A PRELIMINARY STUDY ON BIOLOGICAL NITROGEN REMOVAL PROCESS FOR LOCAL DRINKING WATER TREATMENT

WONG ENG CHEONG

MASTER OF ENGINEERING SCIENCE

LEE KONG CHIAN FACULTY OF
ENGINEERING AND SCIENCE
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By

WONG ENG CHEONG

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ABSTRACT

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Wong Eng Cheong

Greatly input of phosphorus (P) and nitrogen (N) into the water bodies from human activities have caused the arising of eutrophication globally. Limitations of the conventional drinking water treatment processes in removing N has brought the biological nitrogen removal (BNR) to the light and is widely recognized in developed countries with temperate climate. However, knowledge and expertise on the application of this biological treatment process is relatively limited in developing countries, so as Malaysia in tropical region. Thus, this research aims to provide a preliminary study on the establishment of the BNR process for drinking water treatment in the local context.

In the first stage of this study, a lab scale sequencing batch reactor (SBR) fed with synthetic river water and seeded with different seeding sources was operated for the establishment of BNR process. The cultivation period and performance from non-BNR sludge to BNR sludge were studied. The results showed that the SBR seeded solely with activated sludge collected from sewage treatment plant was more promising in terms of N removal after four weeks of cultivation.

In the second stage of this study, a new SBR seeded with the earlier cultivated sludge was operated to investigate the effect of influent chemical oxygen demand (COD) concentration and preanoxic/postanoxic condition on the BNR process performance. From the results, at the influent COD concentration of 45 mg/L in postanoxic condition, the final concentrations of both the COD and NH_4^+-N were at 2 mg/L and 5 mg/L respectively.

In the final stage, the stability and robustness of the process were examined. It showed that the BNR performance was consistent during the prolonged SBR operation with mixed liquor suspended solid (MLSS) and mixed liquor volatile suspended solid (MLVSS) of about 1720 mg/L and 1630 mg/L respectively as well as sludge volume index (SVI) of about 85 mL/g were stably maintained. The robustness of the process was also observed when the system rapidly recovered from an operational failure. These findings suggest that the establishment of BNR process for drinking water treatment is feasible in Malaysia.

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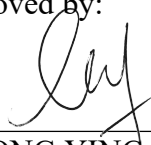
Next, I would also like to express my deepest gratitude to my loving parents and friends for their spiritual encouragement and support throughout the study.

APPROVAL SHEET

This dissertation/thesis entitled "**A PRELIMINARY STUDY ON BIOLOGICAL NITROGEN REMOVAL FOR LOCAL DRINKING WATER TREATMENT**" was prepared by WONG ENG CHEONG and submitted as partial fulfillment of the requirements for the degree of Master of Engineering Science at Universiti Tunku Abdul Rahman.

Approved by:

_____
(Dr. ONG YING HUI)
Date: 15th June 2021
Assistant Professor/Supervisor
Department of Chemical Engineering
Lee Kong Chian Faculty of Engineering and Science
Universiti Tunku Abdul Rahman

_____
(Dr. NG YEE SERN)
Date: 15th June 2021
Assistant Professor/Co-supervisor
Department of Chemical Engineering
Lee Kong Chian Faculty of Engineering and Science
Universiti Tunku Abdul Rahman

LEE KONG CHIAN FACULTY OF ENGINEERING AND SCIENCE

UNIVERSITI TUNKU ABDUL RAHMAN

Date: <u>15th JUNE 2021</u>

SUBMISSION OF DISSERTATION

It is hereby certified that ***WONG ENG CHEONG*** (ID No: **1803997**) has completed this dissertation entitled "<u>A PRELIMINARY STUDY ON BIOLOGICAL NITROGEN REMOVAL FOR LOCAL DRINKING WATER TREATMENT</u>" under the supervision of <u>Dr ONG YING HUI</u> (Supervisor) from the <u>Department of Chemical Engineering, Lee Kong Chian Faculty of Engineering and Science</u>, and <u>Dr NG YEE SERN</u> (Co-Supervisor) from the <u>Department of Chemical Engineering, Lee Kong Chian Faculty of Engineering and Science</u>.

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(*WONG ENG CHEONG*)

DECLARATION

I hereby declare that the dissertation is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

Name <u>WONG ENG CHEONG</u>

Date <u>15th JUNE 2021</u>

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS / ABBREVIATIONS

AMO — Ammonia monooxygenase

NH_3-N — Ammoniacal-nitrogen

NH_4^+ — Ammonium ion

NH_4^+-N — Ammonium-nitrogen

NH_4^+ — Ammonium-oxidizing bacteria

ANAMMOX — Anaerobic ammonium oxidation

BNR — Biological Nitrogen Removal

BOD — Biological oxygen demand

COD — Chemical oxygen demand

DOE — Department of Environment

DO — Dissolved oxygen

DWTPs — Drinking water treatment plants

EDI — Electrode-ionization

HABs — Harmful algal blooms

IWK — Indah Water Konsortium

IX — Ion Exchange

MLSS — Mixed liquor suspended solid

MLVSS — Mixed liquor volatile suspended solid

NO_3^- — Nitrate ion

NO_3^--N — Nitrate-nitrogen

NO_2^- — Nitrite ion

NO_2^--N — Nitrite-nitrogen

NXR — Nitrite oxidoreductase

NOB — Nitrite-oxidizing bacteria

N	Nitrogen
N_2	Nitrogen gas
P	Phosphorus
PAO	Phosphate-accumulating organisms
PHA	Polyhydroxyalkanoate
PHB	Polyhydroxybutyrate
RED	Reverse Electrodialysis
RO	Reverse Osmosis
SBR	Sequencing Batch Reactor
STP	Sewage treatment plant
SND	Simultaneous Nitrification-Denitrification
SVI	Sludge volume index
SBA	Strong base anion
SS	Suspended solids
TON	Threshold Odour Number
USEPA	United States Environmental Protection Agency
WQI	Water Quality Index
WHO	World Health Organization

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Water is an essential element in our daily life. However, poor water quality has drawn the public attention especially in the developing countries (Edokpayi et al., 2017). Rapid urbanization, industrialization, agricultural runoff and improper treated sewage have caused water pollution. These human activities have largely increased the nitrogen (N) and phosphorus (P) contents in the water bodies. This eventually caused eutrophication to the surface water and groundwater (Le Moal et al., 2019).

Eutrophication, a globally recognized environmental issue, can be observed through the increase of algal growth in the water ecosystem. Although eutrophication is a natural occurring phenomenon, the presence of harmful algal blooms (HABs) from human activities have accelerated the eutrophication process (Wurtsbaugh et al., 2019). The increasing presence of HABs and hypoxia have been observed around the world (Sinha et al., 2017). This phenomenon has caused the reduction in water quality and subsequently affected aquatic life.

N-based contaminants such as ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) are reported as primary contributors to the eutrophication (Xue et al., 2016). These contaminants not only cause adverse effects to the environment, but also bring harm to human health, for example, the development of N-nitroso carcinogen compounds and infant methemoglobinemia (Ward et al., 2018). In order to overcome the nutrient pollution in water system, different regulations have been enforced across the globe to control the discharge of N into the water bodies. As listed in Drinking Water Quality Standard of Malaysia and United States Environmental Protection Agency (USEPA), the maximum allowable concentration of ammoniacal-nitrogen (NH_3-N) and nitrate-nitrogen (NO_3^--N) in drinking water system are 1.5 mg/L and 10 mg/L respectively.

N-based contaminants can be removed through physical and chemical methods as well as biological method. Conventional drinking water treatment plants (DWTPs) have been practicing N removal through physical and chemical methods, however, the removal efficiency is not always promising. Thus, biological method which termed biological nitrogen removal (BNR) is being employed as an alternative approach in recent years. And, its application in drinking water treatment has been reported for its effectiveness (Mohseni et al., 2013).

BNR is a two-step process which consists of nitrification and denitrification. Nitrification is an oxidation process of converting NH_4^+ to NO_2^- and NO_3^- in the presence of nitrifying bacteria, whereas a reduction process,

denitrification converts NO_3^- to nitrogen (N_2) gas with the help of denitrifying bacteria (Gerardi, 2002). The alternating aerobic and anoxic conditions of BNR process accelerate the microbial activity which is responsible for N removal.

1.2 Problem Statement

Rivers (surface water) are the most important source for domestic water supply and irrigation (Salmiati and Salim, 2017). According to World Health Organization (WHO) (2017), about 70.7% of the world population has easy access to treated drinking water services, but still the remaining 29.3% has limited access. Figure 1.1 illustrated the river water quality in Malaysia from 2008 – 2018. In 2018, it was found that about 638 river basins in Malaysia that were studied, 56% of the river basin was found clean, 36% was slightly polluted and 8% was categorized as polluted.
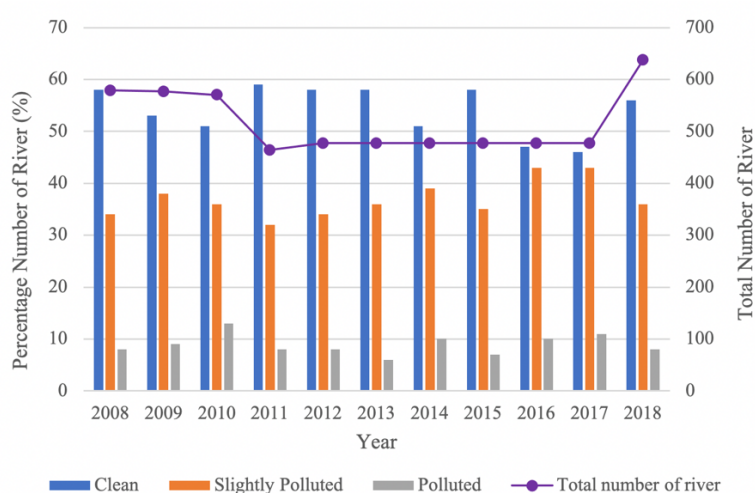
Figure 1.1: River Water Quality in Malaysia from 2008 – 2018 (Adapted from DOE, 2018)

Among the river basins studied, Langat River (Sungai Langat) is the most important water sources in Selangor. However, the growth of industrialization and human activities have led to the deterioration of water quality in Langat River (Juahir et a., 2011). In recent years, several shutdown incidents of water treatment plants at Langat River have been reported due to the excess of ammonia level. Reports have identified that the contamination sources were originated from upstream industrial effluent discharge (Abidin et al., 2018). Meanwhile, the Galing River (Sungai Galing), Kuantan, is one of the most polluted rivers at the east coast of Malaysia. The increase concentration of N-based contaminants was observed in Galing River, which was resulted by rapid urbanization (Kozaki et al., 2016)

Many full scale BNR operating plants have been well-implemented in developed countries for drinking water treatment. However, knowledge and expertise of this process is relatively limited in developing countries such as Malaysia (Hasan and Muhammad, 2020). As the characteristic of drinking water sources vary across the regions, the optimum operational conditions of BNR in local context are essential for its successful application in local drinking water treatment plant.

1.3 Research Objectives

In line with the problem statements, this research aims:

a) To establish a lab scale BNR process for local drinking water treatment by using different seeding sources.

Since there is no BNR process for drinking water treatment in Malaysia, BNR sludge was cultivated using different seeding sources. The acclimatization period of the non-BNR sludge to establish BNR characteristics is important for the operational feasibility.

b) To improve the process performance by looking into initial chemical oxygen demand (COD) concentration and preanoxic/postanoxic conditions.

After the BNR sludge is acclimatized, operational conditions should be further optimized to improve the process performance.

c) To study the stability and robustness of the lab scale BNR process for local drinking water treatment.

The study on the stability and robustness of the process could serve as the reference and guidance for the real BNR operation for drinking water treatment in Malaysia as it is still at its infancy.

1.4   Structure of Dissertation

This dissertation is presented in seven chapters and the contents of each chapter are described below.

Chapter 1 highlights the background and problem statements, as well as the objectives of this research.

Chapter 2 reviews the literature studies and findings from other researchers.

Chapter 3 details the materials and methods used in this research.

Chapter 4 reports about the cultivation of non-BNR sludge to BNR sludge using different seeding sources.

Chapter 5 discusses on the effect of initial COD concentration and preanoxic/postanoxic condition to the BNR process performance.

Chapter 6 shows the stability and robustness of the lab scale BNR process.

Chapter 7 concludes the research findings and recommendations for future studies.

CHAPTER 2

LITERATURE REVIEW

2.1   Water Pollution in Malaysia

The point-source water pollution is originated from sewage treatment plants, livestock and industrial areas whereas the nonpoint-source water pollution is the accumulation from different sources such as the agricultural runoff.

In 2018, Department of Environment (DOE), Malaysia has compiled a data of point sources which focused on wastewater discharged from manufacturing and agricultural industries, wet market, piggery as well as sewage treatment plant. The assessment has focused on three primary parameters, NH_3-N, biological oxygen demand (BOD) and suspended solids (SS), which are the basic benchmark in identifying pollution level in the water. High concentration of BOD promotes rapid oxygen depletion where less oxygen content is available in the water environment that subsequently kills aquatic life. The depletion of oxygen is also caused by the decomposition of algae. High amount of nutrients such as N and P lead to eutrophication and promote algae growth. When algae decompose, the dissolved oxygen in water depletes which further affects the aquatic ecosystem. Table 2.1 shows the distribution of parameters (BOD, SS & NH_3-N) for various point sources of water pollution identified by DOE, Malaysia in year 2018. According to the

statistical data in Table 2.1, high BOD was generated from the sewage treatment plant and piggery, followed by the agro-based industries. Majority of SS and NH_3-N pollutions were originated from sewage treatment plant and piggery compared to other point sources.

Table 2.1: Distribution of Parameters (BOD, SS & NH_3-N) for Various Point Sources of Water Pollution Identified in Year 2018 (DOE, Malaysia)

Sources	BOD		SS		NH_3-N	
	Tons/day	%	Tons/day	%	Tons/day	%
Sewage treatment plant	242	37.0	303	36	162	79
Piggery	217	33.2	450	54	27	13
Manufacturing industries	55	8.4	35	4	5	3
Agro-based industries	133	20.4	39	5	11	5
Wet markets	6	0.9	8	1	0.3	0.1
Total	545	100	909	100	229.3	100

Source: Department of Environment, Ministry of Environment and Water, 2018

2.1.1 Effects of Nutrient Pollution

Eutrophication, a direct effect caused by the high amount of nutrients such as N and P in the water. Excess concentration of nutrients promotes the growth of phytoplankton, a type of microalgae which causes the oxygen depletion in the

water bodies (Bužančić et al., 2016). Moreover, death and decay of algae further reduces the oxygen level in the waterways, produces "dead zones" in the water bodies (Wurtsbaugh et al., 2019).

Other than the environmental effect, high intake of N-based contaminants cause health issue too, such as the development of cancer-causing agent, the N-nitroso carcinogens (Davidson et al., 2011). In addition, exposure of N-based contaminants to infants may lead to brain damage, blue baby syndrome and miscarriage to pregnant women (Omer, 2019). Therefore, it is crucial to remove N-based contaminants from the water bodies.

2.1.2   Stoppages of Water Treatment Plants

The increase cases of river water pollution in recent years have caused the water treatment plants to experience shutdown and directly affected the clean water supply to consumers especially in Selangor state. Langat River, the main water sources in Selangor, has reported eight cases of river pollution in year 2016, as a result of ammonia pollution from unlicensed factories and palm oil spillage. More than one million consumers were affected due to the Langat water treatment plant closure (Edward et al., 2016). In the study of Basheer et al. (2017), the Langat River pollution sources are identified as industrial discharge (58 %), domestic sewage (28 %), construction projects (12 %) and pig farming (2 %).

In year 2019, it was reported about 100 L of benzene contaminant was illegally disposed from a nearby industrial area and from a car workshop into Selangor River (Sungai Selangor). This has caused four water treatment plants to shut down, resulting the water supply disruption for 1.17 million consumers (Alisha, 2019). In December of the same year, Semenyih treatment plant had experienced large-scale operational shutdown due to the disposal of industrial solvent waste into manhole in Bandar Bukit Mahkota, Cheras, affecting about 350 thousand consumers (TheSunDaily, 2019).

In October 2020, Gong River (Sungai Gong) was severely polluted by the illegal scheduled waste disposal from a factory premise. The four major Rantau Panjang water treatment plants closed down when three TON (Threshold Odour Number) was detected and led to the unscheduled water supply disruption, affecting almost one million consumers in the Selangor state (Khairulrijal, 2020). In the following month, another water contamination has been detected in Selangor River, causing water cuts in 1300 areas across the Selangor State. Eight TON was tested at Rantau Panjang water treatment plant and four TON at Phase 1, 2, 3 of the Sungai Selangor water treatment plant (NewStraitsTimes, 2020; TheStar, 2020).

2.2 Drinking Water Quality and Standards in Malaysia

The cases of water pollution are getting worsen as the input of untreated water into the river bodies by irresponsible third parties have largely increased, therefore, local authorities have taken their responsibility in practising the

regulations such as Environmental Quality Act 1974 and Penal Code (Act 574) to reduce water pollution cases. In addition, WHO Drinking Water Quality Guidelines (1993/96/98) was used as the reference source for the drafting of National Standard for Drinking Water Quality, Malaysia (2004). Besides, USEPA was also used for the monitoring of water quality. Both the guidelines stated the threshold concentration of NH_3-N and NO_3^--N in drinking water system are 1.5 mg/L and 10 mg/L respectively. The recommended acceptable value for water quality in Malaysia is as shown in Table 2.2.

Table 2.2: Recommended Water Quality in Malaysia

Parameter	Unit	Acceptable value	
		Raw Water Quality	**Drinking Water Quality**
pH	-	5.5 - 9.0	6.5 - 9.0
Total Dissolved Solids	mg/L	1500	1000
BOD_5, 20°C	mg/L	6.0	-
COD	mg/L	10.0	-
Chloride	mg/L	250	250
Anionic Detergent MBAS	mg/L	1.0	1.0
Ammoniacal Nitrogen (NH_3-N)	mg/L	1.5	1.5
Nitrate-Nitrogen (NO_3^--N)	mg/L	10.0	10.0
Iron (Fe)	mg/L	1.0	0.3
Fluoride	mg/L	1.5	0.4 – 0.6
Hardness	mg/L	500	500
Manganese	mg/L	0.2	0.1

Source: Ministry of Health Malaysia, 2009

In addition, Water Quality Index (WQI) has been practiced in Malaysia for years to evaluate the status of river water quality. It also serves as the basis for the assessment of environmental water quality. Five major classes have been categorized based on the water quality, as shown in Table 2.3. The DOE water quality classification based on Water Quality Index is tabulated in Table 2.4. The index range are calculated based on formula that are termed as sub-index, which will be then categorized into its pollution level according to the index range.

Table 2.3: DOE Water Quality Index Classification

Parameter	Unit	Class				
		I	II	III	IV	V
Ammoniacal Nitrogen	mg/L	< 0.1	0.1 - 0.3	0.3 - 0.9	0.9 - 2.7	> 2.7
BOD	mg/L	< 1	1 - 3	3 - 6	6 - 12	> 12
COD	mg/L	< 10	10 - 25	25 - 50	50 - 100	> 100
Dissolved Oxygen	mg/L	> 7	5 - 7	3 - 5	1 - 3	> 1
pH	-	> 7	6 - 7	5 - 6	< 5	> 5
Total Suspended Solid	mg/L	< 25	25 - 50	50 - 150	150 - 300	> 300
Water Quality Index (WQI)	-	< 92.7	76.5 - 92.7	51.9 - 76.5	31.0 - 51.9	> 31.0

- Class I: Conservation of natural environment; no treatment necessary
- Class II: Conventional treatment; sensitive aquatic species
- Class III: Extensive treatment required; tolerant species; livestock drinking
- Class IV: Irrigation
- Class V: None of the above

Table 2.4: DOE Water Quality Classification based on Water Quality Index

Sub Index & Water Quality Index	Index Range		
	Clean	Slightly Polluted	Polluted
Biochemical Oxygen Demand (BOD)	91 – 100	80 – 90	0 – 79
Ammoniacal Nitrogen (NH$_3$-N)	92 – 100	71 – 91	0 – 79
Suspended Solids (SS)	76 – 100	70 – 75	0 – 69
Water Quality Index (WQI)	81 – 100	60 – 80	0 – 59

2.3 Removal of Nitrogen in Drinking Water Treatment

N-based contaminants in surface water are mainly originated from the improper treated sewage and agricultural runoff. Therefore, controlling N discharged into surface water is a key factor in preventing environmental and health effects from N-based contaminants. To date, N removal in drinking water can be carried out by using physical/chemical and biological methods.

2.3.1 Physical/Chemical Methods

Physical/chemical methods such as sorption, reverse osmosis, electrodialysis have been used to remove N-based contaminants in the drinking water treatment (Mohseni et al., 2013). Ion exchange (IX), a sorption process, is the most commonly used method for NO$_3^-$ removal from drinking water due to its

simplicity, effectiveness and low cost. Figure 2.1 illustrated the schematic diagram of IX process for drinking water.
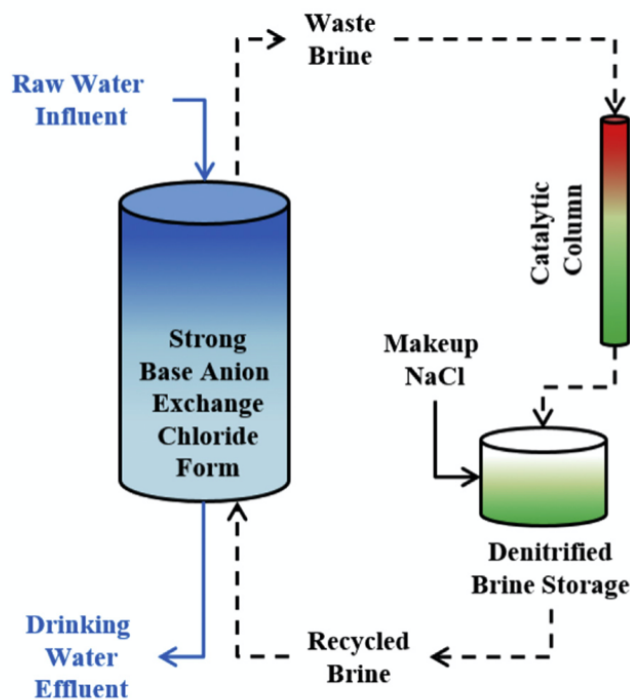
Figure 2.1: Schematic Diagram of Ion Exchange for Drinking Water (Bergquist et al., 2016)

In the IX process, the nitrate-loaded water is passed through a strong base anion (SBA) exchange resins whereby the NO_3^- are absorbed to exchange with chloride ions (Fux et al., 2017; Nujić et al., 2017). In the study of Fux et al. (2017), Purolite A520E and Purolite A300 were used as the IX strong base resins. Purolite A520E is often used for NO_3^- removal from drinking water while Purolite A300 has high operating capacity and good regeneration efficiency. Both the SBA exchange resins achieved more than 90 % of the NO_3^- absorbed on the resins. Among them, Purolite A520E shows slightly better performance as the treated effluent has lower concentrations of

bicarbonate and sulfate ions compared to Purolite A300. The regeneration of IX resins is performed by 8% sodium chloride solution, leaving the spent IX brine containing high NO_3^--N and sodium chloride concentrations.

According to Amini (2018), IX provides effective performance in different water quality from household treatment to drinking water treatment plant. However, IX process possesses side impacts to the environmental and economic costs. The additional by-products such as NO_3^-, chloride, sulfate rich brine require external treatment plants which further incurred extra costs and labour.

In addition, adsorption is also a process that is used in removing impurities such as N-based contaminants from the water bodies, due to its simplicity, cost effective and good removal efficiency (Liu et al., 2018; Dong et al., 2017). It is a process of the solution containing absorbable solute (adsorbate) with highly porous solid surface (adsorbent), whereby the liquid-solid intermolecular forces of attraction cause some adsorbate to be deposited on the adsorbent. In Liu et al. (2018), zeolite has strong adsorption activity on NH_3-N and has been widely applied in water treatment. However, the removal speed with solely zeolite was relatively low. The research found that zeolite supported on dielectrophoresis (DEP) certainly improved the removal speed and efficiency (95 %) compared to unsupported zeolite (66.7 %). This is because the additional of titanium mesh electrode has increased surface area and active adsorption site. However, adsorption speeds up the reactor clogging and requires external cost for regeneration (Crini and Lichtfouse, 2019).

Besides that, reverse osmosis (RO) is also being used to remove NO_3^- from drinking water sources. Figure 2.2 shows the schematic diagram of RO process. In the RO process, the water sources are passed through a semi-permeable membrane by a hydrostatic pressure, leaving the purified water as permeate water while the dissolved organics and inorganics are retained in the concentrate water.
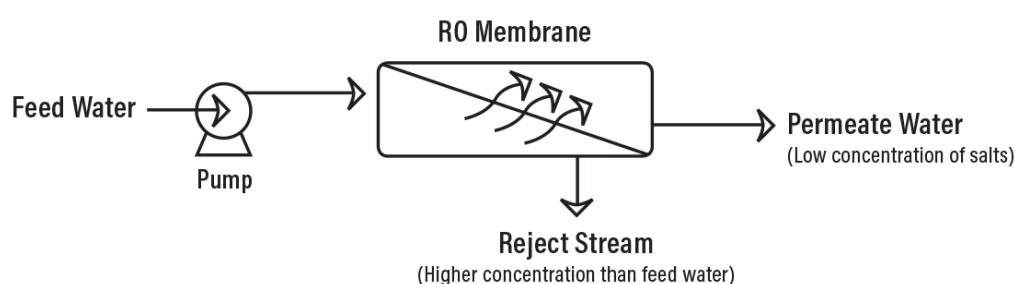
Figure 2.2: Schematic Diagram of Reverse Osmosis

Pirsaheb et al. (2016) conducted a study on RO pilot plant for NO_3^- removal and found that about 90 % of NO_3^- removal efficiency was achieved from the nitrate-loaded water sources (~ 140 mg/L) to permeate water (~ 13 mg/L). However, the study left behind the high NO_3^- concentration in concentrate water (~ 500 mg/L) which has to be disposed with external process. Besides, the removal efficiency of cations and anions such as bicarbonate, sulphate, chloride, sodium and magnesium were more than 90 %.

Other than that, electrodialysis is also applied in drinking water treatment, especially the high recurring problem of salinity in groundwater and surface water (Kunrath et al., 2020). The process transfers ionic compounds that are attracted to the electrodes using electrical potential as driving force by

passing through ion-selective membranes. Figure 2.3 shows the mechanism of electrodialysis.
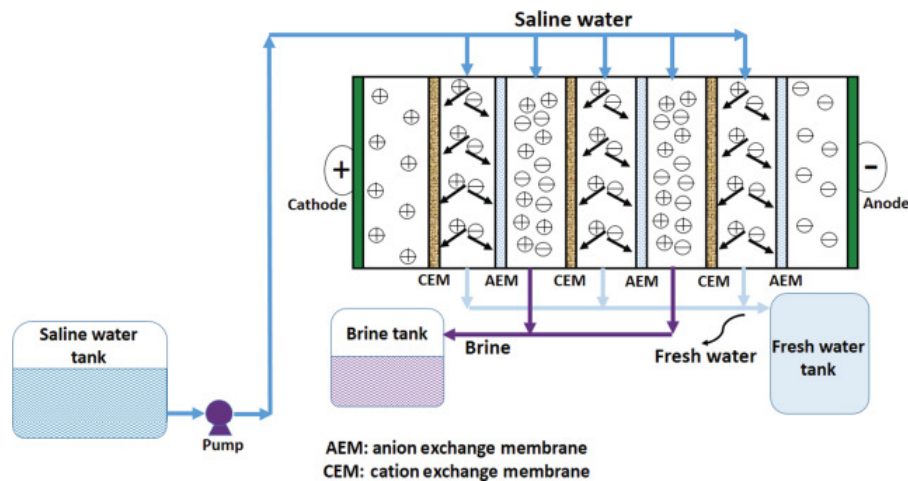
Figure 2.3: Mechanism of Electrodialysis in Water Treatment (Aghaeinejad and Ghasemzadeh, 2017)

Cotruvo (2005) reported that the desalination for drinking water has to evaluate and consider the water characteristics. Both the surface water and groundwater contains contaminants such as microorganisms and toxic elements. The study reported that conventional electrode-ionization (EDI) produces high purity water and remove trace contaminants. It achieved 90 % - 96 % of metal ions (sodium, magnesium, calcium, potassium) and NO_3^- removal. Recent study has showed a promising result in reverse electrodialysis (RED) process in terms of the power generation where the treatment plants are placed near the river and sea for both sources used as influent. However, electrodialysis is not suitable for low solute feed concentration which becomes the major disadvantages (Crini and Lichtfouse, 2019).

2.3.2   Biological Method: Biological Nitrogen Removal (BNR)

BNR has been widely used in developed countries to remove N-based contaminants in the drinking water treatment since the first full-scale plant (80 m^3/h) established at France in 1982. A larger plant (400 m^3/h) was then operated and achieved complete N and organic compounds removal (Rogalla et al., 1990). This achievement has surely cleared the uncertainties and doubt of BNR process in drinking water treatment, making an attention of this treatment process in developing countries. BNR is a process which involves alternating anoxic-aerobic condition, termed as nitrification and denitrification. Figure 2.4 shows the mechanism of typical BNR process.
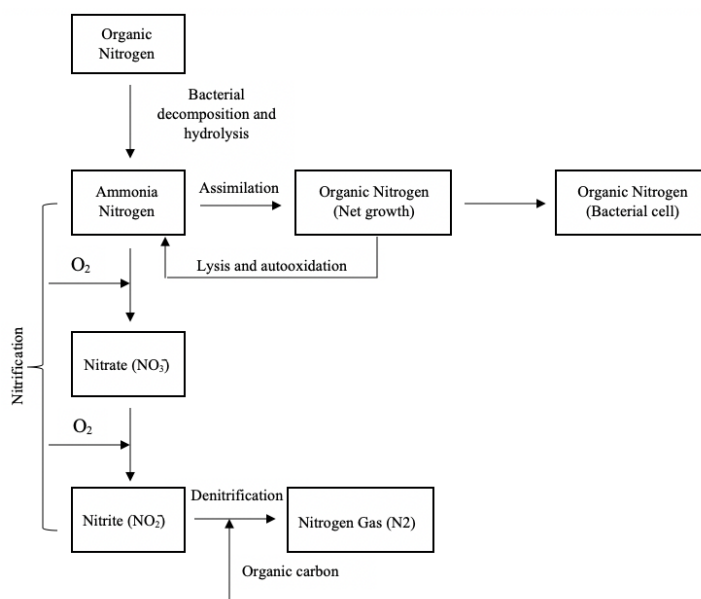
Figure 2.4: Mechanism of Typical BNR Process (Tchobanoglous et al., 2013)

Nitrification is a two-step oxidation process of converting NH_4^+ to NO_2^- by ammonium-oxidizing bacteria (AOB) and converting NO_2^- to NO_3^- by

nitrite-oxidizing bacteria (NOB) (Tchobanoglous et al., 2013). The autotrophic bacteria such as *Nitrosomonas* and *Nitrobacter* use oxygen as electron acceptor and utilize NH_4^+ as energy source. The oxidation of NH_4^+ to NO_2^- requires two steps with hydroxylamine (NH_2OH) as the intermediate (Equations 2.1, 2.2 and 2.3) (Ge et al., 2015).

$$NH_3 + O_2 + 2H^+ + 2e^- \rightarrow NH_2OH + H_2O - energy \qquad (2.1)$$

$$NH_2OH + 0.5O_2 \rightarrow HNO_2 + 2H^+ + 2e^- - energy \qquad (2.2)$$

$$NH_2OH + H_2O \rightarrow HNO_2 + 4H^+ + 4e^- + energy \qquad (2.3)$$
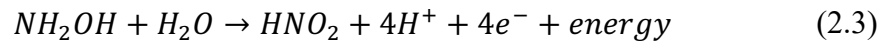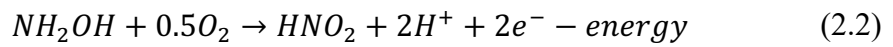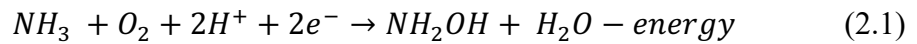
Although NH_4^+ is the energy source for autotrophic nitrifiers, however, not all NH_4^+ in the bacterial cell is nitrified. Some NH_4^+ is used for cell growth where carbon dioxide acts as the carbon source (Equation 2.4) (Gerardi, 2002). The overall oxidation reaction is shown in Equation 2.5:

$$4CO_2 + 4H_2O + NH_4^+ + HCO_3^- \rightarrow C_5H_7O_2N + 3H_2O + 5O_2 \qquad (2.4)$$

$$NH_4^+ + 1.83O_2 + 1.98HCO_3^-$$
$$\rightarrow 0.021C_5H_7O_2N + 0.98NO_3^- + 1.041H_2O + 1.88H_2CO_3 \qquad (2.5)$$
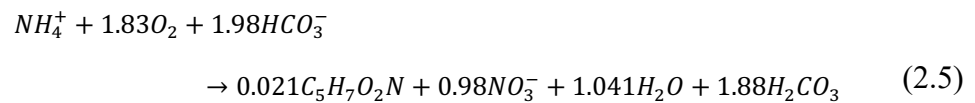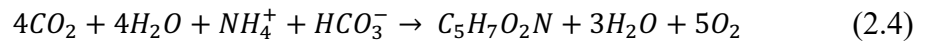
Denitrification involves the NO_3^- reduction to N_2 with the presence of NO_2^- intermediate. *Azopira sp*, *Pseudomonas* and species of *denitrificans* are the examples of responsible denitrifying bacteria that use NO_3^- as electron acceptor and organic carbon as energy source. The reduction reaction is as shown in Equation 2.6:

$$2NO_3^- + 10H^+ + 10e^- \rightarrow N_2 + 2OH^- + 4H_2O \qquad (2.6)$$
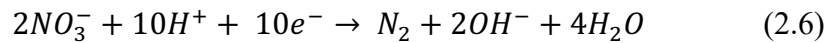
To date, several new BNR systems have been developed to improve the conventional BNR system, such as, simultaneous nitrification-denitrification (SND), shortcut nitrification-denitrification and anaerobic ammonium oxidation (ANAMMOX). These systems were reported to achieve 60% - 100% of total nitrogen (TN) removal with different discharged elements and operating conditions (Zhu et al., 2008).

2.3.2.1   Simultaneous Nitrification-Denitrification (SND)

Simultaneous nitrification-denitrification (SND) is defined as the nitrification and denitrification processes occur in the same reactor (Bueno et al., 2018), where it combines the nitrifying and denitrifying ability to remove N contaminants. Figure 2.5 illustrated the process flow of the SND.
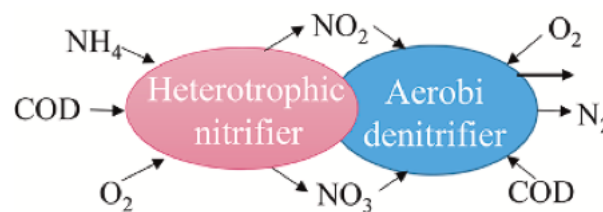
Figure 2.5: Process Flow of Simultaneous Nitrification and Denitrification (Chai et al., 2019)

In a SND system, NH_4^+-N was removed completely in a single suspended sludge sequencing batch reactor. The operation was carried out by 5 phases in a cycle. In the study of Khanitchaidecha et al. (2015), NH_4^+-N was

completely nitrified and the N removal efficiency reached about ~ 65 % in the SND system. However, the denitrification process was ineffective, causing the NO_3^--N to remain in the effluent. The reason behind is caused by the high oxygen content (4 – 5 mg DO/L) and low organic carbon content (< 1.5 C/N ratio).

In addition, since both processes require different oxygen level, dissolved oxygen (DO) plays an important role. Gogina and Gulshin (2016) studied that the TN loss were about 85% in oxidation ditch SND with 0.5 mg DO/L. This showed that the nitrification was taken place concurrently with denitrification at low oxygen level. This was further reported by Zhu et al. (2008) that the SND technology achieved complete TN removal in oxidation ditch and sequencing batch reactor (SBR) with only N_2 as the discharged element.

2.3.2.2   Shortcut Nitrification-Denitrification

Shortcut nitrification-denitrification is a process where NO_2^- is produced as an intermediate in nitrification and reduced to N_2 in the following denitrification process (Hou et al., 2017). Figure 2.6 demonstrated the schematic pathway of the shortcut nitrification-denitrification.

$$NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^- \quad | \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$
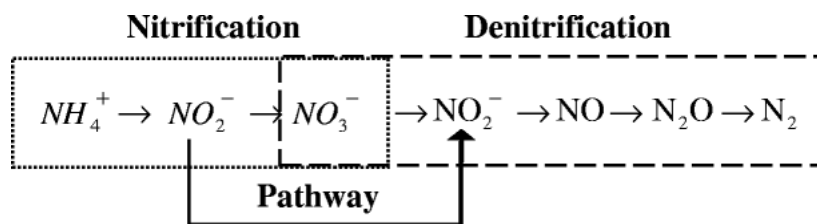
Figure 2.6: Schematic Pathway of Shortcut Nitrification-Denitrification (Wong et al., 2011)

In this process, the inhibition of NOB is crucial because of its ability to oxidize NO_2^- to NO_3^- and caused a complete nitrification (Hou et al., 2017). Gao et al. (2015) and Zhang et al. (2014) demonstrated that the shortcut nitrification-denitrification in SBR achieved 70-90 % TN removal rate with inhibition of NOB through pH range between 7.0 to 7.8 and low dissolved oxygen (1-2 mg/L).

2.3.2.3 Anaerobic Ammonium Oxidation (ANAMMOX)

In ANAMMOX, NH_4^+ is oxidized to N_2 by anaerobic-oxidizing bacteria where hydrazine and hydroxylamine are the intermediate products (Gebus and Halas, 2016; Zhang et al., 2008). Magdum and Kalyanraman (2017) reviewed that the first full scale plant was built as Integrated Fixed-Film Activated Sludge (IFAS) ANAMMOX process in which the AOB are enriched in suspended activated sludge while ANAMMOX bacteria are enriched on a biofilm. Figure 2.7 shows the schematic process of anaerobic ammonium oxidation.
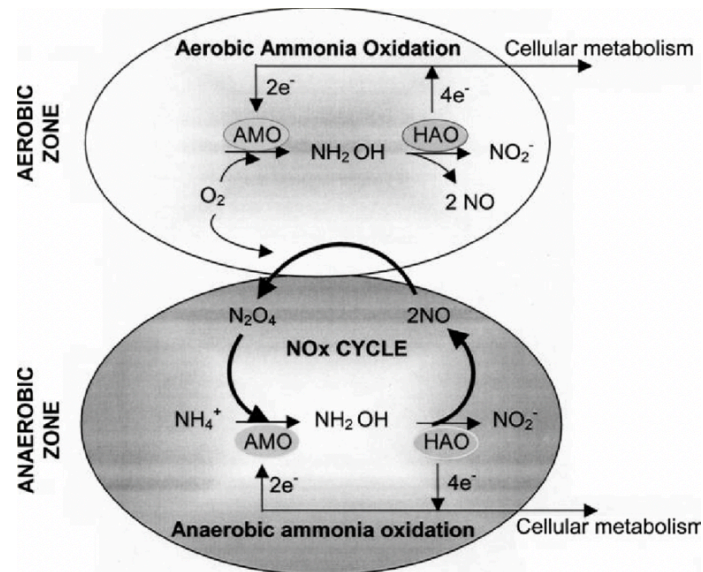
Figure 2.7: Schematic Process of Anaerobic Ammonium Oxidation (Bagchi et al., 2009)

Kokabian et al. (2018) and Wang et al. (2015) reported that ANAMMOX bacteria is more suitable for the process due to their susceptibility to saline water constituent and less oxygen requirement. Besides, the system is enhanced by the drinking water residue in lake sediment as the microbial activity and concentration are improved with the drinking water residue. However, oxygen level more than 0.4 mg/L and NO_2^- concentration of 100 mg/L inhibited the ANAMMOX process. Although ANAMMOX are capable to remove NH_4^+-N, however, there are few limitations in this system, for instance the sensitivity to oxygen and low growth rate of the resident microorganism (Khanitchaidecha et al., 2015). In addition, ANAMMOX-based treatment plants have faced some other issues such as N-based contaminants built up, scaling and foaming issues, as well sludge separation issues which make a challenge for researchers to resolve (Magdum and Kalyanraman, 2017).

2.4   **Factors affecting Nitrogen Removal Process**

Although BNR for drinking water treatment is well-developed in certain countries, however, it is still a challenge to ensure the stability and reliability of BNR in developing countries such as Malaysia. This is mainly influenced by few factors that affect the BNR performance.

2.4.1   **Organic Carbon**

The organic carbon content is important in a biological process because autotrophic and heterotrophic bacteria use them for cell growth. Zielinska et al. (2012) and Chiu et al. (2007) reported that the COD/N ratio has high effect to the microbial population. High COD/N ratio causes the decrease in nitrification efficiency due to the competition of carbon source between autotrophs and heterotrophs in a single reactor. This results in significant drop of AOB population. In contrast, low COD/N ratio promotes partial nitrification, and this causes NO_2^- accumulation. Zielinska et al. (2012) also claimed that only denitrification activity was observed at COD/N ratio of 0.7. However, at COD/N ratio of 6.8, high activity of heterotrophs that utilize oxygen was noticed which favored N removal.
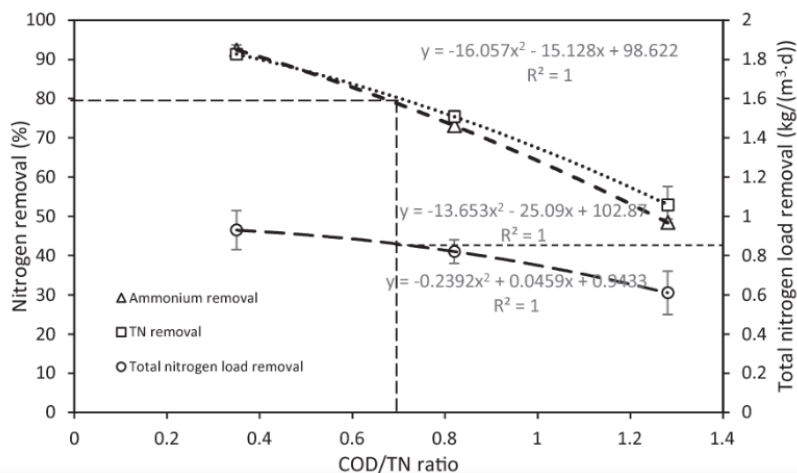
Figure 2.8: Effect of COD/TN Ratio to BNR Performance (Li et al., 2016)

The relationship between COD/TN ratio to N removal was studied by Li et al. (2016). Figure 2.8 shows that approximate 90% of NH_4^+ and TN removal were achieved at COD/TN ratio of about 0.4, while the removal performance decreased as the COD/TN ratio increased.

2.4.2   Alternating Preanoxic/Postanoxic Conditions

In a BNR process, a combination of nitrification and denitrification should be included to complete the objective of TN removal by oxidation and reduction. Preanoxic and postanoxic have become the possible configurations in order to perform a N removal in a single reactor. Preanoxic is referred to the initial contact of wastewater and sludge is an anoxic condition, followed by aerobic condition while postanoxic is an alternating of aerobic-anoxic condition. In Tchobanoglous et al. (2013), preanoxic condition is often used due to the ease of retrofit to existing plants, control of bulking sludge formation, the

production of alkalinity in anoxic zone and the ability of converting existing biological treatment system to N removal in shorter time.

Wang et al. (2015) reported that TN removal efficiency in preanoxic condition is dependent on the recycling ratio and organic carbon supplied, in which the higher ratio will reduce the organic carbon available for denitrification. Besides, the competition between denitrifiers and polyphosphate accumulating organisms (PAOs) also limits the removal efficiencies. In contrast, postanoxic condition is considered through endogenous respiration of heterotrophs for denitrification, but the process is relatively slow. Besides, in the study of Wang et al. (2015) and Marin et al. (2019), about 45% of COD and TN removal efficiencies were achieved at low DO concentration and low carbon source in preanoxic condition. Whereas more than 60% of N removal was obtained with higher DO concentration and prolonged aerobic condition.

2.4.3   pH

pH plays an important role in BNR performance, particularly the nitrification that is highly sensitive to pH change. It is not only affecting the bacterial growth, but also modifying the acid-base equilibrium which further affect the substrate availability for nitrifying bacteria (Le et al., 2019). In several studies, Jaramillo et al. (2018), Amatya et al. (2011), Li et al. (2011), Yuan et al. (2010) and Galí et al. (2007) have suggested that the stable nitrification was achieved at pH range of 7.1 to 9.0 because most activated sludge processes nitrify at

neutral pH. In addition, Tchobanoglous et al. (2013) mentioned that low pH tends to inhibit enzymatic activity while high pH affects organotrophs microbial activity. This was also showed in Jiménez et al. (2011) that inhibition of NOB was noticed at pH below 6.5.

2.4.4 Dissolved Oxygen (DO)

Dissolved oxygen (DO) is a free uncombined oxygen and is one of the most important requirements in BNR because nitrification and denitrification require a different oxygen concentration. Nitrification takes place in aerobic condition while denitrification occurs in anoxic condition. The free molecular oxygen is used as respiration purpose for nitrifying and denitrifying bacteria (Gerardi, 2002). Studies have been conducted to study the effect of DO concentration to the BNR performance. Nitrification is achieved at range of 0.5 to 4.0 mg DO/L but inhibited at DO more than 6.0 mg/L (Gerardi, 2002). In addition, low nitrification activity occurs at DO less than 0.5 mg/L. This is caused by the less oxygen diffusion through the floc particles and oxygen competition by other microorganisms. The nitrification rate increases with increasing DO concentration. Significant nitrification is achieved at 2.0 to 2.9 mg DO/L and maximum nitrification occurs at 3.0 mg DO/L. In contrast, the presence of high oxygen content (> 1 mg DO/L) inhibited the denitrification process and increased NO_2^- concentration (Luo et al., 2016).

2.4.5   Microbial Population

BNR performance is highly dependent to the functional bacteria that has nitrifying and denitrifying ability (Peng and Zhu, 2006). Zielinska et al. (2011) mentioned that the diversity of specific bacterial groups in activated sludge affecting the performance and it is crucial in maintaining the stability of water treatment system. Besides, according to Wittebolle et al. (2008), not only the presence of certain specific species in activated sludge but also community diversity and its dynamic are important indicators of good microbial functionality. *Nitrosomonas* and *Nitrospira* are the two common genera of nitrifying bacteria that oxidize NH_4^+ to NO_2^- and NO_3^- respectively (Ghaly and Ramakrishnan, 2015; Ge et al., 2015). In contrast, facultative anaerobic bacteria reduce NO_3^- to N_2 through NO_2^- (Gerardi, 2002).

In the first step of nitrification, NH_4^+ is oxidized to NO_2^- by ammonium oxidizing bacteria (AOB), a type of Gram-negative bacteria and is catalyzed by ammonia monooxygenase (AMO) as the key enzyme. *Nitrosococcus, Nitrosolobus, Nitrosomonas, Nitrosospira* and *Nitrosovibrio* are the known AOB. Among them, *Nitrosomonas* and *Nitrosospira* are extensively studied (Ge et al., 2015; Kumwimba et al., 2018).

The cultivation of AOB is not easy to accomplish due to high sensitivity to environmental factors such as substrate concentration, temperature, light, pH and oxygen level (Stein, 2019). Although AOB utilize NH_4^+ as energy source, however, high NH_4^+ concentration inhibits the growth

of bacteria such as chemolithotrophs and strictly aerobes due to toxicity (Hommes et al., 2003). However, some special AOB are able to survive in both aerobic and anaerobic conditions (Geets et al., 2006). The NO_2^- formed is further oxidized to NO_3^- by nitrite-oxidizing bacteria (NOB), another type of Gram-negative bacteria via catalysis by nitrite oxidoreductase (NXR) as the key enzyme. *Nitrobacter, Nitrococcus, Nitrospina* and *Nitrospira* are the examples of NOB.

The NO_3^- is then reduced to N_2 in denitrification by facultative anaerobic bacteria. Several genera such as *Alcalihenes, Bacillus* and *Pseudomonas* are the examples of denitrifying bacteria. In addition, *Paracoccus denitrificans* and *Thaurae mechernichensis* are also being reported as the denitrifying species. These bacteria have the capability to degrade cBOD for cellular activity in the absence of free molecular oxygen and carbon source for cellular growth (Gerardi, 2002).

2.5   Summary

Water pollution from point sources and non-point sources have contributed to the increase in BOD, SS and NH_3-N concentrations. These pollutants deteriorate the water quality by causing the oxygen depletion and eutrophication in the water bodies which further affect the aquatic life. In order to reduce the situation of water pollution, regulations and guidelines have been implied for the threshold discharged concentration of unwanted pollutants such N and P to the water bodies. In addition to the regulatory

framework, researchers also play their roles in the development of N-based contaminants removal techniques in the drinking water treatment.

BNR has been recently focused in the drinking water treatment due to its removal efficiency and environmental-friendly characteristic. The combination processes of nitrification and denitrification convert NH_4^+ to harmless N_2 with the help of bacteria. Researchers have discovered several processes such as SND, shortcut nitrification-denitrification and ANAMMOX that can remove N-based contaminants in the drinking water treatment. Each of the processes can be achieved with its different process setup and configurations. Among that, SND is relatively more promising which can be operated in SBR with only N_2 as the discharged element.

CHAPTER 3

MATERIALS AND METHODS

3.1 Sequencing Batch Reactor (SBR)

A lab scale SBR with working volume of 2 L (Appendix C) was set up for the BNR process. At the initial stage of the study, the SBR was seeded with different seeding sources. The successfully acclimatized BNR sludge was later used as the seed sludge for the subsequent studies.

3.1.1 Configuration and Operation of SBR

The SBR was operated at 4 cycles per day with 6 hours each cycle. Each cycle consists of 8 minutes filling phase, 1.5 hours anoxic condition and 3 hours aerobic condition of reaction phase, followed by 1.05 hours settling phase, 15 minutes decanting phase and 2 minutes idling phase. The SBR cycle is illustrated in Figure 3.1.
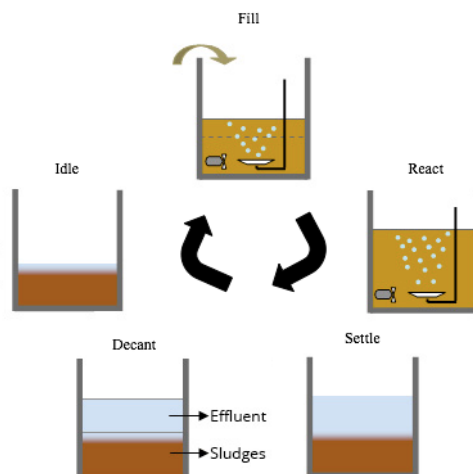
Figure 3.1: Operating Cycle of Sequencing Batch Reactor

The SBR was equipped with an overhead stirrer which consisted of a 4-blades impeller. The mixed liquor was constantly mixed during the alternating anoxic-aerobic conditions in the "React" mode of the SBR. Anoxic and aerobic conditions were maintained by adjusting the air flow from air pump using timer. After the reaction phase, the air pump and stirrer were turned off for the sludge sedimentation. After that, supernatant was discharged. The SBR was allowed for an idle phase before the next cycle started. Then, fresh synthetic river water was refilled into the SBR for the new cycle. The pH was adjusted by adding either 0.5 M hydrochloric acid or 0.5 M sodium hydroxide to the designed pH value. The schematic diagram of SBR is illustrated in Figure 3.2. In addition, the dissolved oxygen was adjusted between 0.2 mg/L - 0.5 mg/L for anoxic phase and 3.1 mg/L - 3.5 mg/L for aerobic phase.
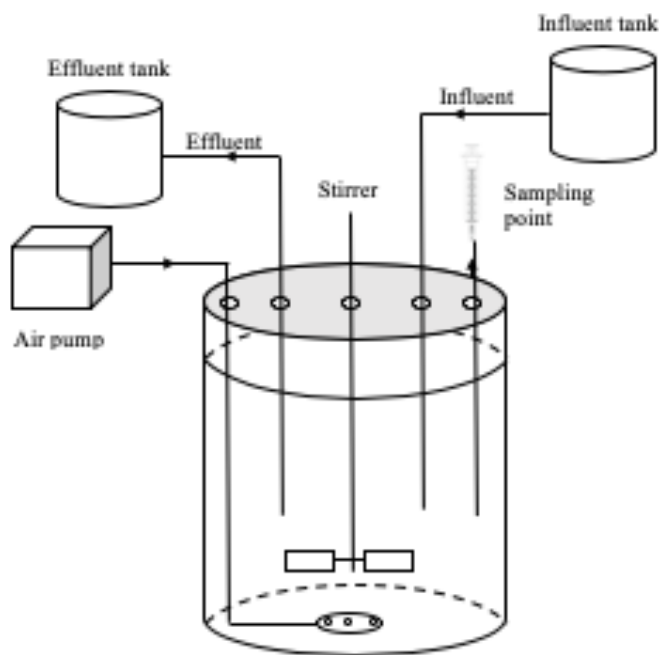
Figure 3.2: Schematic Diagram of Sequencing Batch Reactor

3.1.2   Preparation of Synthetic River Water

In this study, a concentrated synthetic river water was prepared and used as feed. The synthetic river water consisted of 14.0 mg/L sodium acetate, 2.0 mg/L yeast extract, 680 mg/L calcium chloride, 616 mg/L magnesium sulphate heptahydrate, 9.8 mg/L iron (III) chloride, 380 mg/L ammonium chloride and 1.8 mg/L monopotassium phosphate (adapted from Smith et al., 2002). The concentrated solution was autoclaved (Hirayama, HVE 500, Japan) and was diluted accordingly to the designed concentration before feeding into the SBR. The initial concentration of N and COD were then adjusted according to the subsequent experiments designed.

3.2 Experimental Design

Figure 3.3 shows the experimental flow in this study. Three stages were carried out in this research in order to meet the research objectives.
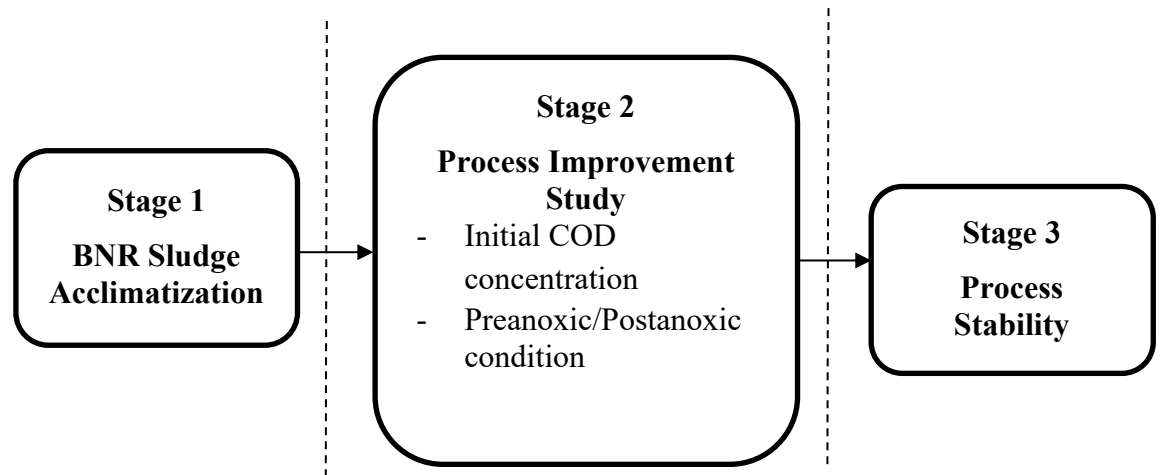
Figure 3.3: Experimental Flow of Research Methodology

3.2.1 BNR Sludge Acclimatization

In order to meet the first research objective listed in Section 1.3, the SBR was seeded with sludge collected from a fish pond and sewage treatment plant (STP) located in Selangor, Malaysia. The SBR was fed with diluted synthetic river water and was operated at the pH ranges of 6.5 to 7.5 at room temperature. The initial mixed liquor suspended solid (MLSS) was set at 1500 to 2000 mg/L while the initial NH_4^+-N and COD concentration were set at 5 mg/L and 15 mg/L. The performance of BNR sludge acclimatization was monitored weekly by measuring the concentration profiles of ammonium-nitrogen (NH_4^+-N), nitrite-nitrogen (NO_2^--N), NO_3^--N and COD.

3.2.2   Effects of Influent COD Concentration and Preanoxic/Postanoxic Conditions on BNR Performance

The successful acclimatized BNR sludge was used as the seed sludge for the subsequent SBR start-up (Stage 2). In this stage, the SBR was operated at room temperature and the pH was manually adjusted to a range between 6.5 to 7.5. The initial MLSS was also designed at 1500 to 2000 mg/L and the initial NH_4^+-N concentration was set as 10 mg/L. The BNR performance was monitored weekly by examining the concentration profiles of NH_4^+-N, NO_2^--N, NO_3^--N and COD. The experimental runs were designed by applying 2^2 factorial design as shown in Table 3.1. The process improvement study was conducted in 4 experimental runs which last for 5 weeks each. Two parameters were focused in this study which are influent COD concentration and preanoxic/postanoxic condition. The high and low levels were denoted as '+' and '-' respectively. The preanoxic condition was started with 1.5 hours anoxic and followed by 3 hours aerobic while the postanoxic condition was first operated with 3 hours aerobic, then followed by 1.5 hours anoxic.

Table 3.1: 2^2 Factorial Design Matrix

Run	Pattern	Influent COD concentration (mg/L)	Preanoxic/Postanoxic Condition
1	$-\ -$	15	Preanoxic
2	$+\ -$	45	Preanoxic
3	$-\ +$	15	Postanoxic
4	$+\ +$	45	Postanoxic

3.2.3   BNR Process Stability

After the operation of all the designed runs, the operating conditions that showed the most significant BNR process was selected for the stability study. The operational period was continued and prolonged for another five weeks to observe the stability and robustness of BNR performance. The process performance was monitored weekly by examining the concentration profiles of NH_4^+-N, NO_2^--N, NO_3^--N and COD.

3.3   Analytical Methods

3.3.1   Determination of Ion Concentration

Weekly sampling was carried out throughout the period of reactor operation for the monitoring of BNR performance. Mixed liquor samples were collected from the SBR at 30 minutes interval in each cycle and subsequently centrifuged (sigma 3-18 K, United Kingdom) for 10 minutes at 10000 rpm. Supernatant of the samples were immediately filtered through 0.45 μm regenerated cellulose syringe filter. The filter samples were further filtered with 0.2 μm PTFE syringe filter prior to the Ion Chromatography analysis. The concentrations of NH_4^+-N, NO_2^--N and NO_3^--N were analyzed using Ion Chromatography (Metrohm 861 Advanced Compact IC, Swiss) (Figure 3.4). For cation (NH_4^+-N) analysis, column Metrosep C4 – 100/4.0 mm with eluent solution consisted of 1.7 mmol/L nitric acid and 0.7 mmol/L dipicolinic acid were used. Whereas for anion (NO_2^--N and NO_3^--N) analysis, column Metrosep

A supp 5 – 150/4.0 mm with eluent solution consisted of 3.2 mmol/L sodium carbonate and 1.0 mmol/L sodium bicarbonate, together with sulphuric acid as chemical suppressor were used.

Figure 3.4: Ion Chromatography, Metrohm 861 Advanced Compact IC

3.3.2   MLSS and MLVSS

MLSS refers to the concentration of suspended solids present in the water sample while MLVSS is the concentration of biomass present in the water sample. Both the MLSS and MLVSS were determined using APHA Standard Method (APHA, 1998).   For MLSS, 20 mL of mixed liquor sample was withdrawn from the SBR and was filtered through a weighed glass-fibre filter paper using a vacuum pump. The residue on the filter paper was then dried in an oven at 105 °C to a constant weight of less than 4 % or 5 mg. The dried filter paper was cooled to room temperature and weighed. The MLSS was then calculated using Equation 3.1:

$$MLSS \left(\frac{mg}{L}\right) = \frac{(final\ weight - initial\ weight)\ x\ 1000}{Volume\ of\ sample\ used\ (mL)} \qquad (3.1)$$

Where;

Initial weight = weight of the glass-fibre filter without sample (mg)

Final weight = weight of the glass-fibre filter with dried residue (mg)

For MLVSS, dried residue from MLSS test was further subjected to a chamber furnace (Nabertherm N 41/H, Germany) at 550 °C for 30 minutes. The ignited filter paper was cooled and weighed. MLVSS was calculated using Equation 3.2:

$$MLVSS\left(\frac{mg}{L}\right) = \frac{(initial\ weight - final\ weight\ )\ x\ 1000}{Volume\ of\ sample\ used\ (mL)} \quad (3.2)$$

Where;

Initial weight = weight of the glass-fibre filter with dried residue from MLSS test (mg)

Final weight = weight of the glass-fibre filter with ignited residue (mg)

3.3.3 Chemical Oxygen Demand (COD)

COD refers to the total measurement of all chemicals (organics & inorganics) in the water or wastewater. The amount of oxygen required to completely oxidize these organic and inorganic compounds to carbon dioxide and water is measured. Mixed liquor samples were collected from the SBR at 30 minutes interval in the reaction period of each cycle. The samples were centrifuged (Sigma 3-38 K, United Kingdom) for 10 minutes at 10000 rpm. Supernatant of the samples were immediately filtered through 0.45 μm regenerated cellulose

syringe filter. The filtered samples (~ 2 mL) were transferred to COD kits prior to the COD analysis. The samples were then heated (HACH DRB 200, United States) at 150 °C for 2 hours and tested using spectrophotometer (HACH 3900, United States) (Figure 3.5).

Figure 3.5: HACH 3900 Spectrophotometer, United States

3.3.4   Sludge Volume Index (SVI)

SVI is a measurement of the settling performance in a mixed liquor sample. The SVI was determined using standard methods (APHA, 1998). A volume of 1 L mixed liquor sample was collected and was transferred into a 1 L measuring cylinder. The sample was let to settle for 30 minutes and the settled volume was measured. The SVI was calculated using Equation 3.3.

$$SVI \left(\frac{mL}{g}\right) = \frac{settled\ volume\ after\ 30\ minutes,\ \left(\frac{mL}{L}\right)}{MLSS\ \left(\frac{g}{L}\right)} \qquad (3.3)$$

3.3.5 NH$_4^+$-N and COD Removal Efficiencies

Both the NH$_4^+$-N and COD removal efficiencies, η was calculated using Equation 3.4, where C_o is the initial NH$_4^+$-N and COD concentrations (mg/L) and C_t is the final NH$_4^+$-N and COD concentrations (mg/L).

$$\eta \ (\%) = \left[1 - \left(\frac{C_t}{C_o}\right)\right] x \ 100\%$$

(3.4)

CHAPTER 4

CULTIVATION OF BNR SLUDGE

In the first stage of this study, three phases of sludge cultivation with different seeding sources were conducted. During Phase 1, the SBR was seeded with aquaculture sludge while in Phase 2, a mixture of aquaculture sludge and activated sludge from STP in the volume ratio of 1:1 was used. In Phase 3, the SBR was solely seeded with activated sludge. As this is a cultivation of non-BNR sludge to BNR sludge, the characteristic of identifying the successful cultivated BNR sludge is mainly based on the N removal performance in terms of nitrification and denitrification activities. The details of different BNR sludge cultivation will be discussed in the following sections.

4.1 Results and Discussion

4.1.1 BNR Sludge Cultivation using Aquaculture Sludge in Phase 1

Figure 4.1 shows the cyclic concentration profiles of NH_4^+-N, NO_2^--N, NO_3^--N and COD in week 1, week 2, week 3 and week 4 of sludge cultivation at aerobic condition using aquaculture as seeding source. The low NH_4^+ oxidation in the first week (Figure 4.1a) could be due to the slow adaptation and NH_4^+ oxidation ability of microbial population in the aquaculture sludge (Lu et al., 2015), resulted in the NH_4^+-N removal efficiency of 14.5 %. The

slight decrease of NH_4^+-N from 5.6 mg/L to 4.9 mg/L at the first 30 minutes of the aerobic phase was due to the basic need of N source for microbial cell growth (Kutvonen et al., 2015; Wang et al., 2016). Other than the oxidation of NH_4^+ by nitrifying bacteria, the decrease of NH_4^+-N concentration could be also caused by the phytoplankton adsorption (Lu et al., 2015). As reported by Lájer (2012), nitrifying bacteria are naturally slow growing bacteria. The substrate consumption rate is higher than the regeneration rate of new nitrifying bacteria at the maximum microbial activity, causing the cellular yield to be relatively low.
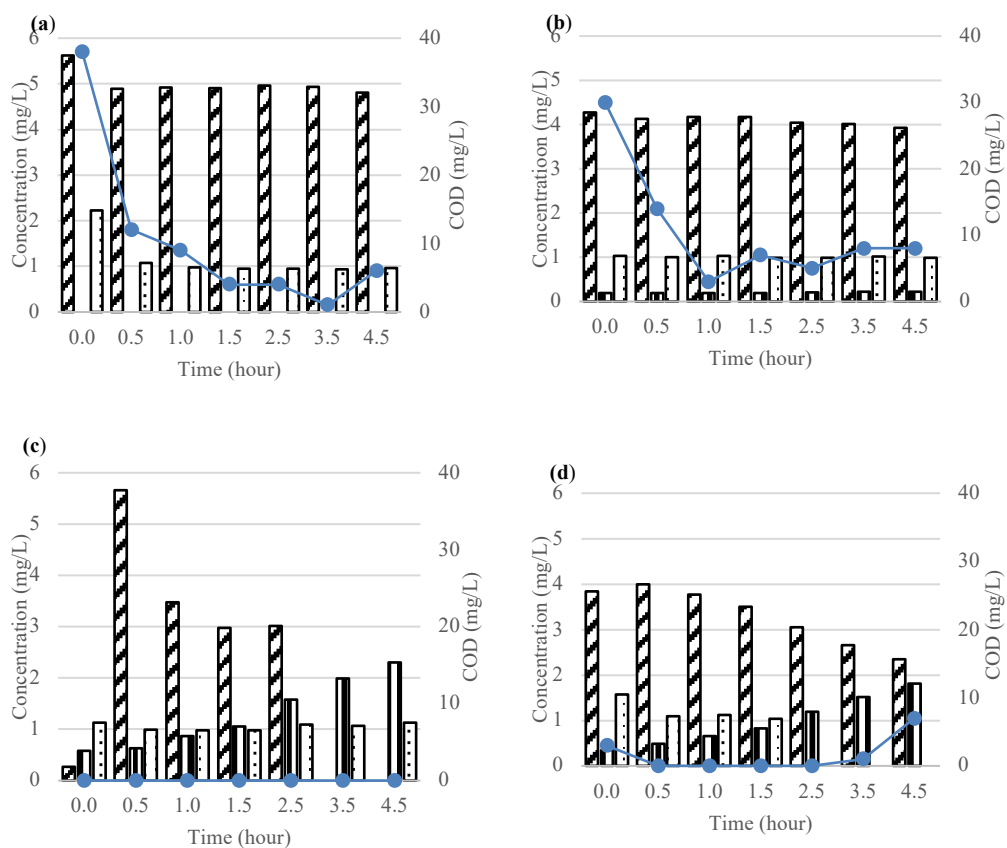
Figure 4.1: Concentration profiles of NH_4^+-N, NO_2^--N, NO_3^--N and COD in one SBR cycle on (a) Week 1; (b) Week 2; (c) Week 3; (d) Week 4 for aquaculture sludge cultivation in Phase 1; (▨) NH_4^+-N; (▥) NO_2^--N; (▦) NO_3^--N; (—●—) COD

From Figure 4.1(a), the microbial activity is yet to reach the exponential phase which showed a low NH_4^+ oxidation. Although the nitrification of NH_4^+ to NO_3^- was inconsistent in the process, there was a noticeable decrease in NO_3^--N, from 2.3 mg/L to 1.0 mg/L in the aerobic phase. The NO_3^--N was nearly dropped to a constant concentration of about 1 mg/L after 30 minutes reactor operation of the cycle. This may due to the presence of some denitrifying bacteria in the sludge that denitrified NO_3^- to N_2. In terms of the COD concentration profile, it showed a decreasing trend from 38 mg/L to 6 mg/L, resulted in 84.2 % COD removal efficiency. The COD removal was not corresponded to the nitrification activity which could be due to the presence of other microbial to carry different roles in the aquaculture (Bentzon et al., 2016). The BNR sludge characteristics were not emerged in aquaculture sludge in week 1.

The slow adaptation of aquaculture sludge endured through week 2 which can be seen from the relatively constant profiles of NH_4^+-N and NO_3^--N, as shown in Figure 4.1(b). The NH_4^+-N removal efficiency reduced from 14.5 % in week 1 to 8.1 %, while about 73.3 % influent COD was utilized at the end of the second week. The reduction in NH_4^+-N and COD removal efficiency could be due to the washed out of sludge which further decreased the microbial population in the system which can be supported by the MLSS and MLVSS analysis in Figure 4.4. In Szabó et al. (2016), the decrease in sludge concentration has led to the reduction of nitrifying bacteria. The washout of slow-growing nitrifying bacteria has slowed down the nitrification process. Besides, there was a traceable concentration of NO_2^--N ($\sim$ 0.2 mg/L) in week 2.

Although NH_4^+-N oxidation was observed in the following weeks, as shown in Figures 4.1(c) and 4.1(d), however, NO_2^--N further accumulated to greater than 1.5 mg/L. The accumulation of NO_2^- intermediate could be due to the limited electron donor such as organic carbon source in the water source that restricted the denitrification activity (Lájer, 2012; Wang et al., 2015).

In the NO_3^--N profile, the minor concentration changed was observed in week 3 (Figure 4.1c) while a reduction trend was seen in week 4 (Figure 4.4d). The observation could be due to the low denitrification activity, as a result of low electron donor availability. Several studies have reported that when acetate is used as carbon source in the influent, it was mainly stored as polyhydroxyalkanoate (PHA) or polyhydroxybutyrate (PHB) for denitrification (Zeng et al., 2003; Wang et al., 2015). However, from the results, it showed that the amount of carbon source supplied was at the lower level for the denitrification activity. The influent carbon source was set at relatively low concentration because this study focuses on the BNR process for the local drinking water treatment where the influent mimics the composition of the river water. As the aquaculture sludge showed relatively low NH_4^+-N removal efficiency (40.4 %) and poor biomass settleability, the Phase 1 was ended after 4 weeks of cultivation.

4.1.2 BNR Sludge Cultivation using Aquaculture Sludge - Activated Sludge in Phase 2

After the termination of Phase 1, Phase 2 was operated at aerobic condition to cultivate the BNR sludge which was seeded with mixture of aquaculture

sludge and activated sludge in the volume ratio of 1:1. The cyclic concentration profiles of NH_4^+-N, NO_2^--N, NO_3^--N and COD were monitored in the selected SBR cycle for a period of 4 weeks, as shown in Figure 4.2.

From Figure 4.2(a), the reduction of NH_4^+-N from 21.9 mg/L to 4.6 mg/L and increment of NO_3^--N from 4.8 mg/L to 7.1 mg/L were observed in the system, indicated the occurrence of nitrification in the system. The NH_4^+-N removal efficiency was 45.8 % which was higher than that in the first week of phase 1 (14.5 %). The large difference of removal efficiency could be due to the addition of activated sludge to the aquaculture sludge that further enriched the microbial community. As mentioned by Xia et al. (2018), nitrifying bacteria are generally about 10 % of the total microbial community in activated sludge. Activated sludge contains high microbial diversity in which bacteria such as nitrifying bacteria, denitrifying bacteria, phosphate-accumulating organisms (PAO) and filamentous bacteria play an important role. However, the increase of NO_3^--N concentration was not corresponding to the drastic dropped of NH_4^+-N concentration which could possibly due to the simultaneous occurrence of denitrification with nitrification, where a portion of NO_3^--N had been converted into N_2. This observation might be due to the high concentration of nitrifying bacteria in the activated sludge (Xia et al., 2018).
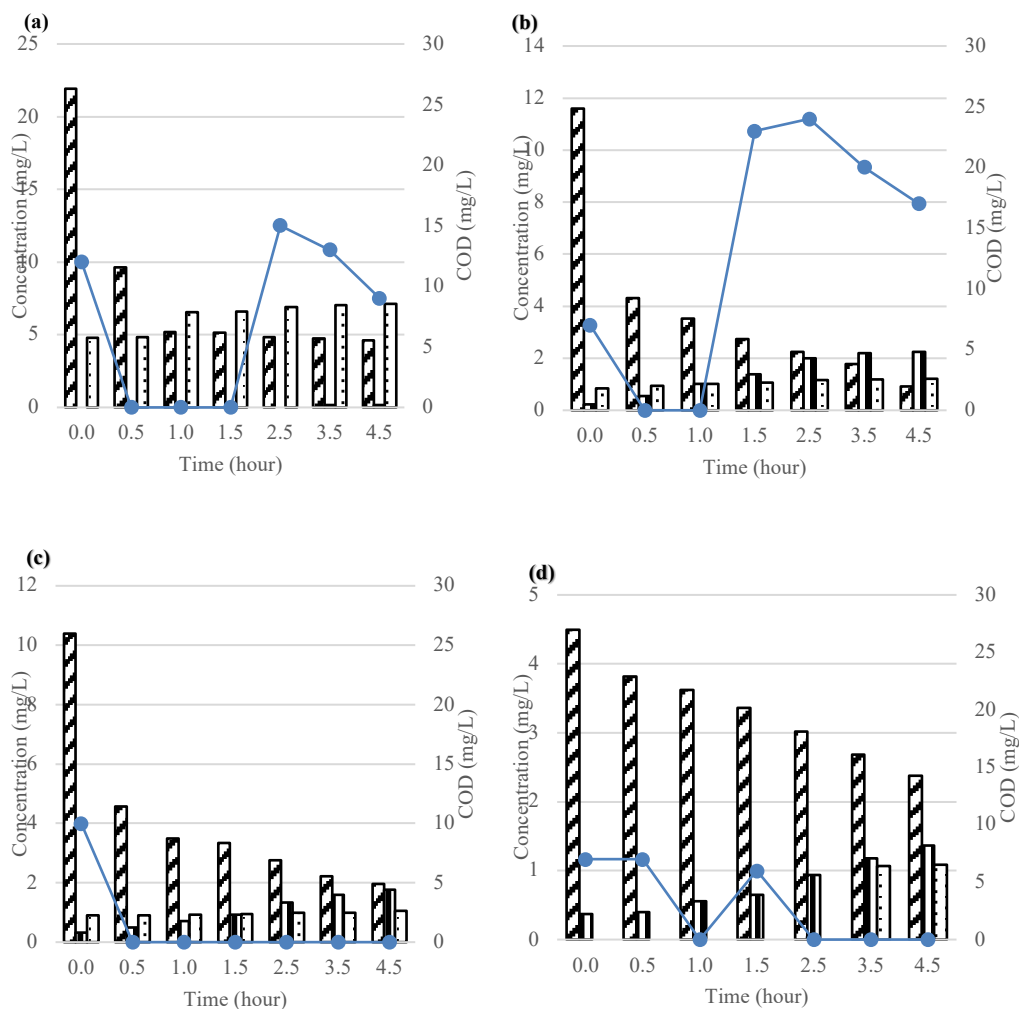
Figure 4.2: Concentration profiles of NH_4^+-N, NO_2^--N, NO_3^--N and COD in one SBR cycle on (a) Week 1; (b) Week 2; (c) Week 3; (d) Week 4 for aquaculture sludge-activated sludge cultivation in Phase 2; (▨▨▨) NH_4^+-N; (▥▥▥) NO_2^--N; (▱▱▱) NO_3^--N; (─●─) COD

For the NO_2^--N profile, the concentration was accumulated from week 2 to week 4, as shown in Figures 4.2(b), 4.2(c) and 4.2(d). Besides the lack of electron donor in the water source, the NO_2^- accumulation could be also affected by the temperature. In Rodríguez et al. (2019), the NO_2^- accumulation was negligible at low temperature of 21 °C. In contrast, NO_2^- accumulation occurred when temperature has increased to 25 °C. This was caused by the low

NOB activity rate to nitrify NO_2^- to NO_3^-. It can be concluded that higher temperature (> 25 ^{o}C) limits the growth of NOB compared to the AOB. In this study, the SBR was operated at room temperature (25 ^{o}C – 27 ^{o}C), causing the NO_2^- accumulation.

For the COD profile, the values were fluctuating over the four weeks. The decreasing trend of COD was observed. The decrease of NH_4^+-N concentration corresponded to the decrease COD concentration indicated that the microbial had utilized the COD for cellular activity. Although the sludge mixture emerged possible SND characteristics with 38.7 % NH_4^+-N removal efficiency on average, however, NO_2^- accumulation has been the drawback of the system, therefore, Phase 2 was ended.

4.1.3   BNR Sludge Cultivation using Activated Sludge in Phase 3

A new run named Phase 3 was started and solely seeded with activated sludge to study the BNR performance. The Phase 3 was operated at alternating anoxic-aerobic condition and monitored periodically. The cyclic concentration profiles of NH_4^+-N, NO_2^--N, NO_3^--N and COD in one selected cycle are detailed in Figure 4.3.
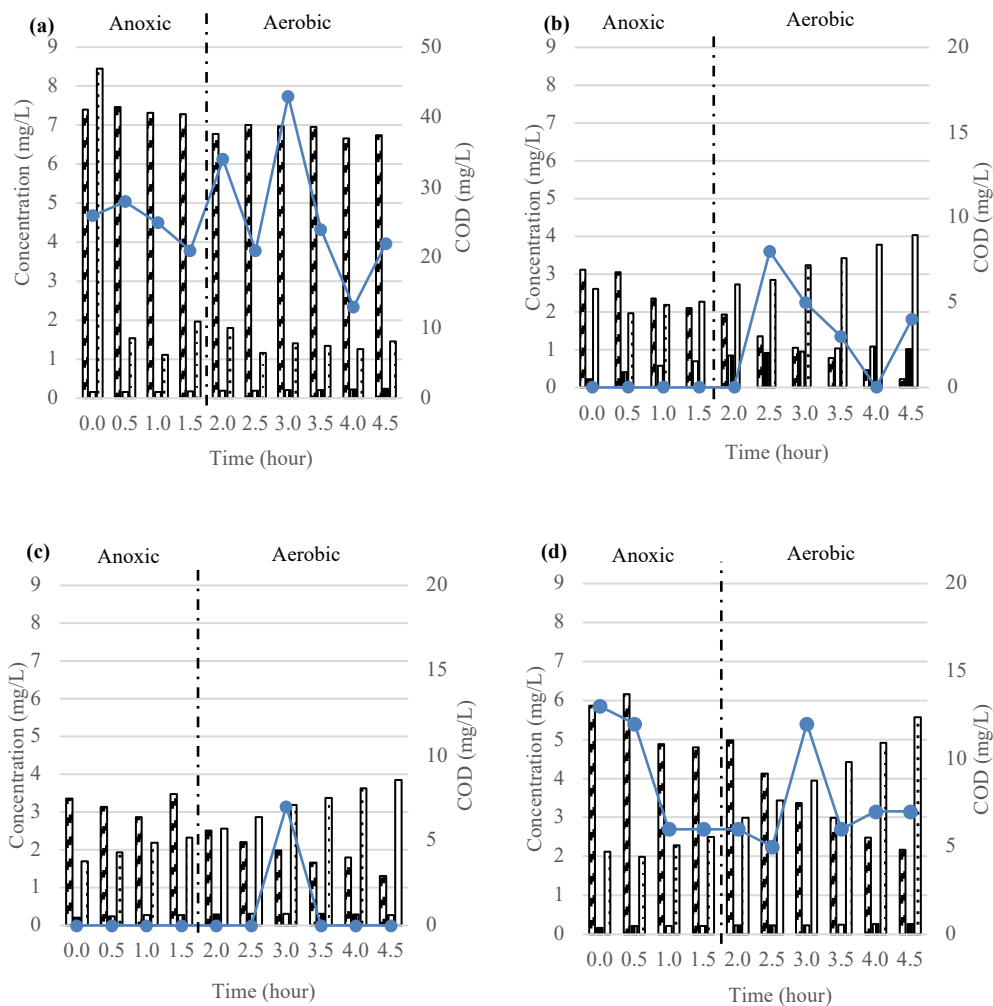
Figure 4.3: Concentration profiles of NH$_4^+$-N, NO$_2^-$-N, NO$_3^-$-N and COD in one SBR cycle on (a) Week 1; (b) Week 2; (c) Week 3; (d) Week 4 for activated sludge cultivation in Phase 3; (▨) NH$_4^+$-N; (▥) NO$_2^-$-N; (▦) NO$_3^-$-N; (─●─) COD

In the first week of the run, the NH$_4^+$-N concentration was about 7.4 mg/L at the anoxic phase while a slight decreasing trend was observed in the aerobic phase. While the inconsistent trend of NO$_3^-$-N was seen in Figure 4.3(a) for both the anoxic and aerobic phase. The observation could be mainly due to the adaption period of microbial community in the activated sludge to the new environment.

After one week of cultivation, BNR characteristic emerged gradually from week 2 to week 4, as shown in Figures 4.3(b), 4.3(c) and 4.3(d). This can be seen from the significant decrease of NH_4^+-N profile throughout the reaction period. The NH_4^+-N removal efficiency was increased from 8.9% in week 1 to 92.9% in week 2 and has reached to a plateau at about 60% in the following weeks. The NH_4^+ not only been utilized by the microbial in the reactor, but also nitrified into NO_3^- as a result of the oxidation process (Gerardi, 2002). In Figure 4.3(b), the decrease of NH_4^+-N concentration along with the NO_3^--N reduction in the anoxic phase and increment in NO_3^--N during the aerobic phase showed that the system was showing the SND characteristics. Decrease of NO_3^--N in the anoxic phase was due to the denitrification that some NO_3^- was denitrified to N_2 while NO_3^--N increased was caused by the nitrification that NH_4^+ was oxidized to NO_3^-. Although the NH_4^+-N profile decreased throughout the reaction period, as shown in Figures 4.3(c) and 4.3(d), the increase of NO_3^--N was noticed in both the anoxic and aerobic phase, indicated the system has a better nitrification activity. As for the COD profile, a decreasing trend was observed.

4.1.4   MLSS, MLVSS and SVI Analysis

The analysis of MLSS, MLVSS and SVI were also monitored to study the sludge performance in terms of biomass concentration and settleability. In the 4 weeks of operational period in phase 1 which was solely aquaculture sludge, the MLSS dropped from around 1700 mg/L in week 1 to about 300 mg/L in week 4. Similar observation was seen in the MLVSS where about 700 mg/L in week 1 was decreased to about 100 mg/L in week 4. The decrease of both the

MLSS and MLVSS suggesting that the sludge has less dense and poor settleability which were in accordance with the SVI analysis of more than 200 mL/g. Besides, MLSS and MLVSS experienced decreasing trend in the phase 2 cultivation of aquaculture sludge and activated sludge. However, the reduction was less than that in phase 1 which can be observed through the SVI analysis from 200 mL/g in week 1 to about 150 mL/g in week 4, indicated the sludge has improved settleability. In the cultivation of activated sludge in phase 3, the SVI showed a dramatically improvement from about 200 mL/g in week 1 to about 90 mL/g in week 4. This showed a good sludge settleability which further affected the performance of N removal as shown in Figure 4.3.
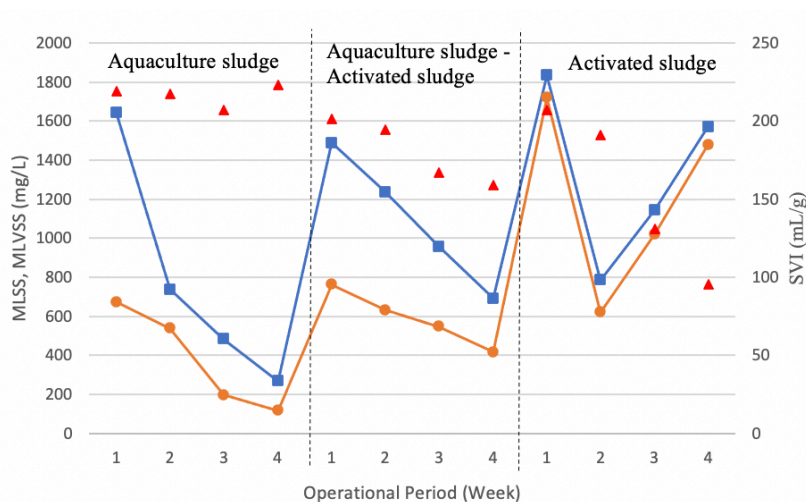
Figure 4.4: Profiles of MLSS, MLVSS and SVI for the runs; (─■─) MLSS; (─●─) MLVSS; (▲) SVI

4.2   Summary

The overall performance in Phase 3 was more promising than in Phase 1 and Phase 2 in terms of N removal characteristics and NH_4^+-N removal efficiency. Phase 3 showed a potential BNR process in SBR with 56.5 % NH_4^+-N

removal efficiency on average compared to Phase 1 and Phase 2 at 40.4 % and 38.7 % on average respectively. The dissolved oxygen was also maintained at 0.2 mg/L - 0.5 mg/L and 3.1 mg/L - 3.5 mg/L for anoxic and aerobic phase respectively. The BNR performance can be further improved by studying the effect of influent COD concentration and preanoxic/postanoxic condition. The parameter studies will be discussed in the following chapters.

CHAPTER 5

EFFECTS OF INFLUENT COD CONCENTRATION AND PREANOXIC/POSTANOXIC CONDITIONS ON BNR PERFORMANCE

After the successful cultivation of BNR sludge in Phase 3, the sludge was used as the seed sludge for the subsequent parameter studies. The effect of influent COD concentration and preanoxic/postanoxic conditions were studied for the improvement of BNR process.

The cultivated sludge in Phase 3 was utilized to start up a BNR process and the starting MLSS was set at about 1500 to 2000 mg/L. There were four runs in this study, Run 1 and Run 2 were operated at preanoxic condition with designed influent COD concentration at 15 mg/L and 45 mg/L respectively. After five continuous weeks of operation for both runs, the operating condition was switched from preanoxic to postanoxic and named as Run 3 and Run 4 respectively.

5.1 Results and Discussion

5.1.1 Monitoring of BNR Performance

The SBR was monitored weekly in all the runs by evaluating the cyclic concentration profiles of COD, NH_4^+-N, NO_2^--N and NO_3^--N, as shown in Figures 5.1 to 5.4.

From Figures 5.1(a), 5.2(a), 5.3(a) and 5.4(a), the COD concentration profiles of the runs showed that about 87 % on average of the influent COD was being utilized. The COD uptake was mainly caused by the microbial community for their cellular activity. The reduction in NH_4^+-N and NO_2^--N concentration with COD uptake were observed at the end of all runs. This observation was correlated to the oxidation of NH_4^+-N and NO_2^--N in the cycles during the runs, as shown in the NH_4^+-N concentration profiles (Figures 5.1b, 5.2b, 5.3b and 5.4b) and NO_2^--N concentration profiles (Figures 5.1c, 5.2c, 5.3c and 5.4c). On the other hand, the increment in concentration is observed for NO_3^--N profile throughout the operational period of all runs, as shown in Figures 5.1(d), 5.2(d), 5.3(d) and 5.4(d). This observation corresponded to the decrement in NH_4^+-N concentration where nitrification takes place.
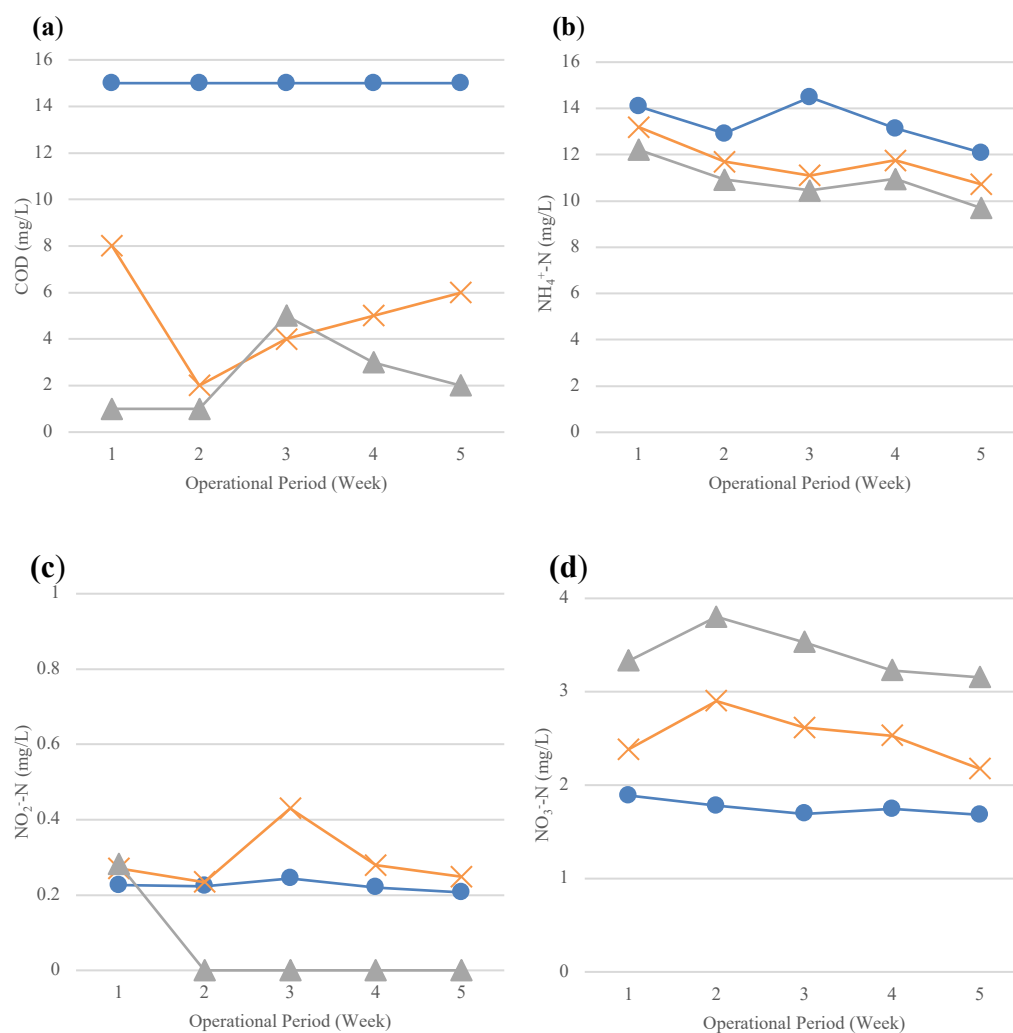
Figure 5.1: Concentration profiles of (a) COD, (b) NH_4^+-N, (c) NO_2^--N and (d) NO_3^--N in the monitored cycles of Run 1 for influent COD of 15 mg/L at preanoxic; (●—) Beginning of anoxic phase; (✕) Beginning of aerobic phase; (▲) End of aerobic phase
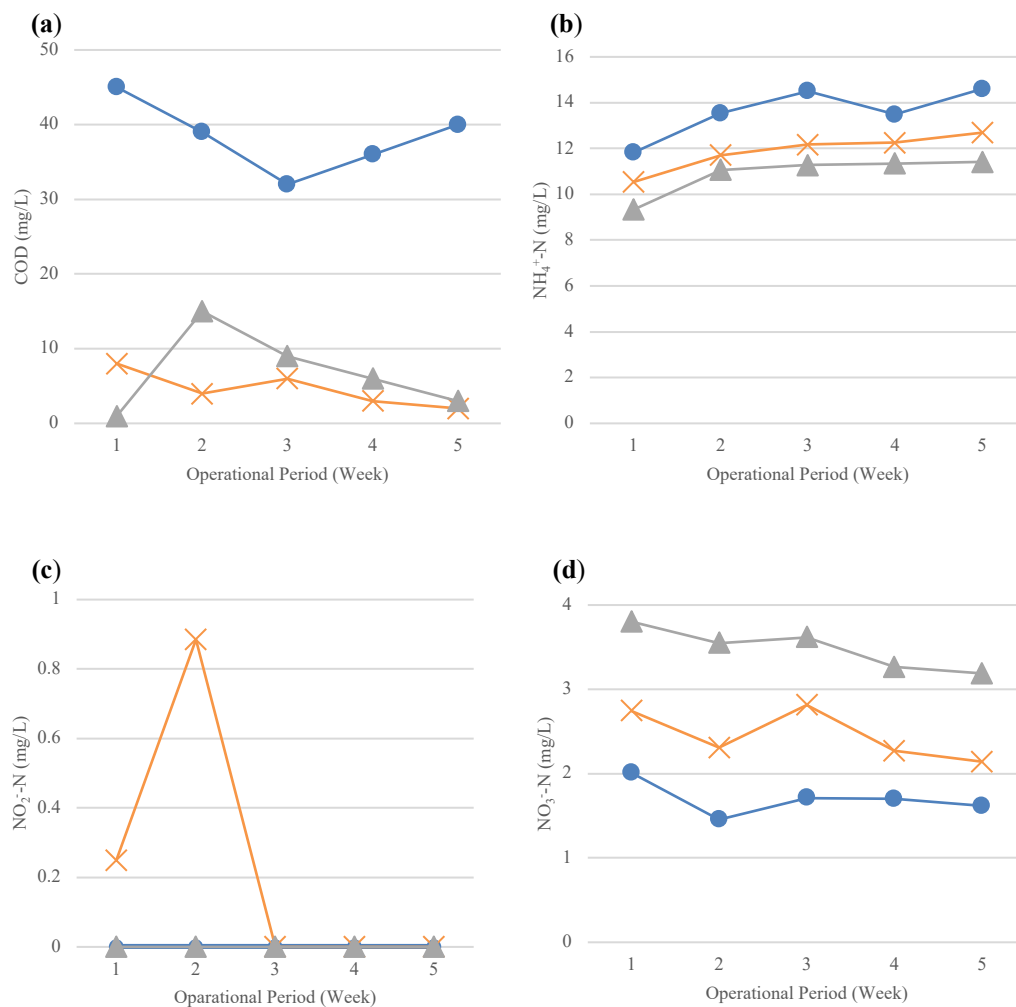
Figure 5.2: Concentration profiles of (a) COD, (b) NH$_4^+$-N, (c) NO$_2^-$-N and (d) NO$_3^-$-N in the monitored cycles of Run 2 for influent COD of 45 mg/L at preanoxic; (——) Beginning of anoxic phase; (✕) Beginning of aerobic phase; (▲) End of aerobic phase
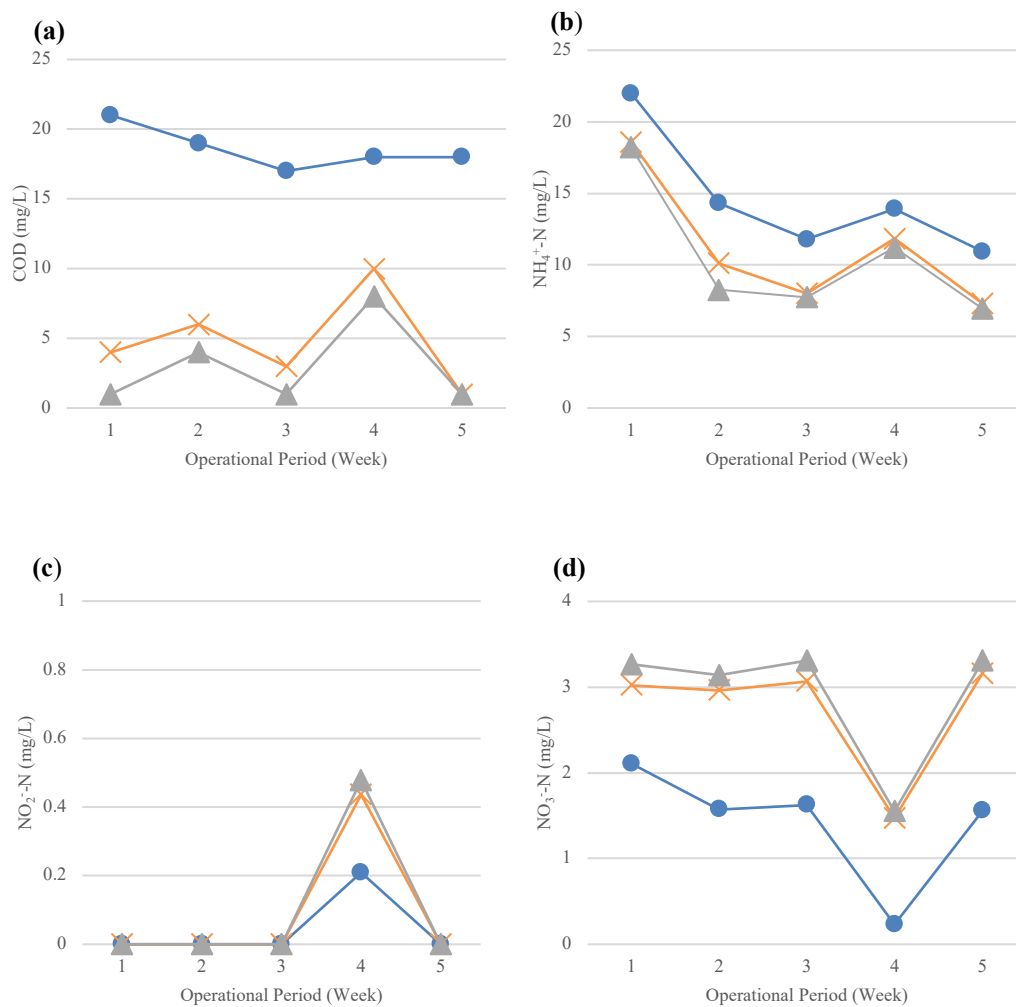
Figure 5.3: Concentration profiles of (a) COD, (b) NH$_4^+$-N, (c) NO$_2^-$-N and (d) NO$_3^-$-N in the monitored cycles of Run 3 for influent COD of 15 mg/L at postanoxic; (●—) Beginning of aerobic phase; (✕) Beginning of anoxic phase; (▲—) End of anoxic phase
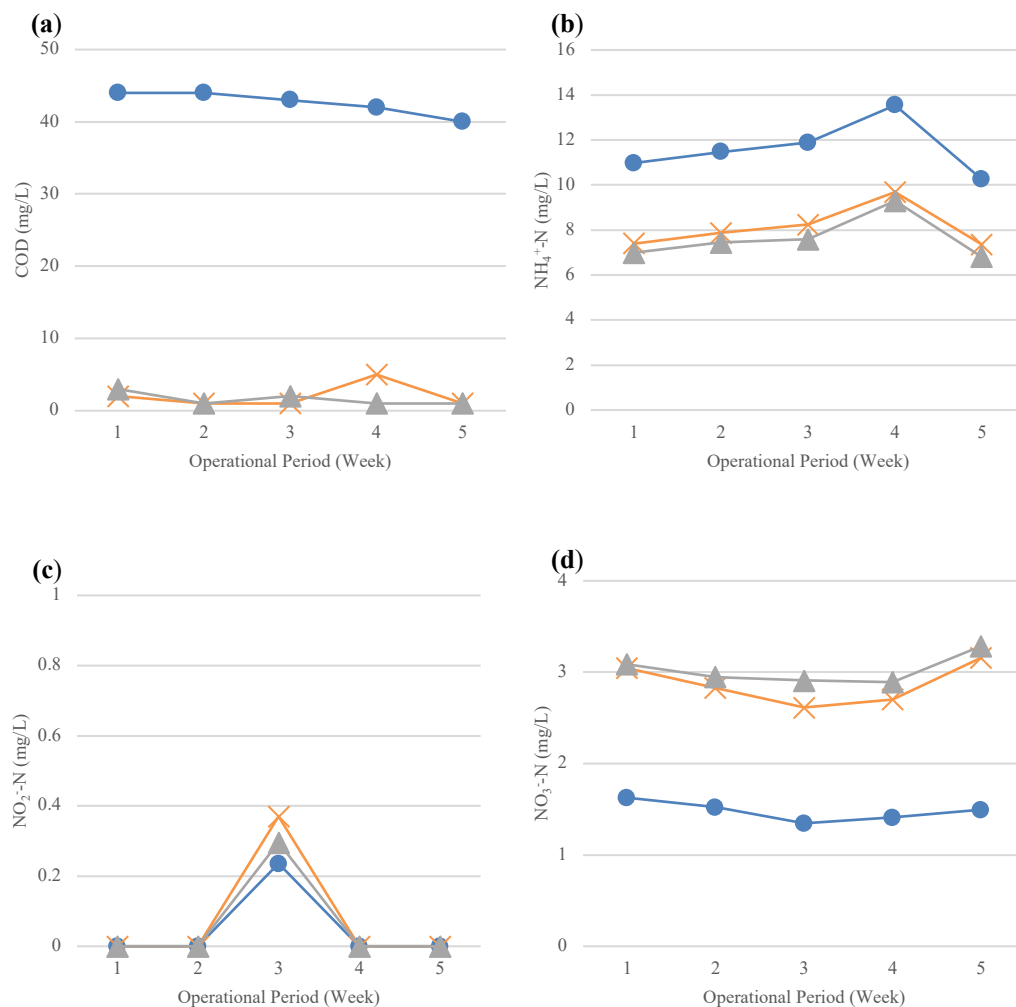
Figure 5.4: Concentration profiles of (a) COD, (b) NH$_4^+$-N, (c) NO$_2^-$-N and (d) NO$_3^-$-N in the monitored cycles of Run 4 for influent COD of 45 mg/L at postanoxic; (—•—) Beginning of aerobic phase; (—✕—) Beginning of anoxic phase; (—▲—) End of anoxic phase

The changes of MLSS, MLVSS and SVI were also monitored periodically. Figure 5.5 shows that the MLSS and MLVSS concentrations experienced fluctuation at the first 4 weeks (Run 1). This was mainly caused by the sludge settleability which can be determined by SVI. The SVI in Run 1 was about 200 mL/g in the first week, indicated that the sludge was less dense and poor settleability. This further resulted in the decreased in MLSS and

MLVSS due to the washout effect. Nevertheless, the MLSS, MLVSS and SVI showed improvements started from week 5, suggesting a better sludge settleability and adaption of microbial in the system. This observation can be further supported by the SVI which was about 85 mL/g at Run 4, indicated the sludge has a better settleability and denser over time. These parameters have direct effect on the BNR performance in terms of the NH_4^+-N oxidation and COD uptake which can be related to the concentration profiles in Figures 5.1 to 5.4.
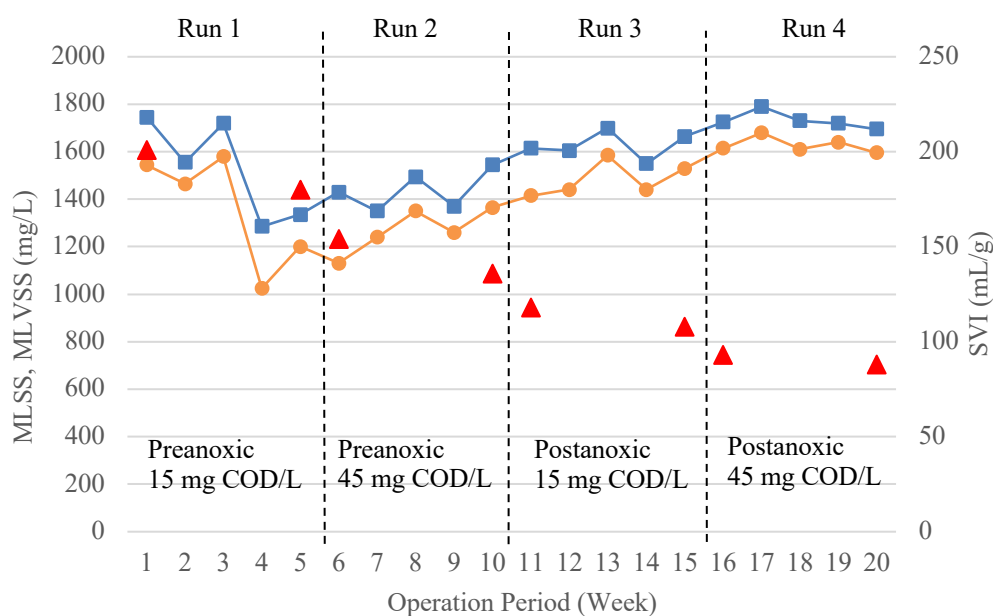
Figure 5.5: Profiles of MLSS, MLVSS and SVI for the runs; (■) MLSS; (●) MLVSS; (▲) SVI

5.1.2 Effect of Influent COD Concentration on NH$_4^+$-N and COD Removal Efficiencies

The effect of influent COD concentration and preanoxic/postanoxic conditions on the BNR performance were analyzed based on NH$_4^+$-N and COD removal efficiencies. Figure 5.6 presents the NH$_4^+$-N and COD removal efficiencies of all runs.
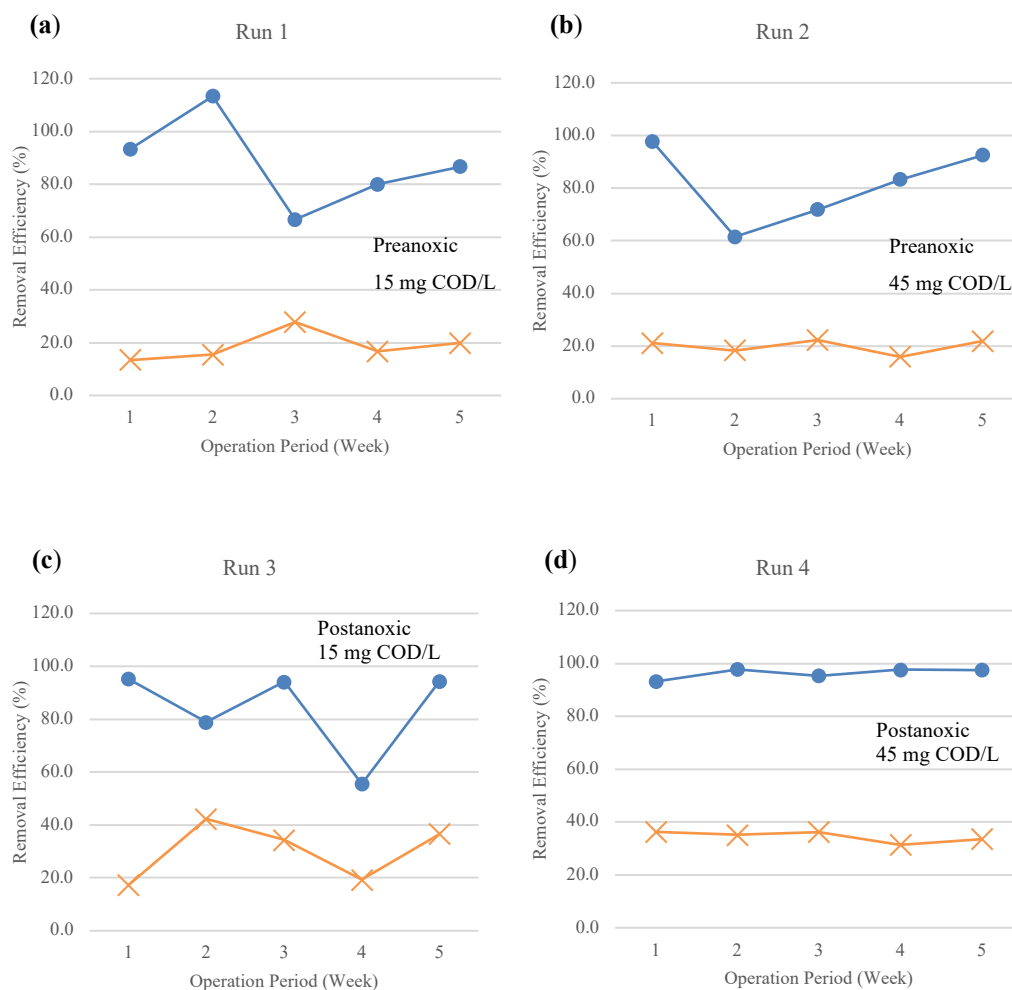
Figure 5.6: Effect of influent COD concentration and preanoxic/postanoxic condition on the removal efficiencies (%) of NH$_4^+$-N and COD; (●—) COD; (✕—) NH$_4^+$-N

5.1.2.1   NH$_4^+$-N Removal Efficiency

Low performance on NH$_4^+$-N removal efficiency was observed for both Run 1 and Run 2. The NH$_4^+$-N removal efficiency in Run 1 increased from 13.4 % to 15.4 % in the first two weeks, as shown in Figure 5.6(a). Then, the removal efficiency was further increased to 27.8 % and decreased in the following week. The decrement could be due to the washout of sludge, resulted from the adaptation of biomass to the new environment and further caused in the reduction of nitrifying bacteria in the system (Szabó et al., 2016). This fluctuation can be supported by the MLSS and MLVSS analysis, as shown in Figure 5.5. The MLSS was decreased from 1720 mg/L in week 3 to 1285 mg/L in week 4. While 1580 mg/L of MLVSS in week 3 was reduced to 1025 mg/L in week 4. The sludge concentration then gradually increased in the following week was observed. Nevertheless, the removal was observed to further increase from 16.1 % to 19.8 % in week 4 and week 5. This indicates that the system is adjusting to stabilize the washout effect.

The NH$_4^+$-N removal efficiency of Run 2 has a slight changed, as shown in Figure 5.6(b). Approximate 21.1% of NH$_4^+$-N removal efficiency with the COD removal of about 97.8 % was achieved at the 1st week of operation which was higher compared to Run 1. This was mainly due to the higher influent COD availability for the nitrifying bacteria as their source of cellular activity. However, the removal efficiency was decreased to 18.3 % in week 2 and increased to 22.3 % in week 3. The system then experienced another dropped in the removal efficiency to 15.9 % in week 4 and then

increased to 21.8 % in the following week 5. This observation could be due to the competition between nitrifying bacteria and heterotrophs in a single reactor as the result of higher COD availability, as well as the competition between nitrifying bacteria for oxygen (Sepehri and Sarrafzadeh, 2019). In contrast, the increment was in line with the COD removal efficiency which is an indication of microbial growth as a result of cell regeneration.

On the other hand, it was found that Run 4 showed better performance than Run 3 whereby about 34.5 % on average was achieved throughout the 5 weeks period. This was due to the stabilization of BNR process to nitrify NH_4^+-N. This was supported by the analysis in Figure 5.5 whereby the MLSS, MLVSS and SVI showed a relatively constant performance. In contrast, inconsistent trend was observed in Run 3 with a relatively large difference of 25.0 % between the lowest (17.3 %) and the highest (42.3 %). As shown in Figure 5.6(c), NH_4^+-N removal efficiency was low at 17.3 % in week 1. However, the NH_4^+-N removal efficiency experienced a drastic increment to 42.3 % in week 2 and decreased to 34.4 % in the following week. This observation could be caused by the competition of COD between the nitrifying bacteria and heterotrophs for their cellular activity, resulted in the difference removal efficiency in week 2 and week 3.

5.1.2.2 COD Removal Efficiency

It is found that the COD removal efficiency of Run 2 (97.8 %) is slightly higher than Run 1 (93.3 %) at the end of five weeks monitored period, as

shown in Figures 5.6(a) and 5.6(b). At the end of the operation period, the influent COD concentration of Run 1 (~ 15 mg/L) and Run 2 (~ 45 mg/L) were reduced to 2 mg/L and 3 mg/L in the effluent respectively. The effluent COD concentrations were complied with the discharged limit stated in Drinking Water Quality Standard of Malaysia. Besides, according to DOE Water Quality Index classification, COD of less than 10 mg/L is classified as Class I and considered environmental friendly.

Run 3 and Run 4 were operated at different influent COD concentrations and the operating condition was switched from preanoxic to postanoxic. In Figures 5.6(c) and 5.6(d), the overall COD removal efficiency of Run 4 was higher compared to Run 3 whereby the COD removal efficiency of Run 4 was stably maintained to more than 90 % throughout the operation period. In contrast, fluctuation was observed in Run 3 where there was an obvious decrease of COD removal efficiency from 94.1 % in week 3 to 55.6 % in week 4 (Figure 5.6c). This was caused by the overflown of the mixed liquor and the sludge was being washout from the SBR. The microbial population could be therefore affected. This was shown in the sludge performance analysis (Figure 5.5) whereby both the MLSS and MLVSS decreased at the 4th week of Run 3. Nevertheless, the system was then restored and recovered by achieving 94.4 % COD removal in week 5 which eventually gives an overall COD removal efficiency of 83.7 % in five weeks operation.

From the comparison made between Figure 5.6(a) and Figure 5.6(b), as well as Figure 5.6(c) and Figure 5.6(d), more than 80 % COD removal

efficiency was achieved for all runs. Run 1 and Run 2 has an average of 85.3 % and 81.4 % COD removal respectively. When the operating condition was switched from preanoxic to postanoxic, the COD removal of Run 3 and Run 4 was 83.7 % and 96.3 % on average respectively. From this study, it was observed that higher influent COD concentration increased the COD and NH_4^+-N removal efficiencies. Zhang et al. (2015) found that higher COD/N ratio between 1.8 to 3.5 suppressed the nitrifying bacteria activity, caused a lower nitrification activity. Besides, Zielinska et al. (2012) reported that higher COD/N ratio decreased the nitrification activity due to the oxygen competition between autotrophs and heterotrophs. In the study of Li et al. (2016), highest nitrogen removal could be achieved at COD/N ratio of 0.4 and decreased when the ratio increased. In this study, low COD/N ratio of 0.8 and 2.4 were used as the imitation to the river water condition and higher COD/N showed better nitrification activity which disobey the findings by the researchers. However, Jenni et al. (2014) found that nitrifying bacteria activity was improved with increasing influent COD/N ratio. This was mainly due to the higher COD concentration favours nitrifying bacteria when sufficient oxygen was supplied in the system.

5.1.3 Effect of Preanoxic/Postanoxic on NH_4^+-N and COD Removal Efficiencies

5.1.3.1 NH_4^+-N Removal Efficiency

Run 3 showed a higher performance than Run 1, whereby an average 30.0 % NH_4^+-N removal efficiency was achieved in Run 3 throughout the operation

period (Figure 5.6c) compared to 18.6 % on average of NH_4^+-N removal efficiency in Run 1 (Figure 5.6a). In contrast, a stable removal efficiency of about 34.5 % on average was maintained in Run 4 compared to the low NH_4^+-N removal efficiency of approximately 19.9 % on average in Run 2.

5.1.3.2   COD Removal Efficiency

Figures 5.6(a) and 5.6(c) show that the COD removal efficiency of Run 1 was slightly lower than Run 3 at the end of 5th week operation period which was 86.7 % and 94.4 % respectively, whereby the influent COD concentration of Run 1 (~ 15 mg/L) and Run 3 (~ 18 mg/L) were reduced to 1 mg/l and 2 mg/L respectively. Besides, with comparison between Figures 5.6(b) and 5.6(d), the overall COD removal efficiency of Run 4 was higher than Run 2. The COD removal efficiency for both Run 2 and Run 4 were stably maintained at approximately 81.6 % and 96.4 % respectively in the five weeks of operation period. With 81.6 % COD removal efficiency, the effluent COD concentration of Run 2 has an average of 6.8 mg/L while the effluent COD concentration of Run 4 was 1.6 mg/L on average with 96.4 % COD removal efficiency.

It was observed that the COD removal at the postanoxic condition between Run 2 and Run 4 was increased by 18.3 %. In contrast, a decrement by 1.88 % between Run 1 and Run 3 was obtained at the preanoxic condition which was caused by the system disturbance in Run 3 which resulted from the sludge washout, as supported and illustrated in Figure 5.5.

From the study, it showed that postanoxic condition has a better performance than the preanoxic condition. Preanoxic condition is often being used due to the ability of increasing alkalinity in the anoxic zone for the nitrification activity which will stably maintain the pH and increase nitrification activity (Tchobanoglous et a., 2013). However, nitrification is pH-sensitive of ranges 6.5 - 8.0 and uses alkalinity during the oxidation process. As the alkalinity decreases, it lowers the pH and further limits the nitrification activity. Besides, a recent study by Alagha et al. (2020) found that about 99 % COD and nitrogen removal efficiencies could be achieved in the postanoxic condition by increasing settling time and decreasing filling time of the SBR to provide a longer anoxic zone for the system. Therefore, by increasing the anoxic zone in postanoxic condition, the alkalinity could be maintained as well.

5.1.4   Denitrification Activity

In this study, the nitrification activity is rather obvious than denitrification activity because denitrifying bacteria are known to compete for carbon supplied. The low denitrification rate could be due to the carbon deficiency in the system (Alagha et al., 2020; Li et al., 2020). The electron donor required in the denitrification was supplied by the endogenous death and lysis of biomass (Marin et al., 2019). Due to the generation of microaerophilic condition, the denitrification rate was limited, led to the significant nitrification activity in the system. Additional carbon source has to be supplied to the system in order to improve the denitrification rate. (Wang et al., 2015; Marin et al., 2019).

5.2 Summary

From the experimental results, it showed that the influent COD concentration and preanoxic/postanoxic had effects on the COD and NH_4^+-N removal efficiency. At influent COD concentration of 45 mg/L in postanoxic condition (Run 4), both the removal efficiencies were significant. This could be due to the sufficient oxygen supplied despite of the high COD/N ratio and postanoxic condition which allowed the system to restore its alkalinity. Furthermore, the effluent COD concentrations has fulfilled the standard discharged limit imposed by the Drinking Water Quality Standard of Malaysia.

CHAPTER 6

BNR PROCESS STABILITY

After the process improvement study, Run 4 (45 mg COD/L at postanoxic condition) showed the most significant BNR performance and was selected for the stability study. The process was continued for another five weeks to evaluate the stability of the BNR performance.

6.1   Results and Discussion

Figure 6.1 shows the cyclic concentration profiles of COD, NH_4^+-N, NO_2^--N and NO_3^--N in the selected run (Run 4) for 10 operation weeks. The COD profile shows a decreasing trend from the beginning to the end of the reaction phase, as illustrated in Figure 6.1(a). The influent COD concentration of 43.3 mg/L on average has reduced to 2.7 mg/L on average in the effluent throughout 10 operation weeks which has stably maintained the COD removal at more than 90 % throughout the Run 4, particularly in the extended operational period, as shown in Figure 6.2. This indicates that the microbial community in the system is constantly utilizing the COD for microbial cell growth (Tchobanoglous et al., 2013). Nevertheless, the effluent COD concentration of the prolonged period also complies with the discharged limit imposed by the Drinking Water Quality Standard for Malaysia.

For NH_4^+-N, a reduction in concentration from average 11.6 mg/L to 6.7 mg/L was observed throughout the operation weeks, as shown in Figure 6.1(b). This leads to a NH_4^+-N removal efficiency of 41.7 % on average in the 10 weeks, as illustrated in Figure 6.2. This was attributed to the nitrification of NH_4^+ to NO_2^- and NO_3^- by the nitrifying bacteria as energy source for cell respiration (Gerardi, 2002). The system demonstrates a constant NH_4^+ nitrification.
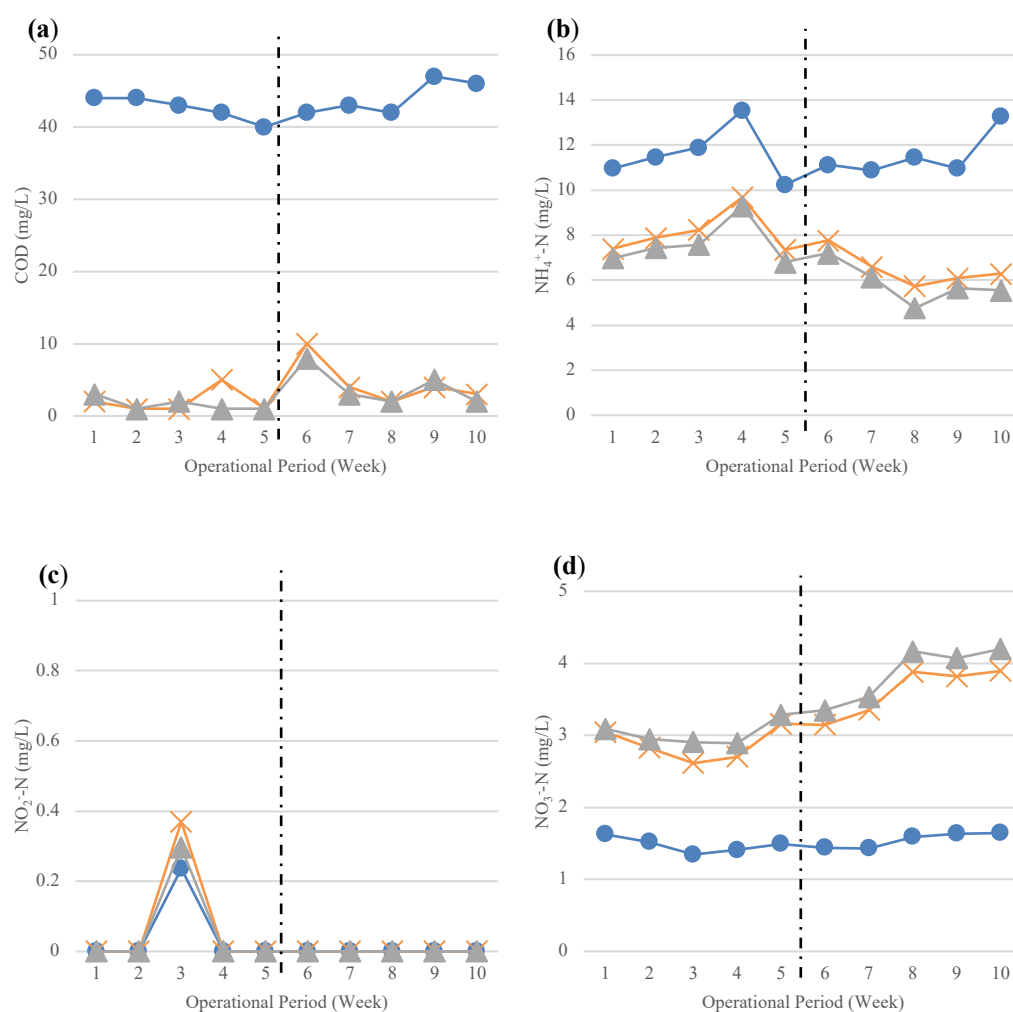
Figure 6.1: Concentration profiles of (a) COD, (b) NH_4^+-N, (c) NO_2^--N and (d) NO_3^--N in the selected run for stability study; (—•—) Beginning of aerobic phase; (—✕—) Beginning of anoxic phase; (—▲—) End of anoxic phase
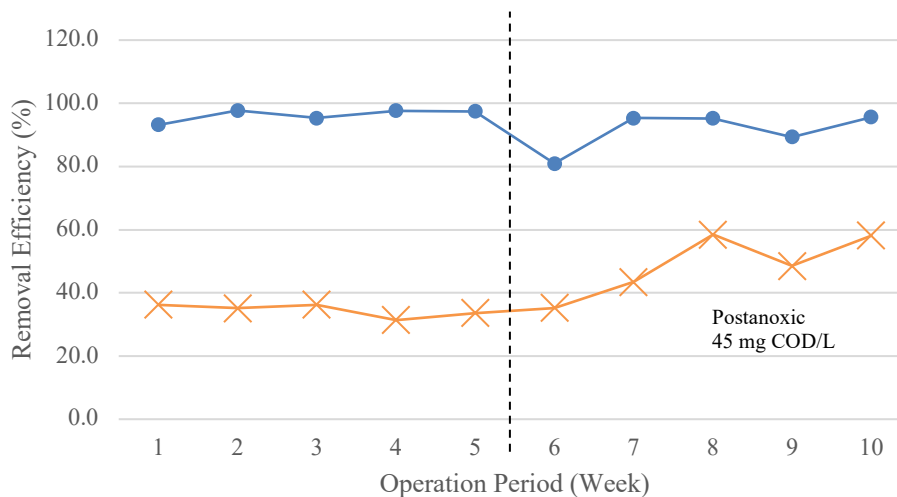
Figure 6.2: Removal efficiency (%) of COD and NH$_4^+$-N for stability study; (•—) COD; (✖) NH$_4^+$-N

As the result of NH$_4^+$ oxidation, the NO$_2^-$-N shows zero concentration for most of the operation period and the NO$_3^-$-N concentration is found to increase throughout the weeks, as shown in Figures 6.1(c) and 6.1(d). However, it was found that there is a spike in NO$_2^-$-N concentration in week 3. This could be mainly due the microbial population in the system has experienced a surrounding change caused by the power trip in the lab. After few days of recovery period, the NO$_2^-$-N concentration was found to drop and maintained at zero, suggesting that the system can response to the changes and readjust to stable operation. Furthermore, the increase in NO$_3^-$-N concentration over time, indicates that the system is continuing nitrified. The nitrification activity in the system is relatively stable. This is mainly due to the lack of electron donor in the system that restricts the denitrification activity (Wang et al., 2015; Lájer, 2012). In addition, the MLSS and MLVSS were stably maintained around 1720 mg/L and 1630 mg/L respectively in the Run 4

throughout the 10 operational weeks, suggesting that the sludge settleability and microbial adaption had improved in the system. This observation can be further supported by the SVI analysis of Run 4 which was about 85 mL/g, indicates that the sludge has a better settleability and dense, as shown in Figure 6.3.
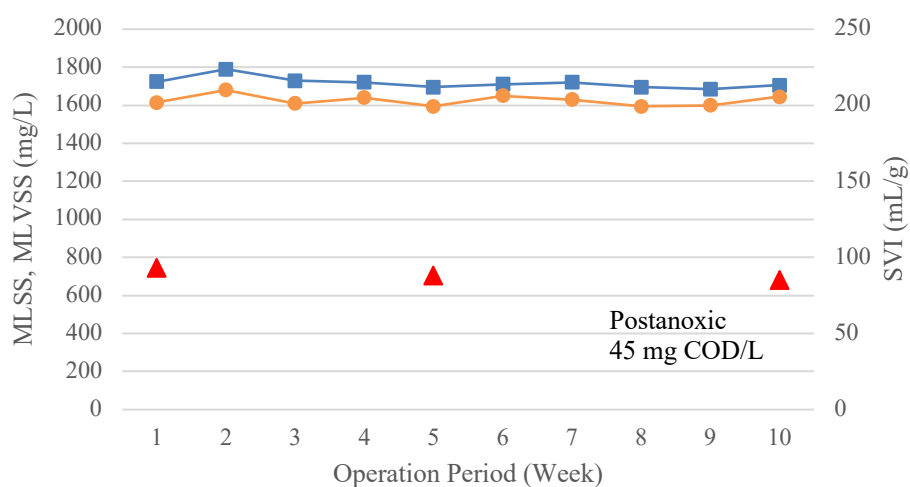
**Figure 6.3: Profiles of MLSS, MLVSS and SVI for stability study; (■)
MLSS; (●) MLVSS; (▲) SVI**

6.2   Summary

The experimental results showed that the BNR characteristics was consistent during the prolonged study of operation period. The MLSS, MLVSS and SVI was stably maintained in a continuing operation period. The effluent COD was complied with the regulations. In addition, the robustness of the process was observed with rapid recovery when the system experienced an operational failure.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

The conclusions from the research findings are as follow:

a) BNR sludge was successfully cultivated in a lab scale BNR process by using conventional activated sludge from sewage treatment plant. This study demonstrated the possibility in retrofitting the existing conventional drinking water treatment process into BNR process in Malaysia. A non-BNR sludge was successfully acclimatized to establish the BNR performance within 4 weeks of cultivation period.

b) The experimental findings found that both initial COD concentration and preanoxic/postanoxic conditions affected the COD and NH_4^+-N removal efficiencies for the BNR process. The final concentrations of COD and NH_4^+-N at 45 COD mg/L in postanoxic condition have achieved 2 mg/L and 5 mg/L respectively. Besides, the MLSS and MLVSS were achieved at around 1720 mg/L and 1630 mg/L respectively while SVI was stably maintained at about 85 mL/g.

c) The BNR process operated at 45 COD mg/L in postanoxic condition was relatively stable in terms of the COD and NH_4^+-N removal efficiencies in

the prolonged operation period. Besides, the dissolved oxygen was consistent in the anoxic and aerobic condition that ranges between 0.2 mg/L - 0.5 mg/L and 3.1 mg/L - 3.5 mg/L respectively. Nevertheless, the robustness of the process was also noticed when the system experienced an operational change.

7.2   Recommendations

Few recommendations for possible future studies are suggested as follows:

a) To optimize the BNR performance in drinking water treatment by studying different operating parameters such as pH, temperature, DO and carbon source in order to maximize the COD and NH_4^+-N removal efficiencies. For example, nitrification is highly pH sensitive and pH ranges between 6.5 – 9 have showed a stable nitrification activity. Furthermore, temperature between 20 °C – 30 °C can be evaluated to study the BNR performance and overcome the NO_2^- accumulation caused by high temperature. In addition, DO ranges between 1.0 – 6.0 mg/L can be studied as both nitrification and denitrification require different oxygen content. Nevertheless, higher COD/N ratio is also necessary to enhance the denitrification activity.

b) To replace the synthetic river water with real river water in the future studies to give a clearer view on the BNR process performance in local drinking water treatment. The operating conditions in lab scale should mimic the conditions of actual plant to bridge the gap between the lab

scale and full scale. Then, the robustness of the process on the real river water can be evaluated.

c) To explore the biodiversity and growth kinetics of microbial population present in the activated sludge by studying the growth conditions, for example, temperature, pH and carbon source. The interaction between growth condition and microbial population in the BNR process can be also evaluated to identify the optimum condition for microbial growth.

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APPENDICES

APPENDIX A: Biological Ammonia Removal in Developed Countries

Processes	Locations	Influent NH_4^+-N concentration (mg/L)	Reduction Percent (%)
Biological filters			
Pilot-scale (aerated system)	France	> 2.5	≈100
Full-scale (Aerated system)	France	≤ 4	97.5
	France	≤ 1	> 95
	France	0.3 – 0.5	> 83
Pilot-scale	Great Britain	3.0	82.5
Fluidized bed			
Pilot-scale	Great Britain	≤ 2-2.5	≈100
Pilot-scale	Great Britain	≤ 0.83	100
Full-scale	Germany	-	0.05
Rapid sand filters			
Pilot-scale	France	0.08 – 0.68	66 (average)
Full-scale	France	0.5	100
Full-scale	France	0.34	≈100
Full-scale	Germany	1.0	≈100
Full-scale		1.3	50
Microbial active GAC			
Full-scale	Germany	0.33	94
Pilot-scale	Germany	1.53	95.4
Full-scale	France	0.17	> 70.6
Full-scale	France	1.36	78
Soil-aquifer treatment			
Full-scale	France	1.45 (average)	100
Full-scale (bank filtration	Germany	0.34	89

*GAC = Granular activated carbon

APPENDIX B: Biological Nitrate Removal in Developed Countries

Processes	Locations	Influent NO_3-N concentration (mg/L)	Reduction Percent (%)
Biological filters			
Full-scale	France	13.5	50
Ful-scale	France	37.9	84
Pilot-scale	Great Britain	-	78 – 100
Fluidized bed			
Pilot-scale	Great Britain	14	100
Ful-scale	Great Britain	15	63
Rotating biological contactor			
Ful-scale	United States	60 - 80	91 – 93
Soil-aquifer treatment			
Ful-scale	Netherlands	22.5	72
Ful-scale	Germany	-	75
Autotrophic denitrification			
Pilot-scale	France	18.1	90 – 100
Pilot-scale	France	109 – 168	78 – 85
Pilot-scale	Netherlands	65 – 70	> 90

APPENDIX C: Setup of Lab-scale SBR