PERFORMANCE ENHANCEMENT OF ANAEROBIC AMMONIUM OXIDATION REACTOR START-UP AND OPERATION WITH THE EXTERNAL HYDRAZINE ADDITION

by

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LIST OF ABBREVIATIONS

$(N_2H_5)HSO_4$	Hydrazinium sulphate
AMO	Ammonium oxygenase
Anammox	Anaerobic ammonium oxidation
AOB	Ammonium oxidizing bacteria
ATP	Adenosine triphosphate
BCA	Bicinchoninic acid
$C_6H_{12}O_6$	Glucose
CANON	Completely autotrophic nitrogen removal over nitrite
CAS	Conventional activated sludge system
CO_2	Carbon dioxide
$CoCl_2$	Cobalt chloride
COD	Chemical oxygen demand
$CuSO_4$	Copper sulphate
DI	Deionised water
DO	Dissolved oxygen
DOE	Department of Environment
EDTA	Ethylenediaminetetraacetic acid
EPS	Extracellular polymeric substances
EQA	Environmental Quality Act
EQR	Environmental quality report
FA	Free ammonia
FBR	Fixed/Fluidized bed reactor
$FeSO_4$	Iron sulphate
FNA	Free nitrous acid

H_2O	Water
H_3BO_3	Boric acid
HAO	Hydroxylamine oxidoreducatse
HDH	Hydrazine dehydrogenase
HRT	Hydraulic retention time
HZS	Hydrazine synthase
IC	Inhibition percentage
IP	Inhibitory percentage
K_2HPO_4	Di-potassium phosphate
KCl	Potassium chloride
KH_2PO_4	Potassium di-hydrogen phosphate
$KHCO_3$	Potassium hydrogen carbonate
MBR	Membrane bioreactor
MF	Membrane filter
MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
$MnCl_2$	Manganese Chloride
N_2	Nitrogen
N_2H_4	Hydrazine
N_2O	Nitrous oxide
Na_2MoO_4	Sodium molybdate
Na_2SeO_4	Sodium selenate
Na_2SO_4	Sodium sulphate
NaCl	Sodium chloride
NaOH	Sodium hydroxide

$NaNO_2$	Sodium nitrite
NH_2OH	Hydroxylamine
NH_3	Ammonia
NH_4Cl	Ammonium chloride
NH_4-N	Ammonium nitrogen
$NiCl_2$	Nickel chloride
NIR	Nitrite oxidoreductase
NLR	Nitrogen loading rate
NO	Nitric oxide
NO_2-N	Nitrite nitrogen
NO_3-N	Nitrate nitrogen
NOB	Nitrite oxidizing bacteria
NOR	Nitric oxic reductase
NOS	Nitrous oxide synthase
NRA	Nitrate reductase
NRE	Nitrogen removal efficiency
NRR	Nitrogen removal rate
NXR	Nitrate oxidoreductase
O_2	Oxygen
OLR	Organic loading rate
PBS	Phosphate buffer solution
PN	Protein
PS	Polysaccharide
RBC	Rotating biological contactor
RPM	Rotation per minute

SBR	Sequencing batch reactor
SHARON	Single reactor system for high ammonium removal rate over nitrite
SRT	Sludge retention time
Tem	Temperature
TN	Total nitrogen
UASB	Upflow anaerobic sludge blanket reactor
UBF	Upflow biofilter
UV-VIS	Ultraviolet-visible spectrophotometry
VSS	Volatile suspended solids
WTP	Water/wastewater treatment plant
WWTP	Wastewater treatment plant
$ZnSO_4$	Zinc sulphate

LIST OF SYMBOLS

$\%$	Percentage
Cu^{2+}	Copper ion
H^+	Hydrogen ion
NH_4^+	Ammonium ion
NO_2^-	Nitrite ion
NO_3^-	Nitrate ion
r_1	Anammox activity and time t_1
r_2	Anammox activity and time t_2
$^{\circ}C$	Degree Celsius
€	Euro
μL	micro litre
μ_{max}	The maximum specific growth rate
μmol	micro molar
μ_{obs}	Anammox activity over time
A	Absorbance value
Ar	Argon
B	Weight of empty filter paper
b_{AN}	Decay rate
c	Concentration of the solution
C	Weight of filter paper from MLSS test
d	day
D	Weight of filter paper after furnace ignition
e^-	Electron
E	Weight of filter paper plus sample

exp	Exponential
ε	Milimolar extinction coefficient
F	MLVSS valued in the SBR
g	grams
G	MLSS value in the effluent
HRT	Hydraulic retention time
kg	kilograms
KHz	Kilohertz
K_I	Non-substrate inhibition constant
K_{ie}	Edwards inhibition constant
K_S	Half saturation constant
l	Length of solution cell
L	Litre
m	metre
M	Molarity
m^3	metre cube
mg	milligrams
mmol	mili molar
nm	nano metre
NRR	Nitrogen removal rate
NRR_{max}	Maximum nitrogen removal rate
R11	Reactor inhibited with 1100 mg N/L substrate
$R11_H$	Recovering reactor inhibited with 1100 mg N/L substrate with hydrazine addition
R13	Reactor inhibited with 1300 mg N/L substrate

$R13_H$	Recovering reactor inhibited with 1300 mg N/L substrate with hydrazine addition
$R9$	Reactor inhibited with 900 mg N/L substrate
$R9_H$	Recovering reactor inhibited with 900 mg N/L substrate with hydrazine addition
R_a	Starvation reactor with the presence of ammonium
R_h	Starvation reactor with the presence of hydrazine
R_n	Starvation reactor with the presence of nitrite
S	Substrate concentration
t	Time of inhibition/starvation
TAN	Total ammonia nitrogen
t_{HL}	Half life time
TN_{eff}	Total nitrogen concentration in effluent
TN_{in}	Total nitrogen concentration in influent
TNN	Total nitrite nitrogen
$t_{reaction}$	Reaction time
t_{total}	Total batch time
VSS_t	VSS value after inhibition/starvation
Y_1	NRR after inhibition
$Y_{control}$	NRR before inhibition

KESAN PENAMBAHAN HIDRAZIN TERHADAP PENGAKTIFAN DAN OPERASI REAKTOR YANG MENJALANKAN PROSES PENGOKSIDAAN AMMONIUM SECARA ANAEROBIK (ANAMMOX)

ABSTRAK

Pengoksidaan ammonium secara anaerobik (Anammox) adalah salah satu rawatan biologi yang digunakan secara meluas. Bagaimanapun, pengaktifan sistem Anammox mengambil masa yang panjang. Tambahan pula, bakteria Anammox juga sensitif terhadap turun naiknya nilai substrat dimana ia mudah mengalami proses perencatan dan kebuluran. Objektif pertama kajian ini adalah bagi memperkayakan bakteria Anammox dalam reaktor kelompok penjujukan (SBR) dengan isipadu 8L. Benih enapcemar bagi objecktif ini diperolehi daripada reaktor yang menjalankam proses separa nitrifikasi dan sebuah reaktor anaerobik. Bagi 75 minggu pertama, substrat yang diberikan, dinaikkan secara beperingkat iaitu dari 100 - 900 mg N/L. Substrat yang disediakan (amonium + nitrit) berada dalam keadaan nisbah seimbang (1: 1). SBR utama mencapai lebih daripada 90% penyingkiran nitrogen dalam tempoh 14 minggu. Objektif kedua kajian ini adalah untuk mengkaji kesan tambahan hidrazin dalam membantu pengaktifan reaktor Anammox. Kesan 5 kepekatan hidrazin berbeza dalam mengaktifkan reaktor Anammox (0, 5, 10, 15, 20 mg / L) dikaji. SBR dengan tambahan 10 mg/L hidrazin didapati mengambil masa 7 minggu untuk mencapai (kecekapan penyingkiran nitrogen) NRE sebanyak 86%. SBR tanpa penambahan hidrazin mengambil masa 11 minggu dimana NRE sebanyak 83.5% sahaja dicapai. Objektif ketiga kajian ini dilakukan untuk menilai kesan perencatan substrat terhadap bakteria Anammox dan keupayaan hidrazin untuk membantu proses pemulihan. 3 kepekatan substrat yang dikaji adalah 900, 1100 dan 1300 mg N/L. Peratusan perencatan (IP%) yang diperolehi untuk reaktor dengan kepekatan substrat 900, 1100 dan 1300 mg N/L selepas 28 hari perencatan adalah 27, 38 dan 75%. Model perencatan substrat (Edwards Model) didapati dapat memberikan penjelasan lebi baik berkaitain impak perencatan substrat. Pemalar ketepuan separuh (Ks) dan Pemalar perencatan (K_{IE}) yang diperoleh ialah 361.62 mg/L dan 731.3 mg/L. Dalam proses pemulihan, reaktor yang dengan tambahan hidrazin menunjukkan keupayaan untuk pulih secara mampan jika dibandingkan dengan reaktor tanpa tambahan hidrazin. Keputusan terbaik diperoleh dari reaktor yang direncat dengan 900 mg N/L dan dipulih dengan tambahan hyrazin ($R9_H$) dimana NRE sebanyak 94% dicapai manakala keputusan terendah dicapai oleh R13(tanpa hidrazin) dengan NRE sebanyak 80%. Objektif terakhir kajian ini menilai kesan keadaan kebuluran berbeza terhadap pemulihan bakteria Anammox. Tiga keadaan kebuluran yang dikaji adalah keadaan kebuluran dengan kehadiran ammonium (R_a), nitrit (R_n), dan hidrazin (R_h). Kadar kematian yang setelah 15 hari untuk R_a, R_n, dan R_h adalah 0.032/hari, 0.042/hari dan 0.019/hari. Akhir sekali, keupayaan pemulihan yang terbaik dan terburuk selepas proses kebuluran ditunjukkan oleh reaktor R_h dan R_n di mana kadar pertumbuhan yang diperoleh adalah 0.092/hari dan 0.011/hari.

PERFORMANCE ENHANCEMENT OF ANAEROBIC AMMONIUM OXIDATION REACTOR START-UP AND OPERATION WITH THE EXTERNAL HYDRAZINE ADDITION

ABSTRACT

Anaerobic ammonium oxidation (Anammox) is one of the widely used biological treatments to remove nitrogenous compounds from wastewater. Despite this, Anammox system has long start-up period and the Anammox bacteria can easily undergo inhibition or starvation due to fluctuation of feed in wastewater treatment plants. In the first part of the study, Anammox bacteria were enriched in a sequencing batch reactor (SBR) with a working volume of 8L. In 75 weeks the substrates were increased step wise from 100 – 900 mg-N/L. The substrates provided (ammonium + nitrite) were in equimolar balance of 1:1. The parent SBR achieved more than 90% of nitrogen removal efficiency (NRE) in 14 weeks. The second part of the study was to investigate the effect of external hydrazine addition on aiding the start-up of Anammox reactor. Effects of 5 different externally added hydrazine concentration on reactor start-up were studied (0, 5, 10, 15, 20 mg/L). According to the results obtained, SBR with 10 mg/L hydrazine addition only took 7 weeks with an NRE of 86%. However, the SBR with no hydrazine addition took 11 weeks to stabilize with a NRE of 83.5%. The third part of the study was done to evaluate the effect of substrate inhibition on Anammox bacteria and ability of external hydrazine addition to aid the recovery of Anammox bacteria. 3 substrate concentrations were studied which were 900, 1100 and 1300 mg-N/L. The outcomes show that the inhibition percentage (IP%) obtained for reactors with substrate concentration of 900, 1100

and 1300 mg-N/L after 28 days of inhibition were 27, 38 and 75%, respectively. It was found that the substrate inhibition model (Edwards model) is the best model to represent the inhibition towards Anammox bacteria. Half saturation constant (K_s) and inhibition constant (K_{IE}) obtained were 361.62 mg/L and 731.3 mg/L, respectively. During the recovery studies, reactors that had hydrazine addition showed better recovering capabilities compared to the one without hydrazine addition. The best result in terms of NRE was obtained from the reactor inhibited with 900 mg-N/L and recovered in the presence of hydrazine ($R9_H$) which was 94%. Evidently, the best growth rate was also obtained from reactor $R9_H$ at 0.22/day and the lowest growth rate was obtained for R13 which was 0.07/day. The final part of this study was to evaluate the effect of different starvation condition on Anammox bacteria and its recovery. Three different starvation were studied which were starvation with the presence of ammonium (R_a), nitrite (R_n), and hydrazine (R_h). The decay rates calculated after 15 days for R_a, R_n, and R_h were 0.032/day, 0.042/day and 0.019/day, respectively. Finally, the best and the worst recovering capabilities after starvation were shown by reactor R_h and R_n where the growth rate value tabulated were 0.092/day and 0.011/day, respectively.

CHAPTER 1

INTRODUCTION

1.1 Background

This chapter provides information on the importance of this research. Brief explanation on nitrogen pollution of river waters, the effects of this pollution and the need for effective water treatment to remove nitrogenous compounds are discussed. The chapter is finally concluded by lining out the problem statements, objectives and the organization of the thesis.

1.1.1 River water pollution trend

Till to this date, the drinking or tap water resource of Malaysia comes from the rivers around the nation. However, recent data obtained suggest that the river water quality in Malaysia has been on decline (Department Of Environment, 2017). Rapid growth in industrial sectors and population has given rise to more river pollution. Major causes of river pollution are industrial and domestic sewage, waste from agriculture and animal farming, food processing facilities wastes, mining wastes and others (Sasakova et al., 2018). According to the 2016 Environmental Quality Report (EQR) by Department of Environment, Malaysia (DOE), the number of clean rivers has been on decline since 2007. Figure 1.1 depicts the trend of river water quality in Malaysia from 2005 to

2016. A more alarming trend is that there is increase in pollution due to nutrients particularly ammoniacal nitrogen in the rivers. It is widely known that ammoniacal nitrogen was not listed in Standard A and Standard B of EQA until the year 2009 and was only included in 2009 (Department Of Environment, 2017). The Standard A and B for discharging industrial wastewater into water bodies can be found in Table 1.1. The negligence in not including ammoniacal nitrogen into the standard discharge schedule has led to several problems such as eutrophication and wastewater treatment plant closure. For instance, Sungai Langat and Johor Water Treatment Plants was temporarily closed in 2015 and 2017 due to sudden elevation in ammoniacal nitrogen concentration in the Semenyih river and Sembrong Dam (Abidin et al., 2018).

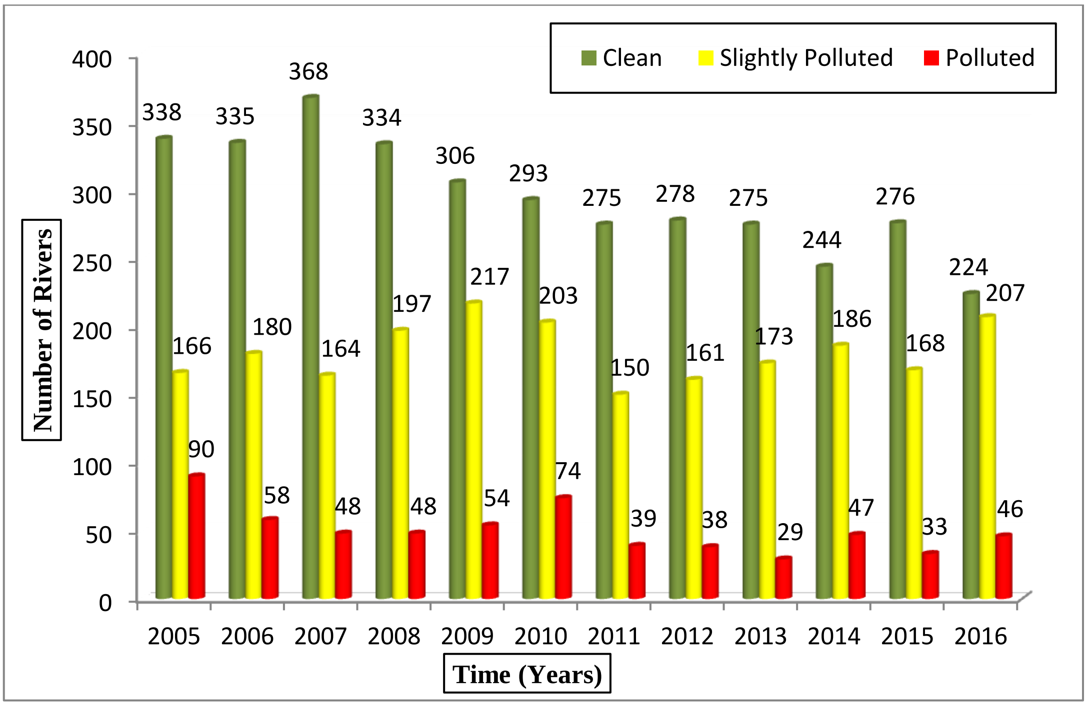

Figure 1.1: Trend of the river water quality in Malaysia from 2005 – 2016 (Department of Environment, Malaysia, 2017)

Table 1.1: Environmental Quality Act 1974, Environmental Quality (Sewage), Regulations 2009, Second Schedule (Regulation 7), Acceptable Conditions of Sewage Discharge of Standards A and B (approved after January 1999)

Parameters	Unit	Standard	
		A	**B**
BOD_5 at 20 ^{0}C	mg/L	20	50
Suspended Solids	mg/L	50	100
Oil and Grease	mg/L	1.0	10
Ammoniacal Nitrogen	**mg/L**	**10**	**20**

* Sources: Environmental Quality Report, Department of Environment, Malaysia, 2017.

Figure 1.2 shows the trend of rivers that have been polluted by ammoniacal nitrogen from 2005 to 2016. As of 2016, it is estimated that about 231.35 tonnes/day of ammonia load is discharged into the rivers of Malaysia (Department Of Environment, 2017). The main sources of ammoniacal nitrogen pollution in Malaysia are from sewage, agriculture, manufacturing industries such pharmaceutical and fertilizer productions and domestic wastes (Department Of Environment, 2017). The breakage of weight of the ammoniacal nitrogen pollution source is given in Figure 1.3. Even though, the DOE has a strict discharge limit, ammoniacal nitrogen pollution has been on the rise as seen in Figure 1.2. One of the main reasons for this to occur is that, the industries stated above continues to not adhere to the discharge limits that have been set by DOE (Abidin et al., 2018). Moreover, continuous increase in pollution of rivers and closures of wastewater treatment plants (WTP) does not only impact the daily life of the public but also increases the cost of water treatment and maintenance of WTPs. It was reported that the Federal Government of Malaysia had spent RM25 million for rehabilitation of polluted rivers from the year 2011 – 2014 (Drainage and Irrigation Department of Malaysia, 2014).

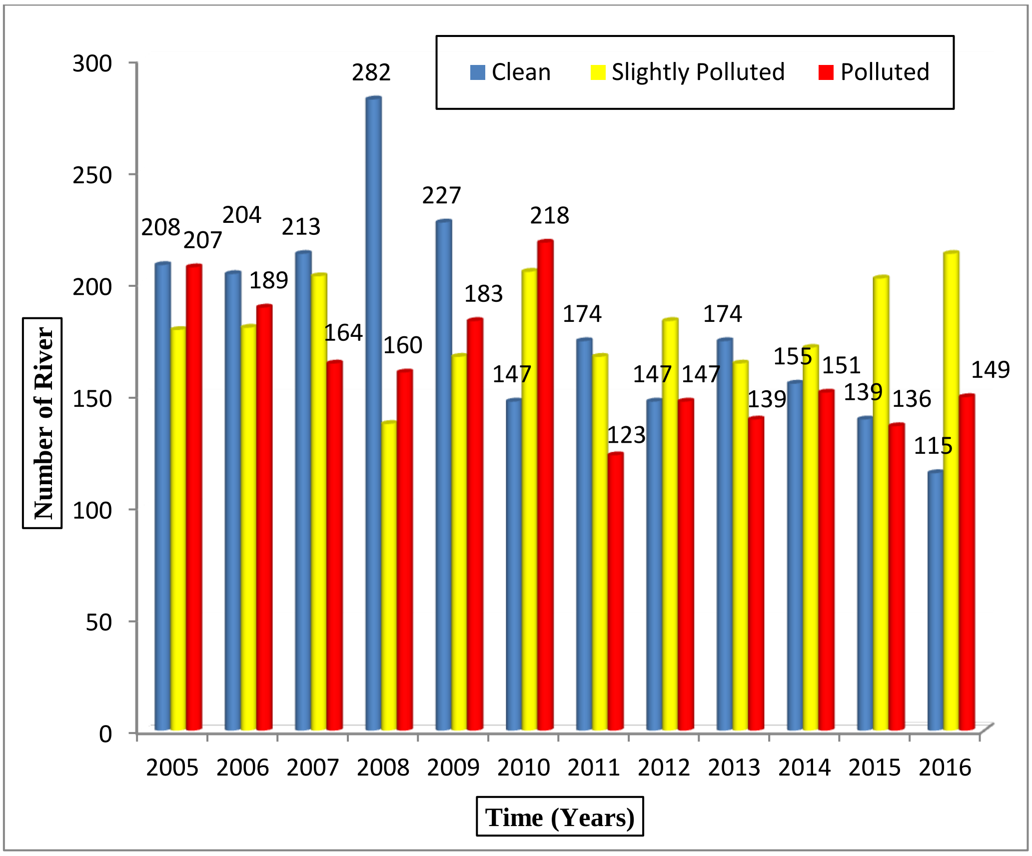

Figure 1.2: **The trend of rivers in Malaysia being polluted by ammoniacal nitrogen from 2005 – 2016 (Department of Environment, Malaysia, 2017)**

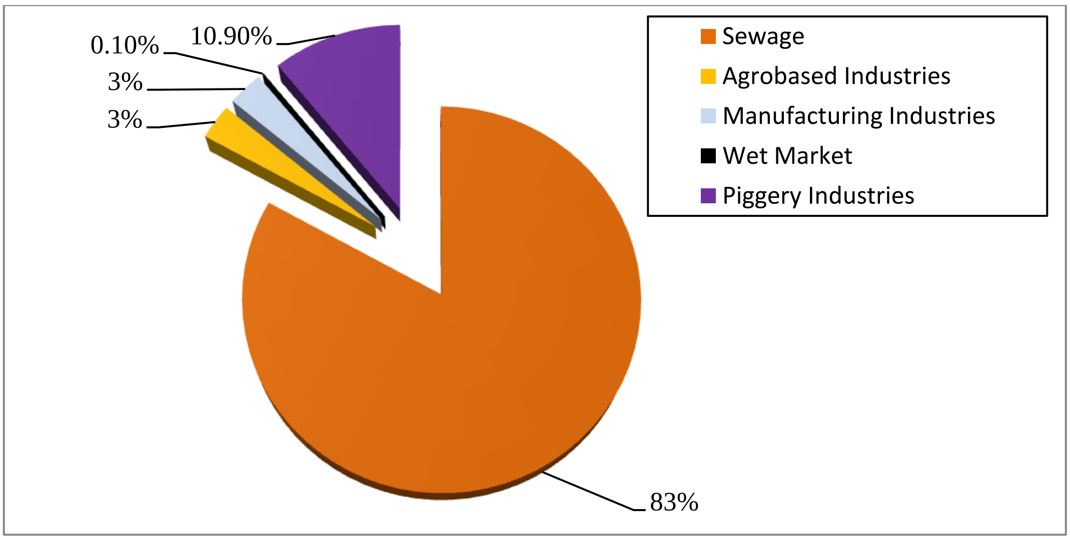

Figure 1.3: **Ammoniacal nitrogen loading estimation according sources of pollution in Malaysia, 2016 (Department of Environment, Malaysia, 2017)**

Therefore, a more comprehensive method of treating the wastewater or river water in regards to removal of ammonia should be implemented. Currently water and wastewater treatment plants use physical, chemical and biological treatment method to remove nitrogen (Ye et al., 2019). However, in recent years biological treatment has been favoured to remove nitrogen from waters and wastewaters because it can be removed more comprehensively with limited by-products being produced (Ye et al., 2019). Biological treatment also is said to be more comprehensive because most of the time the stringent ammonia discharge limits (10 – 20mg/L) could be achieved effectively with reduction of operation cost (Zhu et al., 2008). Above all, biological treatments also reduce the amount of external chemical usage which might be more detrimental to humans and aquatic lives in a long run.

In regards to biological treatment, various methods have been found and used to treat wastewater containing nitrogenous components. Among those methods used are conventional biological treatment, partial nitrification + denitrification, and anaerobic ammonium oxidation (Anammox process). Each and every method has its own advantages and disadvantages. However, recent advances have suggested that the Anaerobic Ammonium Oxidation (Anammox) should be considered to be one of the main choices in treating nitrogenous wastewater. One of the major reasons for the preferences of Anammox is because no organic carbon addition is needed, since ammonia is used as electron donor for nitrite reduction. In addition, Anammox process does not need aeration as well because this process occurs in an anaerobic environment (Ye et al., 2019). Thus, this helps to reduce the operation cost of a WTP. Evidently, the points stated above shows that biological treatment methods such as Anammox could pave the way for better water and wastewater treatment capability.

1.1.2 Problems associated to ammoniacal nitrogen in the rivers

Problems associated with the presence of high ammoniacal nitrogen content in river can be grouped into 3 major categories which are environmental, health and cost. The most concerning environment effect is the eutrophication. The presence of high amount of ammonia in the surface waters can lead to increase in the amount of nitrate in the water due to natural nitrification process (Xia et al., 2018). Nitrate is the main source of algal bloom which predominantly leads to eutrophication and drop in water quality due to depletion of oxygen (Hosseini, 2016). Due to eutrophication, water bodies will become shallow and in the long run this leads to reduction in fresh water resources and flooding.

Secondly, the availability of high ammoniacal nitrogen in the rivers is very much toxic to the aquatic life. Ammonia is chronically toxic towards the aquatic life because it affects the reproductive capabilities and in some cases cause death (Hosseini, 2016). In regards to human, the ammonia can uncouple the oxidative phosphorylation process where this will lead to inhibition and depletion of adenosine triphosphate (ATP) in the brain (Camargo and Alonso, 2006). Severe lack of ATP can damage the brain activities in a long run. Besides that, ingestion of high amount of ammonia can lead to internal organ burns as well (Semerjian et al., 2018; USEPA, 2007).

Finally, increase in the ammoniacal nitrogen in water bodies increases the cost for remedial action. It is reported from the year 2000 the combined cost of European economy on ecosystem, climate and health impact due to nitrogen pollution has increased from 70 to 320 billion euro (Van Grinsven et al., 2013). Out of this total cost, normally 60% of it was used to improve and remedy human health due to nitrogen pollution, 35% to improve the ecosystem health and the balance 5% was used to reduce the green house gas effects due to nitrogen pollution. The money spend on this equates to a welfare loss of in between 150 – 740 euros/person (Van Grinsven et al., 2013). Due to this, it is important to treat the wastewaters with high nitrogen concentration before it is discharged into water bodies.

1.2 Problem Statement

One of the biggest problems faced by worldwide water and wastewater treatment plants is achieving the standard discharge level of ammoniacal nitrogen which is in the range of $\leq$ 20 mg/L (Khin and Annachhatre, 2004; Ye et al., 2019). To make matters worse, most conventional water treatment plants are not fitted or designed with nitrogen removal facilities and this leads to water being polluted by nitrogenous compounds like ammonia (Jetten et al., 2002). Apart from that, the wastewater sources that contain high nitrogen content have increased in line with increase of population and industrial revolution. Wastewater originating from industries such as dairy farms, food processing, fertilizer manufacturing, slaughter house and landfill leachate contain greater amount of nitrogen load nowadays (Xia et al., 2018). Thus, to make sure these wastewaters are treated properly and the nitrogen discharge limits are attained, an efficient and comprehensive treatment needs to be developed and implemented.

In most cases chemical and physicochemical methods are still preferred as the treatment method for ammonium removal. However, chemical and physicochemical treatment method depends on criteria's such as cost benefit analysis, energy requirement, chemical dosage, familiarity with operational procedures and finally impact on environmental sustainability (Rajasulochana and Preethy, 2016). The biggest problem in using chemical and physicochemical methods are that they are not cost effective and this continues to be a major factor for industries to disregard attaining the discharge limit (Sedlak, 2018). Due to this, the world is moving towards using biological treatment because biological treatment is cheaper and has higher nitrogen removal efficiency (Karthikeyan and Joseph, 2007; Sedlak, 2018). Thus, nitrogen removal via biological means needs to be studied more so that a clear understanding on designing and developing highly efficient biological treatment method can be implemented at industrial level in Malaysia.

Thus, Anammox is considered to be one of the most effective method in removing nitrogen from wastewater because of no external carbon source is needed, more energy efficient, and above all has higher nitrogen removal compared to the other biological treatment methods (Ye et al., 2019). However, Anammox has its disadvantages which can jeopardize the removal efficiency. The biggest problem of achieving a stable and efficient Anammox process is the need of long start-up period. One of the main factors that affect Anammox start-up time is the slow growth of Anammox bacteria where the cell doubling time is 7-20 days (Kartal et al., 2012; Miodonski et al., 2019). Another setback is that Anammox populations cannot be cultivated using conventional microbiological techniques such as petri dish culturing which makes it harder to obtain pure cultures that can be used as seed sludge (Kumar et al., 2017). Finally, Anammox bacteria has low ability to out-grow other autotrophic organisms such as the nitrifiers and algae at the very early stage of start-up and this normally leads to Anammox bacteria being washed out from the system (Ibrahim et al., 2016). Due to the circumstances stated above, multiple studies have been carried out to tackle and reduce long start-up periods of an Anammox reactor. However if providently observed, so far all the measures taken to reduce the start-up time focuses on operational parameters where the biochemical pathway of Anammox bacteria was not considered (Miodonski et al., 2019). This leads to the question, on how does manipulation of the biochemical pathway helps in reducing the start-up time of Anammox reactor. There is still a big research gap in regards to this problem.

Maintaining optimum operational condition of a wastewater treatment plant is very much complicated because most of the time the volume and the properties of the wastewater will vary on a daily basis. Thus, the complex nature of industrial wastewater and sewage composition is normally associated with the inhibition of Anammox process. To worsen things, the recovering capability of Anammox bacteria after inhibition are mostly compromised due to the nature of the wastewater composition as well (Jin et al., 2012b; Zhao et al., 2018). Even though, there are many constituents that can inhibit Anammox bacteria, ammonium and nitrite remain as the most important inhibition source to be considered and studied because both these elements are the major substrates for Anammox process. Above all, the inhibition of substrate is normally only studied as a separate entity (ammonium or nitrite) and not as a whole which provides a scarcity on knowledge on this part.

Finally, studies on starvation and recovery after starvation of Anammox process and bacteria remains limited. Starvation happens in wastewater treatment plants when large fluctuations of wastewater flow and composition occur regularly. This induces a famine period for the bacteria which could last from a few days to few months (Ye et al., 2018). Besides fluctuation of feed flow and feed composition, starvation condition could also be induced by closure of waste water treatment plant for maintenance (Ma and Wang, 2018) and storage of sludge/biomass that is due to be transported or used as seed sludge (Ji and Jin, 2014). The biggest problem associated with starvation is the difficulty to maintain cell viability. The main reason for this to happen is because the cell decaying process will kick start so as to maintain partial bacterial activity during starvation period (Wang et al., 2018b). Thus, ways of maintaining Anammox cell viability during starvation needs to be explored more as to reduce the recovery time of an Anammox reactor that was subjected to starvation.

Though various ways to overcome slow start-up time of Anammox reactor, reduce inhibition effects and increase cell viability during starvation has been studied, manipulation of the biochemical pathway of the Anammox bacteria continues to be discarded especially the usage of hydrazine (Miodonski et al., 2019). Hydrazine is known to be the most important product in Anammox process as oxidation of hydrazine releases 4 electrons to be used for energy production (Kumar et al., 2017). Thus, it is quite alarming that so far addition of hydrazine has not been considered to help reduce start-up time, enhance recovering capabilities of inhibited Anammox bacteria and reduce cell decay during starvation. Furthermore, these problems could not only be tackled by manipulating the Anammox process but also by choosing the correct unit operations. In this case, the usage of Sequencing Batch Reactor (SBR) should be considered. SBR is considered to be one of the most effective unit operations that can be used to effectively treat high strength nitrogen and phosphorus wastewater (Rajab et al., 2017). Moreover, the usage of SBR in treating multiple types of wastewater especially Palm Oil Mill Effluent (POME) in Malaysia has been steadily increasing (Liew et al., 2015). As a summary, the gaps of research in regards of start-up, inhibition, starvation and recovering capability of Anammox bacteria remains to be tackled and studied on a more holistic manner.

1.3 Objectives

The primary aim of the study is to achieve an enriched and efficient Anaerobic Ammonium Oxidation (Anammox) system that can treat high strength nitrogenous wastewater using Sequencing Batch Reactor (SBR) while studying the effects of hydrazine in helping to reduce the effects of inhibition and starvation. The specific objectives are:

- To develop an enriched Anammox system that has stable and efficient nitrogen removal capabilities in a sequencing batch reactor.
- To investigate the effects of external hydrazine addition on the duration of start-up period of Anammox process.
- To study the substrate inhibition onto Anammox bacteria and the role of externally added hydrazine in aiding the recovery process.
- To elucidate the correlation effects of ammonium, nitrite and hydrazine in maintaining cell viability of Anammox bacteria during starvation and recovery after starvation period

1.4 Scope of Study

This study revolves around enrichment of Anmmox culture in a SBR (parent reactor) and on how hydrazine can help in tackling the start-up problems and aiding recovery of Anammox bacteria after substrate inhibition and starvation. Thus, this study consists of four major parts**.** The first part is where enrichment of anammox culture was done using a SBR. During enrichment the substrate concentration (ammonium + nitrite) was increased from 100 to 900 mg/L nitrogen with the ammonium:nitrite being 1:1. In this part, the research was limited to monitoring reactor performances in regards to Nitrogen Removal Efficiency (NRE), biomass production, hydrazine accumulation, nitrous oxide (N_2O) emission and production of nitrate plus Heme C extraction. These details are essential in understanding the Anammox enrichment process before further experiment can be carried out.

In the start-up study, the effects of different externally added hydrazine concentration in reducing the start-up time of an Anammox reactor is investigated. The time taken for the reactor to stabilize and achieve more than 80% nitrogen removal efficiency was monitored. Hydrazine accumulation and nitrous oxide emission in the start-up reactors were also closely monitored throughout the study.

Once the enrichment of Anammox culture is successful, the Anammox cultures were subjected to long term substrates inhibition. This experiment was done using 2L SBR whereby the Anammox cultures was obtained from the 8L main SBR. 3 different substrates concentration was used to study the inhibition effect on Anammox bacteria. In this part, the inhibition kinetics in terms of removal of both substrates was also studied to give a proper insight into total nitrogen inhibition on Anammox. Finally, the ability of hydrazine to aid recovery of the inhibited Anammox cells was studied as well.

Finally, Anammox bacteria from 8L main SBR were subjected towards different type of starvation condition. Presence of minute amount of ammonium, nitrite or hydrazine during starvation in maintaining cell viability and in aiding the recovery process after starvation was investigated. This was important, because any biological wastewater treatment plants are normally subjected to starvation condition on a yearly basis. Without proper knowledge on maintaining cell viability during starvation, the whole WTP operation can be disrupted.

The entire findings of this research are limited to lab scale only. Further research is required to implement this technology at pilot plant or real plant scale. However, the parameters studied in this work could form a strong basis to upscale the process to pilot plant or real plant scale in the future.

1.5 Organization of Thesis

There are five major chapters in this thesis. Chapter 1 (Introduction) is a brief introduction on river pollution trend in Malaysia and the increasing ammonium pollution problems in rivers of Malaysia. It also includes brief explanation on current biological treatment used to remove ammonium or nitrogen from wastewater. Compact information on Anammox is provided as well. Besides that, the need for this study to be done (problem statement), the objectives of this research, and scope of the research as well the arrangement of the thesis are also mentioned in this chapter.

In Chapter 2, a thorough literature review regarding this topic was provided. This chapter comprises of technical terms and aspects regarding Anammox process which was obtained from previous studies done on Anammox process and bacteria. Besides that, the application of SBR for wastewater treatment, development of Anammox start-up and enriched culture, factors of inhibition, starvation studies, as well as recovering capabilities were also discussed.

Chapter 3 (Materials and Methods) provides the information about the materials and methods used in carrying out this research for analyzing purpose. The raw material, analyzing methods and equipments, and kinetic modelling are discussed in detail in this particular chapter.

Meanwhile in Chapter 4 (Results and Discussions), the results obtained in this research were elaborately explained. The first part of the chapter revolves around the enrichment of Anammox culture and sustaining them for a long period of time to achieve stable and optimum nitrogen removal. Next, effects of long term substrates inhibition and capabilities of hydrazine to aid in recovery of Anammox bacteria that were subjected to long term inhibition was studied. In this same part, the inhibition kinetic using two different models were also provided and discussed. This is followed by starvation studies where the ability of Anammox bacteria to recover after certain period of time under different starvation condition was discussed. The connection between cell decay and degradation of extracellular polymeric substances was also discussed in detail.

The final chapter which is Chapter 5 (Conclusion) will revolve around the conclusions drawn from this study as it is reported. The conclusions were made based on the discussions made in Chapter 4. These conclusions would able to determine whether the objectives of this study are met or not. Some recommendations were also given for future works based on the current study done. The shortages found in this research could be addressed in the upcoming works to further enhance the treatment method in order to optimize nitrogen removal from wastewater.

CHAPTER 2

LITERATURE REVIEW

2.1 Biological treatment

Biological wastewater treatment is a process that uses microorganisms to help with the decomposition of organic and inorganic substances that are found in wastewaters. Though on the surface it looks much simpler, most of the time the biological treatment processes contain complex levels of biochemical interactions and pathways. Thus, in this part of the literature review, various biological treatment methods are discussed and dissected.

2.1.1 Conventional process

Generally, nitrogenous compounds exist in wastewater in the form of ammonium. Conventional biological removal of nitrogenous compounds from wastewater comprises of two-step process. First is the nitrification involving autotrophic bacteria and the second is the denitrification which involves heterotrophic bacteria. Nitrification process involves Ammonium Oxidizing Bacteria (AOB) which oxidizes ammonium into nitrite (Equation 2.1) and Nitrite Oxidizing Bacteria (NOB) which oxidize the nitrites into nitrates (Equation 2.2) (Verhoeven et al., 2018). This is followed by the denitrification process which will reduce the nitrate to dinitrogen gases in the presence of various electron donors such as methanol and acetate (Equation 2.3) (Capodici et al., 2018; Zhu et al., 2008).

$NH_3 + 0_2 \rightarrow NO_2^- + 3H^+ + 2e^-$ **(2.1)**

$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-$ **(2.2)**

$5C_6H_{12}O_6 + 24NO_3^- + 24H^+ \rightarrow 30\ CO_2 + 12N_2 + 42H_2O$ **(2.3)**

Though conventional process is still the very much popular choice in treating nitrogenous wastewaters, there are many disadvantage of this process. The first one is that since the conventional nitrification and denitrification process are carried out by two different organisms with different conditions, two different setup of reactors had to be designed and operated at different time spaces (Marin et al., 2016; Zhu et al., 2008). Besides that, high level of external carbon source had to be added during denitrification process to achieve high removal efficiency. High amount of aeration was also needed during nitrification process because 1g of ammonium is oxidized by 4.2g of oxygen (Bruce and Perry, 2001). Both of these factors lead to high operating cost. Above all, conventional biological treatment process requires high retention time or large volume to achieve complete nitrogen removal (Zhu et al., 2008). The factors stated above, forced the scientific community to find or develop alternative biological treatment processes so that a more efficient and low cost process can be adapted.

2.1.2 Partial nitrification and denitrification

Combination of partial nitrification and denitrification is a short cut process of a conventional biological treatment process. This process is also known as short route nitrogen removal and illustrated in Figure 2.1. This happens whereby, during nitrification process, the ammonia will only be oxidized into nitrite by inhibiting the growth of NOB (Ge et al., 2015). Due to this, it will not undergo full oxidation until nitrate is obtained. This process uses nitrite as the intermediate product instead of nitrate.

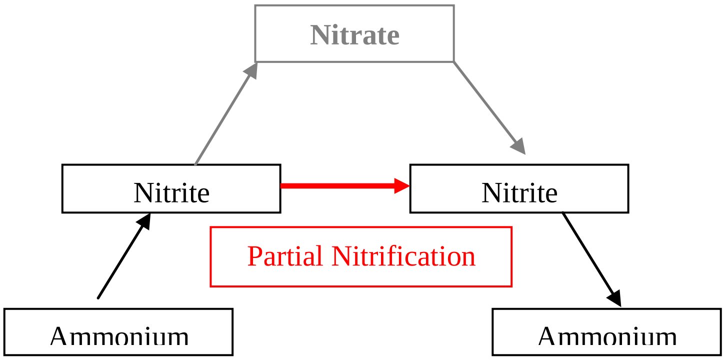

Figure 2.1: The short cut route for nitrogen removal using partial nitrification

Recently it was demonstrated that both the processes could be undertaken in a single reactor by controlling operational parameters such as dissolved oxygen (DO), pH, substrate load, aeration patterns and sludge retention time (SRT) (She et al., 2016). This combined process in a single reactor is called Single reactor system for High Ammonia Removal Rate over Nitrite or SHARON. The advantages of this process is that it reduces the oxygen usage by 25%, reduces the external carbon addition by 40% and increases nitrogen removal efficiency during denitrification by 1.5 – 2.0 folds (Peng and Zhu, 2006; She et al., 2016). Just as any other biological treatment process, this short-cut process has its disadvantages as well. Even though partial nitrification process can be achieved at low DO concentration, but the low DO causes lower COD degradation and enhances sludge bulking. Bulking of sludge could lead to maintenance issues of wastewater treatment plant (WTP) as well as the treatment capability could be jeopardized. Besides that, for SHARON to efficiently work, the reactors need to be operated at temperatures higher than the room temperature (35˚C) (Volcke et al., 2007). The economic compatibility of running the reactors at higher temperature to achieve optimum and efficient nitrogen removal must always be taken into consideration as to not increase the operational cost. Thus, the need to find a better suited process in removing nitrogen by biological means continues.

2.1.3 Anaerobic ammonium oxidation (Anammox)

As pointed out above, the limitations of other biological treatment methods has led to new methods being developed or discovered continuously. One of those new methods discovered to eradicate the shortcomings of other biological treatment methods is Anaerobic Ammonium Oxidation (Anammox) process. Anammox process was discovered in 1995 where the Anammox bacteria oxidizes the ammonium into nitrogen gas (N_2) using nitrite as the electron acceptor (Kumar et al., 2017; Mulder et al., 1995). In short, Anammox bacteria combine both ammonium and nitrite to produce hydrazine (N_2H_4). This hydrazine will later be oxidized in to nitrogen gas (N_2) and water (H_2O) (Karthikeyan and Joseph, 2007; Kumar et al., 2017). The interesting fact here is that Anammox bacteria actually have the ability to synthesize hydrazine as an intermediate product and metabolize it to be used as an energy source (Schalk et al., 1998). Five major genera of bacteria that is associated with the ability to carry out Anammox process are *Kuenenia, Brocadia, Anammoxoglobus,* *Jettenia, and Scalindua* (Jetten et al., 2009). Anammox bacteria catabolism starts with the reduction of nitrite (NO_2^-) to nitric oxide (NO) by a nitrite oxidoreductase (NIR). Then the hydrazine synthase enzyme (HZS) forms hydrazine (N_2H_4) by combining ammonia (NH_4^+) with NO. Later the N_2H_4 will be oxidized to dinitrogen (N_2) by hydrazine dehydrogenase (HDH) where four electrons are released for production of adenosine triphosphate (ATP) (Kartal et al., 2011). The most interesting part here is that hydrazine is the intermediate product of Anammox and it is considered to be extremely toxic plus it inhibits majority of the bacteria used in biological treatment except Anammox bacteria (Qiao et al., 2016). Figure 2.2 depicts the different pathways that Anammox bacteria can undergo under different substrate availability (Park et al., 2017)

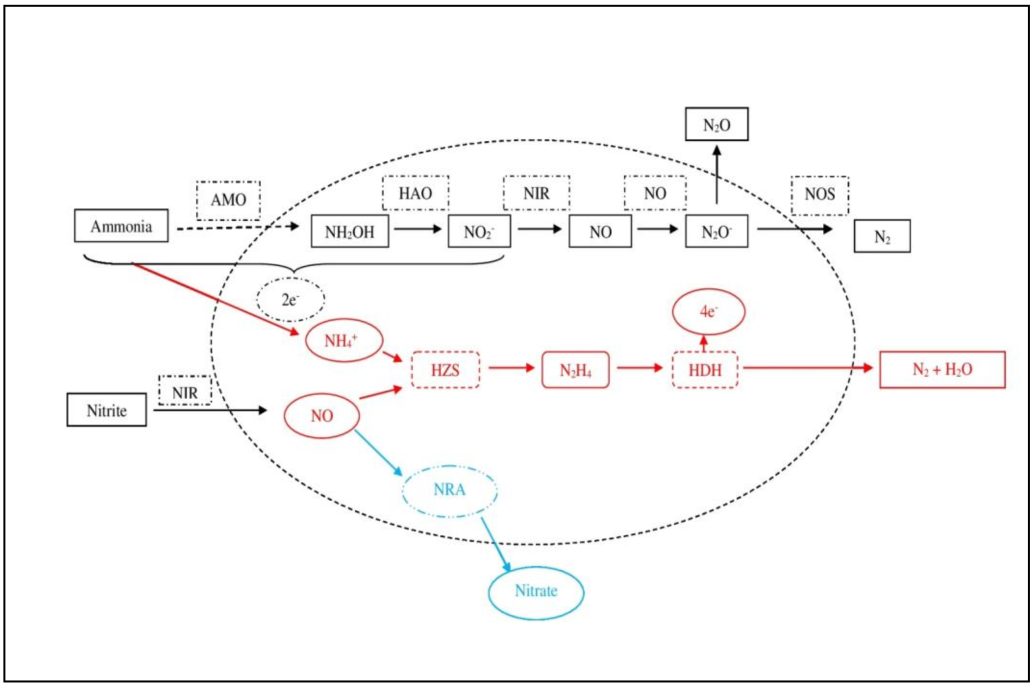

Figure 2.2: Metabolite pathways of Anammox process (Park et al., 2017) (AMO: Ammonium Oxygenase; NIR: Nitrite Oxidoreductase; HAO: Hydroxylamine Oxidoreductase; NOR: Nitric Oxide Reductase; NRA: Nitrate Reductase; NOS: Nitrous Oxide Synthase; HZS: Hydrazine Synthase and HDH: Hydrazine Dehydrogenase).

Key factors that need to be regulated to achieve an optimum and efficient Anammox process are substrate concentration, reactor configuration, dissolved oxygen (DO), pH, and SRT. Currently, Anammox process is preferred in biological treatment because it has higher nitrogen removal rate, lower operational cost and requires lesser space for operation (Van der Star et al., 2007; Zhao et al., 2017). Above all it does not need addition of external source of carbon as it has the ability to fix carbon dioxide (CO_2) (Zhu et al., 2008). Anammox process also produces low biomass yield which reduces the amount of biomass sludge being produced because for every gram of ammonium oxidized only 0.11g of volatile suspended solids (VSS) is accounted for (Fux, 2003; Kumar et al., 2017). This also helps to reduce the cost of sludge treatment and management in a WTP.

In addition, Anammox process can be designed and developed to operate in a single reactor and this helps to reduce the operational hours as well. Anammox process can be combined with partial nitrification process to remove ammonia and it is known as Completely Autotrophic Nitrogen removal over Nitrite or CANON. The capability of Anammox process to treat nitrogenous wastewater efficiently and reduce operational cost at the same time makes it a no brainer to be used commercially in WTPs. However, limited number of countries only has adapted this method in recent years. In Malaysia, Anammox treatment has not been adapted yet so far. Thus, this condition begs to add on the knowledge of starting up and operating an Anammox reactor to optimize the nitrogen removal.

2.2 Advantages and Disadvantages of Anammox Process

Just like any other biological treatment methods, Anammox do have its fair share of advantages and disadvantages. One of the advantages of using Anammox process is that it reduces the energy usage for aeration by approximately 58% (Hauck et al., 2016; Strous et al., 1999a). Besides that, the need of addition of organic carbon is eliminated entirely as Anammox bacteria are chemoautotroph (Kumar et al., 2017). Chemoautotroph microorganisms has the ability to oxidize inorganic compound to produce energy source and at the same time utilizes carbon dioxide to produce new cells (Mara and Horan, 2003). Both the advantages stated above helps to reduce the cost where only 1.55€/kg N_{elimin} is spend when Anammox process is used compared to conventional nitrification/denitrification process which needs 3.10€/kg N_{elimin} (Lackner et al., 2014). Apart from that, the amount of sludge produced by Anammox process also is much lower compared to the one produced by other biological treatment process (Kumar et al., 2017). But, above all, the Anammox process has the ability to achieve high nitrogen removal efficiency because it reaches effluent discharge target on a frequent basis (Yang, 2012). Another important advantage is that, Anammox process helps to reduce the CO_2 gas emission because Anammox bacteria uses CO_2 gases as their carbon source for growth purpose (Ye et al., 2019).

Though, Anammox process has the capability to be highly efficient, it still has its disadvantages. The biggest problem for Anammox bacteria is that it has a very slow growth rate where the doubling time could vary in between 7 –

20 days (Ali and Okabe, 2015; Kartal et al., 2012). This complicates things during the start-up of the reactor because long start-up time is required before a stable and efficient Anammox process can be established. Secondly, Anammox bacteria are very much sensitive towards the changes in substrate (ammonium and nitrite) concentration which could lead to inhibition and starvation if not monitored properly (Kartal et al., 2012). Construction cost for replacing conventional nitrogen removal wastewater treatment plants (WWTP) to Anammox treatment plants will cost more as the implementation of process control, installation of plant and maintenance of sensors such as pH and DO sensor are more dynamic and advanced (Lackner et al., 2014). Finally, the knowledge on Anammox process especially on pure culturing the Anammox bacteria is still scarce as conventional culturing method such as petri dish culturing has failed so far (Kartal et al., 2012; Mulder et al., 1995). Thus, it is important to find ways and methods to eliminate or reduce the disadvantages in order to establish Anammox treatment plant.

2.3 Factors Affecting Anammox Bacteria Enrichment and Process Start-Up

In this part, various factors that will affect the enrichment process and start-up period of an Anammox reactor are discussed thoroughly. A comparison table done on different start-up studies is provided at the end of the part as well.

2.3.1 Seed sludge

Seed sludge plays a vital role in Anammox bacteria enrichment and process start-up. The normal way of enriching an Anammox reactor is by using Anammox seed sludge or non-Anammox seed sludge (Suneethi and Sri Shalini, 2015). It is very much important to select the correct seed sludge so as to reduce the start-up time of an Anammox reactor (Suneethi and Sri Shalini, 2015). As a whole Anammox seed sludge are very much preferred because normally a stably running Ananmmox reactor will contain significant amount of Anammox bacteria. By obtaining seed sludge from an Anammox reactor, not only the start-up time can be reduced but several Anammox reactors can be initiated simultaneously as well (Suneethi and Sri Shalini, 2015). So far, reactors enriched with Anammox seed sludge could be started-up within 25 – 50 days (Bae et al., 2015; Guo et al., 2016). On the contrary, Anammox reactors enriched using non-Anammox seed sludge had longer start-up time which were in the range of 43 – 92 days (Connan et al., 2016; Wang et al., 2013).

Besides start-up time, the efficiency of nitrogen removal varies according to the type of seed sludge used for enrichment purposes as well. The normal Ammonium Removed: Nitrite Removed: Nitrate Produced ratio of a stably running Anammox reactor is 1:1.31:0.26 (van der Star, 2008). The reactors enriched with Anammox seed sludge normally provides the result stated above. However when seed sludge from sewage and digester were used to enrich an Anammox reactor, the ratios of substrate removal and nitrate produced were 1:1.02:0.23 and 1:1.19:0.25, respectively (Date et al., 2009). Nonetheless, recent