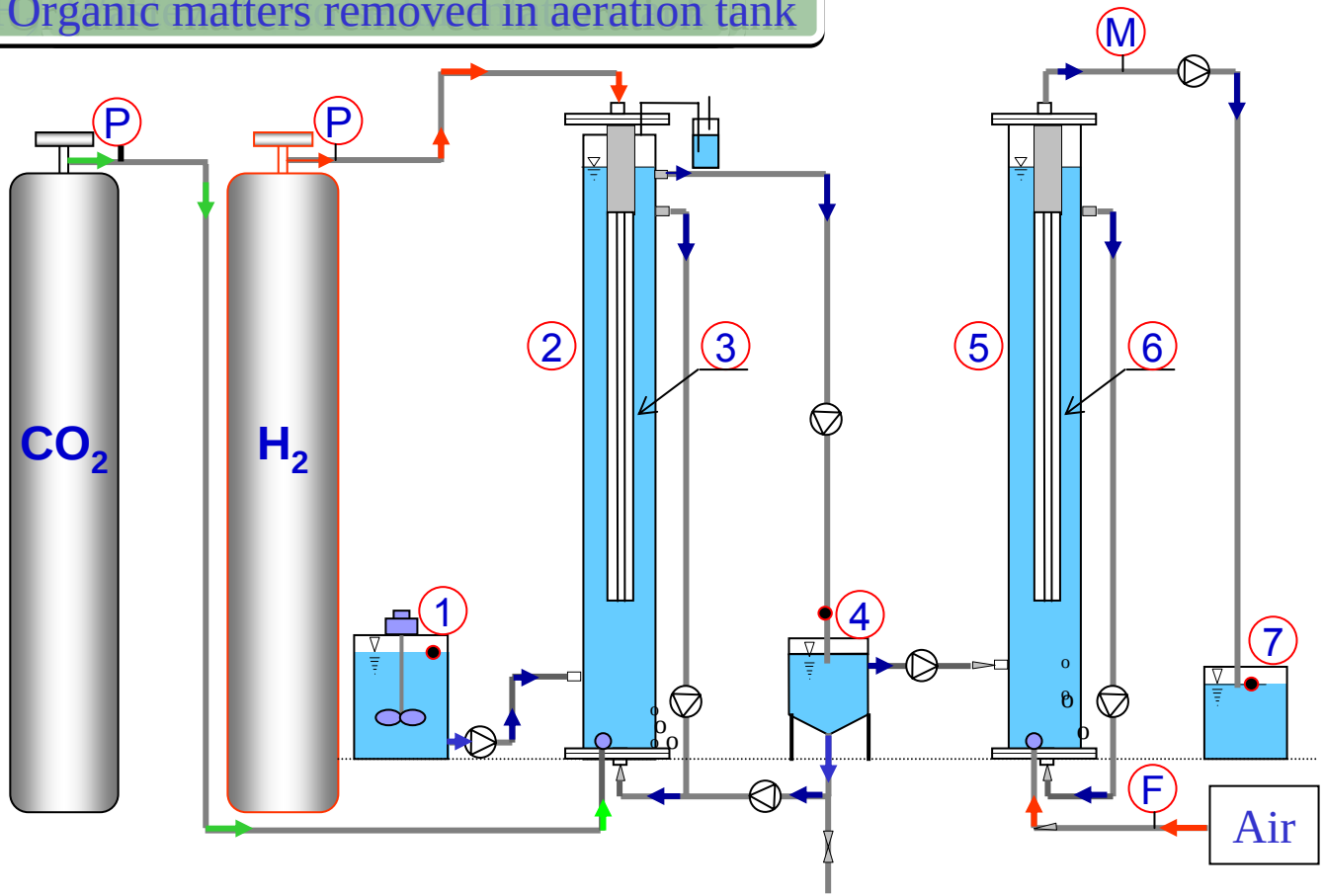
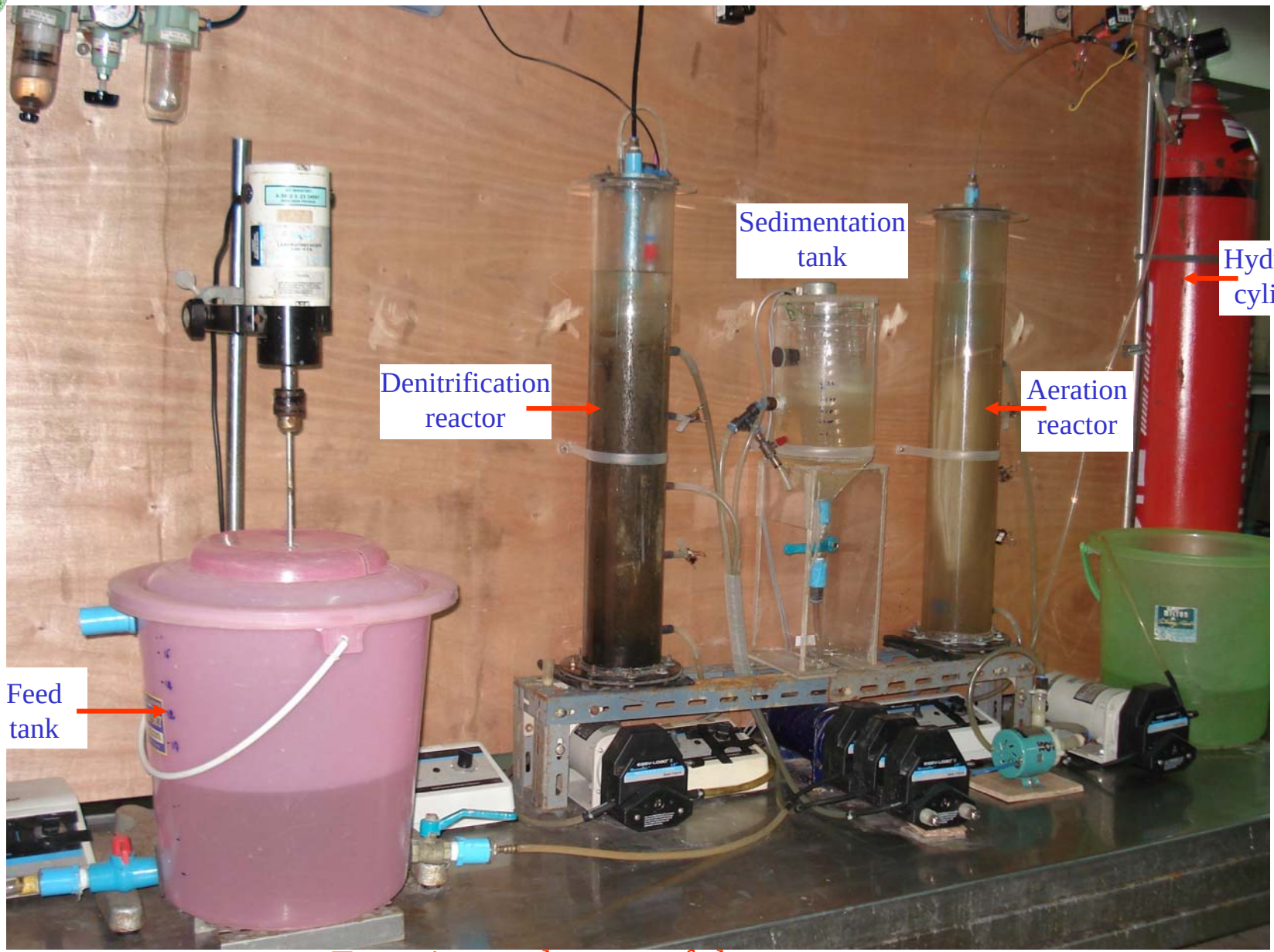
METHODOLOGY

☞ Diagram of denitrification – aeration system

Organic matters removed in aeration tank



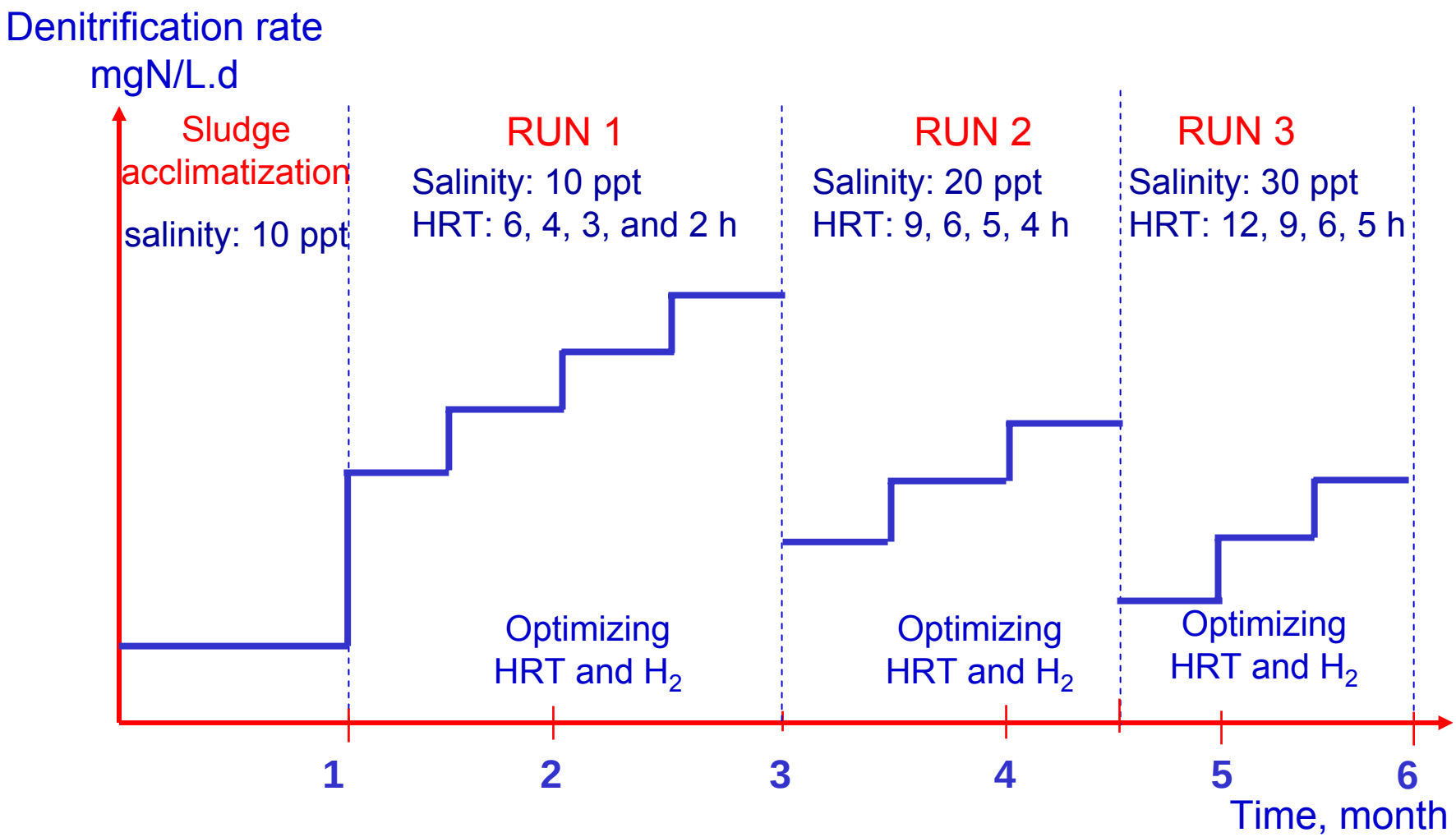
1. Feed tank
2. Denitrification tank
3. Membrane diffusing H_2
4. Sedimentation tank
5. Aeration tank
6. Membrane sucking treated wastewater
7. Effluent tank
- Sampling point
F. Flow meter
M. Manometer



Experimental setup of the system

METHODOLOGY

Experimental runs



METHODOLOGY

Analytical methods

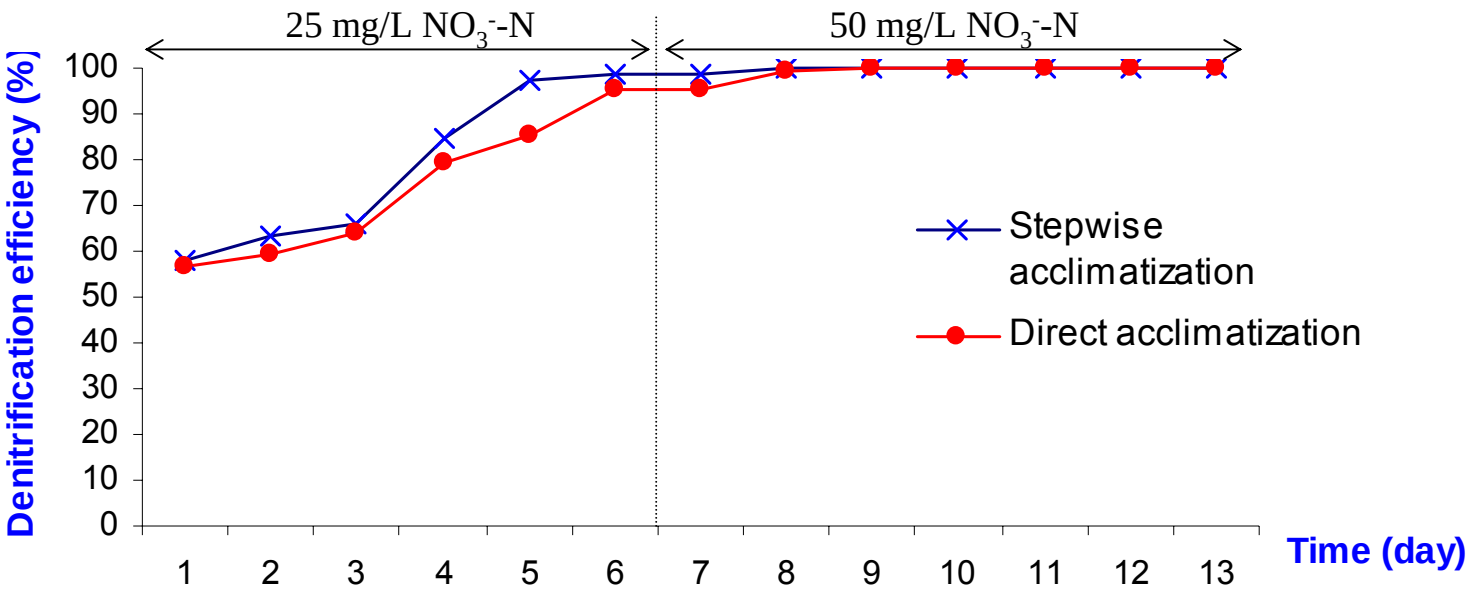
Parameters	Analytical method	Analytical equipment	Sampling point
DO	-	DO meter	Aeration tank
pH	-	pH meter	S1, S2, S3
TOC/DOC	High-temperature combustion	TOC analyzer	S1, S2, S3
ORP	-	Redox meter	Denitrification tank
NO_2^--N	Colorimetric	Spectrophotometer	S1, S2, S3
NO_3^--N	Cadmium Reduction	Spectrophotometer	S1, S2, S3
MLVSS	Filtration-Evaporation-Weighting	-	Denitrification tank, S1

Source: APHA et al., 1999.

S1: influent
S2: effluent of denitrification tank
S3: effluent of aeration tank

RESULTS AND DICUSSION

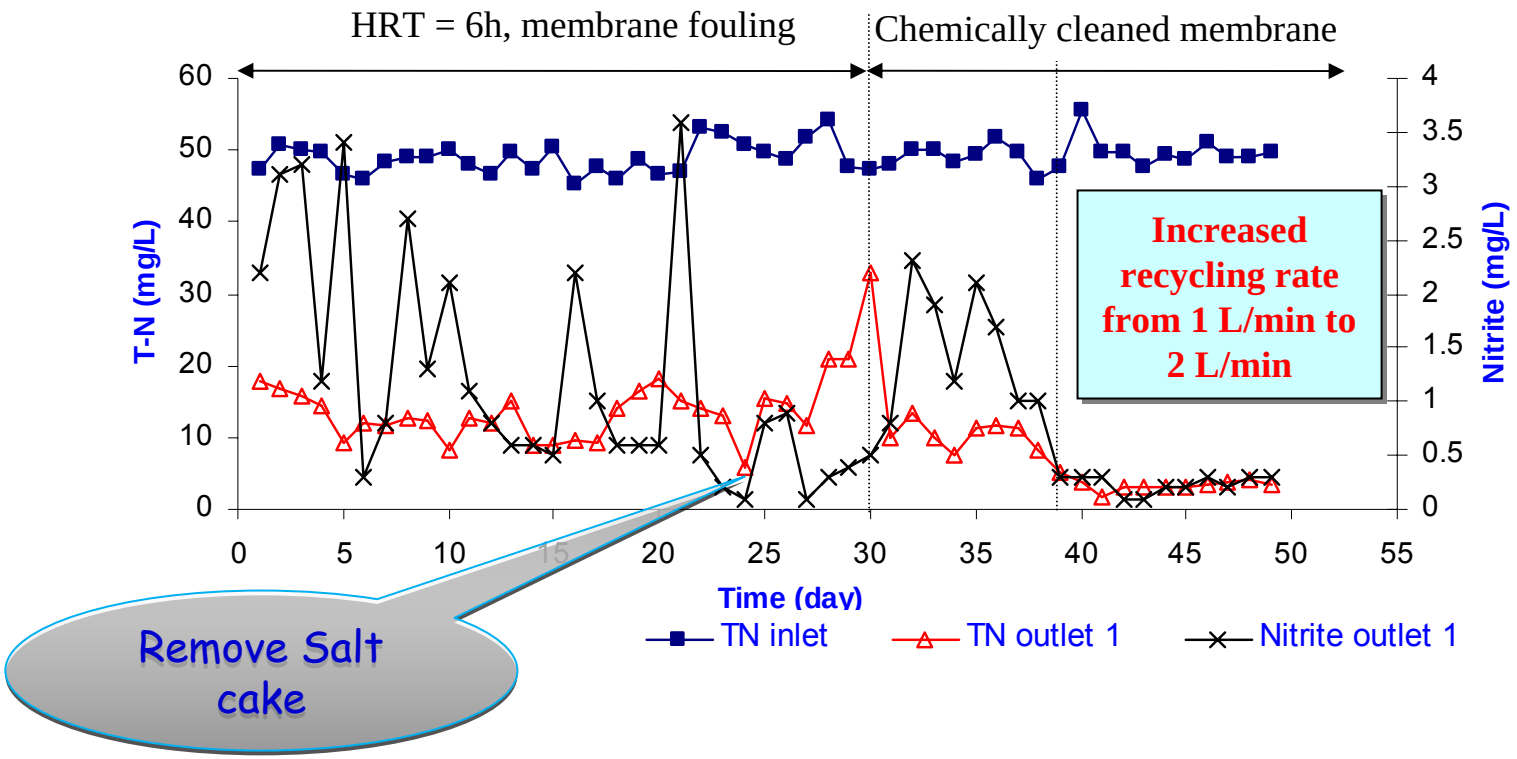
☞ Sludge acclimatization



- Results of two setups (with and without adding salinity) were similar.
- Denitrification efficiency reached to 100% after 8 days
- Direct acclimatization was more efficient than stepwise acclimatization

RESULTS AND DICUSSION

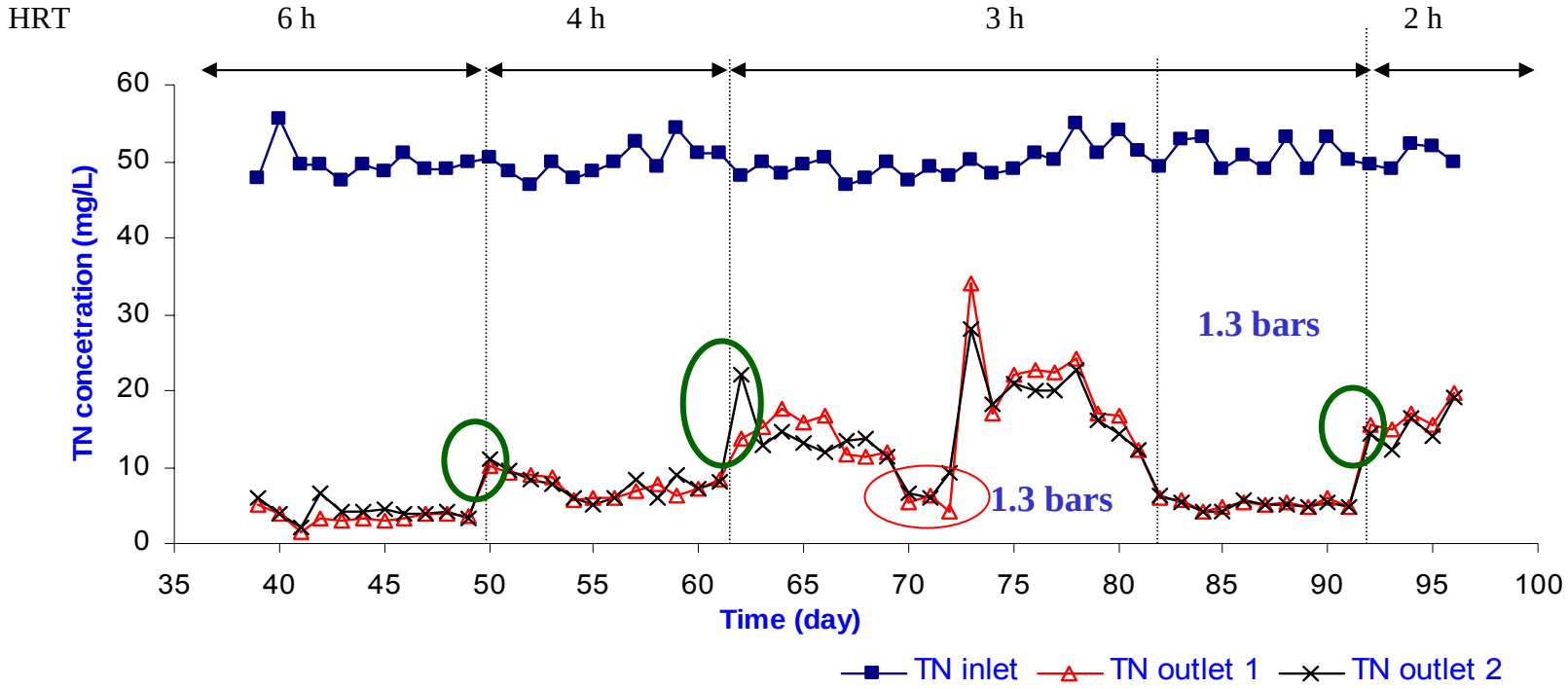
- Effect of membrane fouling and recirculating flow rate on denitrification



- Membrane fouling caused reduction of denitrification.
- Increasing recirculating flow rate from 1L/min to 2 L/min increased denitrification efficiency and reduced nitrite in the effluent.

RESULTS AND DICUSSION

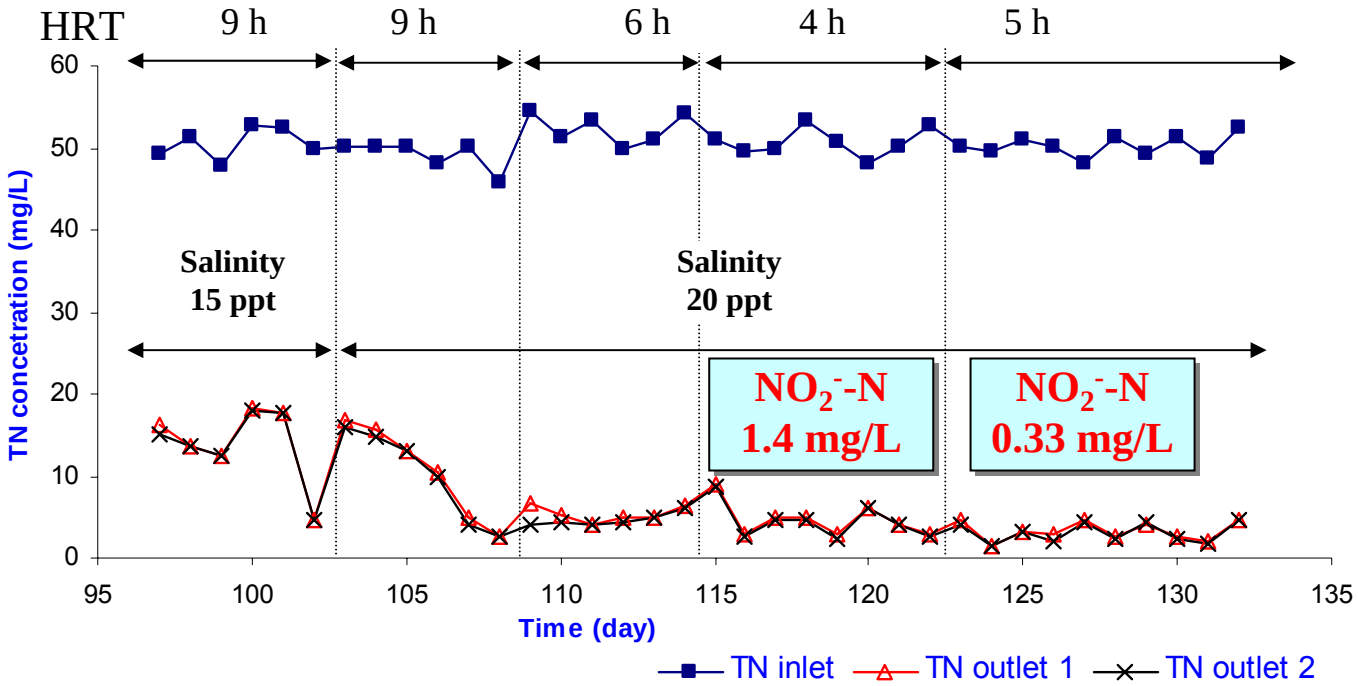
Denitrification of run 1



- Total nitrogen outlet increase when nitrogen loading suddenly increased.
- Optimum HRT = 3 h
- Denitrification efficiency of 89.8% and denitrification rate of 365.7 g/m^3.day were achieved in denitrification tank

RESULTS AND DICUSSION

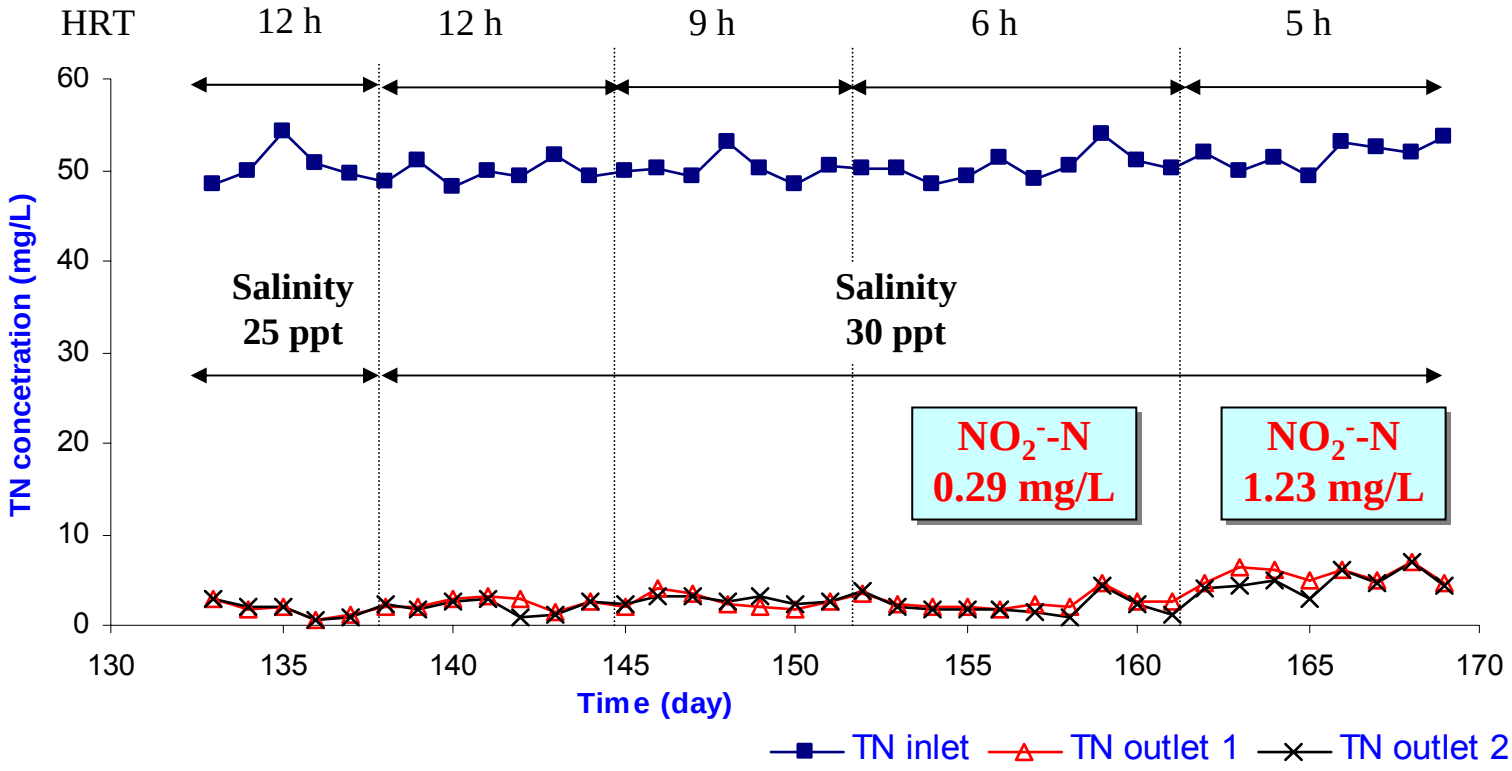
Denitrification of run 2



- Bacteria had good ability to adapt to increase of salinity
- Optimum HRT of 5 h
- Denitrification efficiency of 93.5% Denitrification rate of 225.2 $g/m^3.day$

RESULTS AND DICUSSION

Denitrification of run 3



- No distinct increase of nitrogen effluent in HRT from 12h to 6h due to long retention time
- Optimum HRT = 6 h
- Denitrification efficiency of 95% Denitrification rate of 191.3 g/m^3.day

RESULTS AND DICUSSION

☞ Nitrite accumulation

Wastewater	HRT	Efficiency of D tank (%)	Nitrite (mg/L)		Nitrite removal efficiency of A tank (%)
			Outlet 1	Outlet 2	
Saline (10 ppt)	3	89.8	0.54	0.10	**81**
Saline (20 ppt)	5	93.5	0.33	0.19	**42**
Saline (30 ppt)	6	95.0	0.29	0.21	**28**
Fresh (Hung, 2006)	3	91.4	0.10	0	**100**

- Nitrite effluent from denitrification tank was higher than that in fresh wastewater case.
- The removal of nitrite in aeration tank was too low in comparison with fresh wastewater case

RESULTS AND DICUSSION

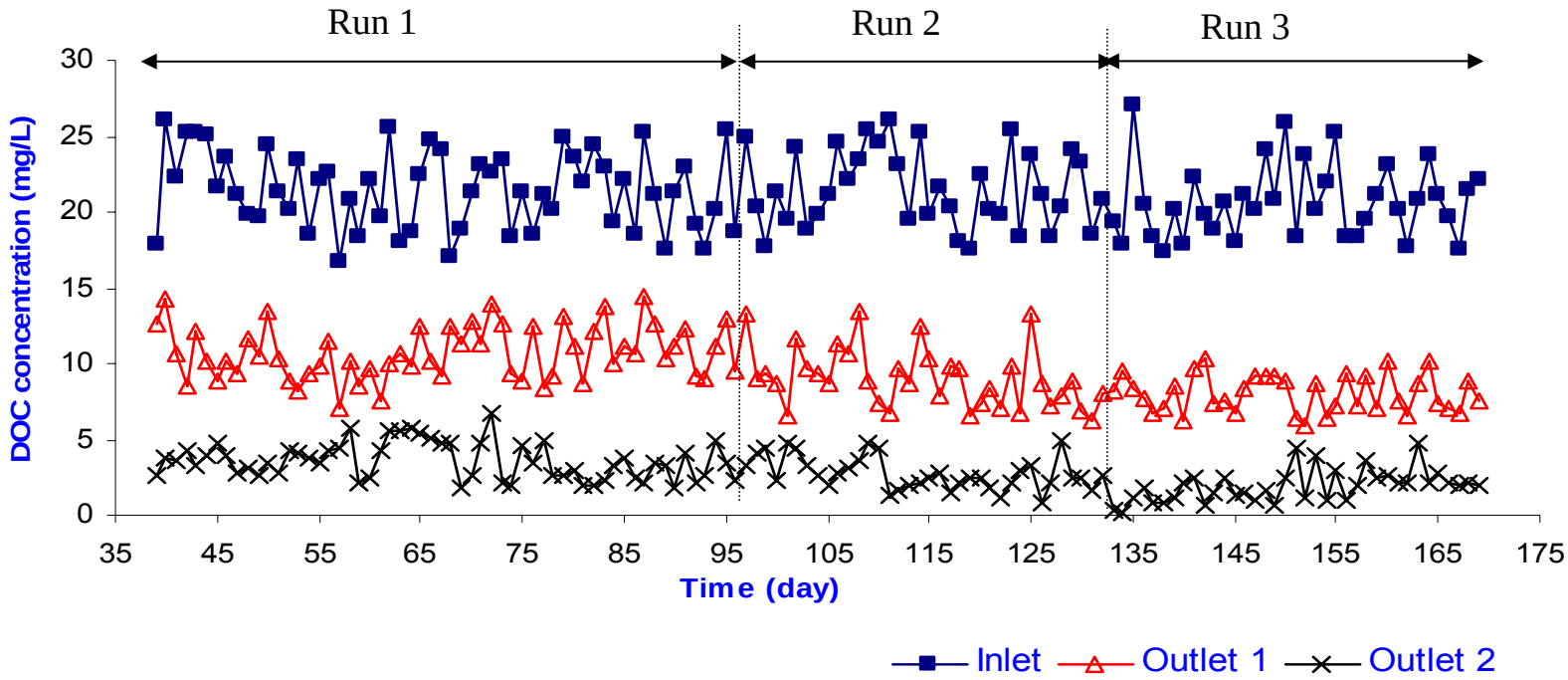
☞ Nitrogen removal of total system

Wastewater	Denitrification rate (g/m^3. day)		Denitrification efficiency (%)		Reference
	D tank	Total system	D tank	Total system	
Saline (10 ppt), 3h	365.7	366.8	89.8	90.0	
Saline (20 ppt), 5h	225.2	226.2	93.5	93.9	
Saline (30 ppt), 6h	191.3	193.2	95.0	95.9	Current study
Fresh (HRT = 3h)	363.7	365.0	91.4	91.5	Hung (2006)

- Little nitrogen amount was removed by assimilation of bacteria in aeration tank
- Result of run 1 was similar to fresh wastewater case at HRT of 3h
- Optimum HRTs increased when salinity increased

RESULTS AND DICUSSION

☞ DOC removal and involvement of heterotrophic denitrification



- DOC removal in denitrification was from 45 – 63%
- Heterotrophic denitrification took place in the nitrogen removal process, resulting in high biomass yield, from 0.42 to 0.48 g cells/g NO_3^--N

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RESULTS AND DICUSSION

Water quality after treatment

Parameter	Inlet	Outlet			Safety level
		Run 1	Run 2	Run 3	
pH	6.9 - 7.2	7.6-8.1	7.6-7.9	7.6-8.0	6.5-8.3 Blancheton, 2000
DOC (mg/L)	21.2-21.5	2.8	2.5	2.3	-
NO_3^--N (mg/L)	48-54	5.0	2.9	1.9	<50 Lucas and Southgate, 2003
NO_2^--N (mg/L)	0-1.1	0.1	0.2	0.2	<0.6 Lucas and Southgate, 2003
SS (mg/L)	-	0	0	0	<15-200 Jewell and Cummings, 1990

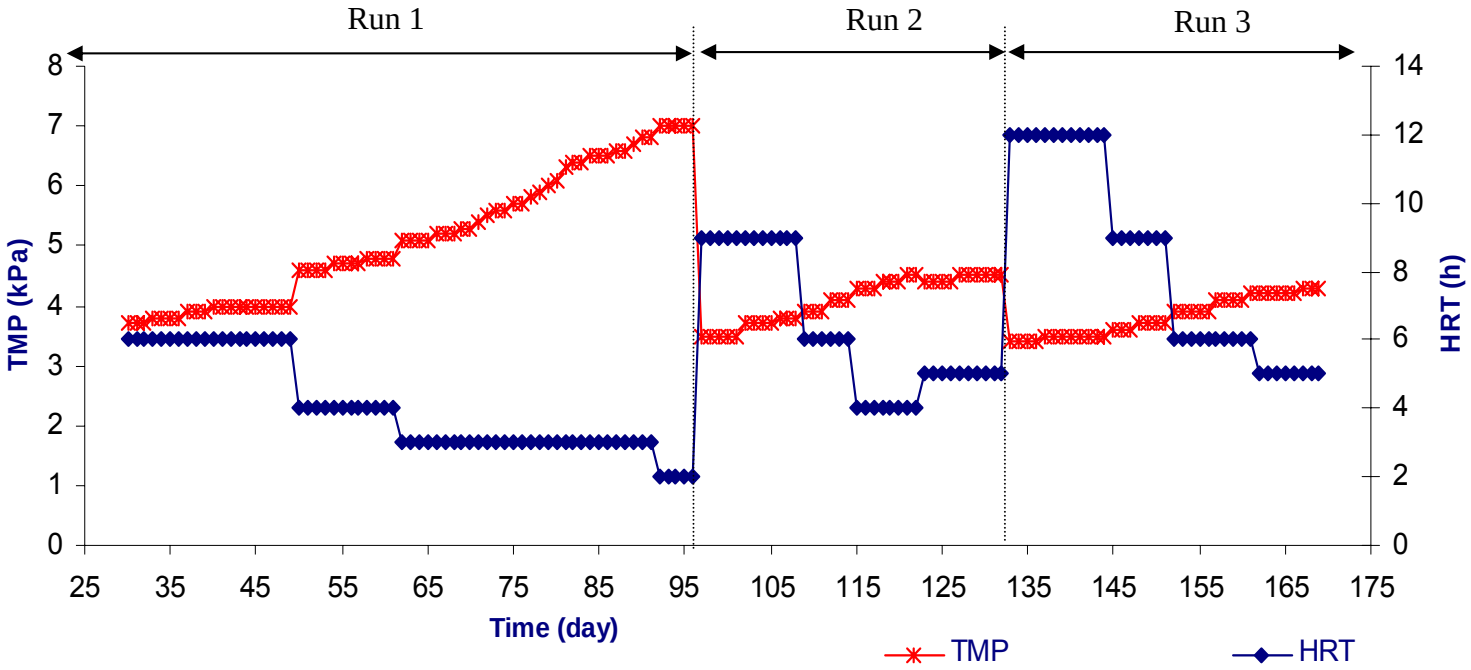
- All the parameter were lower than safety level many times
- The treated wastewater can be recycled to aquaculture pond

RESULTS AND DICUSSION

- Membrane fouling in denitrification tank
  - Membrane fouling caused reduction of denitrification at the beginning of run 1 since membrane in acclimatization stage (no CO_2 supplied) was continuously used in run 1 without cleaning
  - High recirculating flow rate (2 L/min) and CO_2 reduced the fouling
  - Diffusing membrane can be used up to one month without effect of fouling on denitrification efficiency

RESULTS AND DICUSSION

- Membrane fouling in aeration tank



- Membrane fouling was not found since the permeate flux was lower than membrane filtration capacity and biomass concentration was low.

RESULTS AND DICUSSION

- H_2 utilization and cost analysis

Type of denitrification	Cost for 1 g removed NO_3^--N (Baht/g)		Cost for 1 m^3 wastewater (Baht/m^3)		Reference
	Theory	Reality	Theory	Reality	
Heterotrophic (methanol)	0.49	0.52 – 0.96	22.1	23.4 – 44.8	-
Autotrophic (hydrogen) fresh wastewater	0.44	0.78	19.8	34.8	Hung (2006)
Autotrophic (hydrogen) saline wastewater	**0.43**	**0.52 – 0.71**	**19.7–20.6**	**23.6 – 33.7**	**Current study**

- Hydrogen utilization rate was from 0.426 to 0.583 g H_2/g N higher than theoretical value of 0.357 g H_2/g N
- Hydrogen utilization efficiency was from 61 to 84%
- The cost for treating 1 gram nitrate and 1 m^3 wastewater was comparable to using methanol as electron donor

CONCLUTIONS AND RECOMMENDATION

Conclusions

- Direct acclimatization was more efficient than stepwise acclimatization
- Optimum hydraulic retention time (HRT) of run 1, 2 and 3 was 3h, 5h and 6h.
- Denitrification rate of total system was 366.8, 226.2 and 193.2 g/m^3.day for these HRTs.
- Heterotrophic denitrification took place in nitrogen removal, resulting in high biomass yield.
- Water quality of treated wastewater was very good at optimum HRTs.
- Hydrogen utilization efficiency was from 61 to 84%. Cost for hydrogen was comparable to heterotrophic denitrification
- Diffusing membrane was not fouled within one month of operation.

CONCLUTIONS AND RECOMMENDATION

Recommendation

- Measure dissolved H_2 to find its effects on denitrification
- Investigate diversity and abundance of microbial communities
- Conduct batch experiment to study in detailed kinetic.
- Run the system with different concentrations of nitrate and organic matters
- Develop membrane fouling model for the system at different salinities

Thank You