THE EFFECT OF AERATION PATTERN ON GRANULAR AMMONIA OXIDIZING BACTERIA DEVELOPMENT AND PERFORMANCE

MUHAMAD SHAIFOL BIN MOHD YUSOF

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by

MUHAMAD SHAIFOL BIN MOHD YUSOF

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LIST OF SYMBOL

	Symbol	Unit
MLSS	Mixed liquor suspended solid	mg/L
MLVSS	Mixed liquor volatile suspended solid	mg/L
V	Settling velocity	m/s

LIST OF ABBREVIATIONS

AMO	Ammonia mono-oxygenase
Anammox	Anaerobic ammonium oxidation
AOB	Ammonia oxidizing bacteria
BOD	Biochemical oxygen demand
COD	Chemical oxygen demand
CO_2	Carbon dioxide
DO	Dissolve oxygen
FA	Free ammonia
FNA	Free nitrous acid
HAO	Hydroxylamine oxidoreductase
MLSS	Mixed liquor suspended solid
MLVSS	Mixed liquor volatile suspended solid
NOB	Nitrite oxidising bacteria
NOR	Nitrite oxidoreductase
O_2	Oxygen
PN	Partial nitrification
SBR	Sequencing batch reactor
SRT	Solid retention time

TSS	Total suspended solids

KESAN CORAK PENGUDARAAN TERHADAP GRANUL BAKTERIA PENGOKSIDAAN AMMONIA PERKEMBANGAN DAN PRESTASI

Absrak

Nitrifikasi separuh oleh granul aerob selalunya dijalankan pada pengudaraan yang tetap. Dalam kajian ini, kesan pengudaraan terhadap nitrifikasi separauh granul bakteria pengoksidaan ammonia telah dikaji di dalam tiga reaktor kelompok urutan (R1-R3) yang berbeza. Kesan pengurangan pengudaraan terhadap pengranulan telah dikaji di dalam Reaktor 1 sementara kesan nitrifikasi separuh dikaji dalam Reaktor 1, Reaktor 2 dan Reaktor 3. Hasil kajian menunjukkan Reaktor 1 berjaya mencapai 41 % penyingkiran ammonia dengan purata diameter 1.55 mm. Kepekatan nitrit di dalam efluen R1 ialah 158 mg/L. Masa mendakan memainkan peranan yang penting terhadap proses pengranulan dimana ia akan bertindak sebagai daya ricih. Tempoh mendakan telah dikurangkan dari lima minit ke tiga minit seterusnya kepada dua minit dan akhir sekali tempoh mendakan dikurangkan kepada satu minit. Kepekatan biojisim di dalam Reaktor 1 berkurang sepanjang kajian ini dilaksanakan dan kepekatan tersebut mula stabil pada hari ke-57. Pada masa yang sama, Reaktor 2 mencapai 50 % penyingkiran ammonia dengan purata diameter granul 2.77 mm dan Reaktor 3 mencapai 42 % penyingkiran ammonia walaupun mempunyai kepekatan biojisim yang rendah. Kepekatan nitrit yang dikesan dalam efluen Reaktor 2 dan Reaktor 3 masing-masing ialah 360 mg/L dan 242 mg/L.

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Abstract

Partial nitrification (PN) of aerobic granule usually carried out in constant aeration rate. In this study, the effect of reduction in aeration toward granular ammonia oxidizing bacteria (AOB) was studied in three different columns of sequencing batch reactor (SBR) (R1-R3). The effect of reducing aeration on granulation process was studied in Reactor 1 while the effect on PN was studied in Reactor 1, Reactor 2, and Reactor 3. Results show that Reactor 1 achieve 41 % of ammonium removal with average granule diameter of 1.55 mm. The concentration of nitrite at the effluent is 158 mg/L. The settling time plays an important role on the granulation process where it will acst as shear force. The settling time of Reactor 1 was reduced from five minutes to three minutes followed by two minutes and finally to one minute. The biomass concentration in Reactor 1 initially decrease and start to stabilize at day 57. Meanwhile, Reactor 2 achieved 50 % of ammonium removal with 2.77 mm average granule diameter and Reactor 3 achieved 42 % ammonium removal despite low biomass concentration. The concentration of nitrite presence in the effluent of Reactor 2 and Reactor 3 are 360 mg/L and 242 mg/L respectively.

CHAPTER ONE

INTRODUCTION

1.1 Wastewater Treatment

In 2014, Malaysia generated around 2.97 billion cubic meters per year of wastewater which mainly comes from the municipal and industrial sector (Shaari et al., 2014). The wastewater needs to be treated before discharging into the environment. This because it will pose a chemical and biological health risks to irrigation and communities that have prolonged contact with the untreated wastewater (Mukhtar et al., 2013). According to the Environmental Quality (Sewage) Regulations 2009, the treated wastewater are categorized into two classifications which is Standard A and Standard B. Standard A applicable to discharges into any inland waters within catchment areas while Standard B is applicable to any other inland waters or Malaysian waters. The characteristic of both classifications are shown in Table 1.1 (Department of Environment Ministry of Natural Resources and Environment, 2010).

Wastewater treatment plant is divided into four stages which are preliminary treatment, primary treatment, secondary treatment and tertiary treatment. In the first stage, the coarse solids and other large materials that will damage and disturb the equipment and operational of subsequent treatment are removed. This is important in order to ensure the subsequent treatment plant operate smoothly. The wastewater later will enter primary treatment plant. During this stage, 25 to 50% of the incoming biochemical oxygen demand (BOD), 50 to 70% of the total suspended solids (TSS) and 65% of the oil and grease are removed (Food And Agriculture organisation of United Nation, 1992).

Table 1.1: Acceptable conditions of sewage discharge of standards A and B

	Parameter	Unit	Standard	
			A	B
	(1)	(2)	(3)	(4)
(a)	Temperature	°C	40	40
(b)	pH Value	-	6.0-9.0	5.5-9.0
(c)	BOD5 at 20°C	mg/L	20	50
(d)	COD	mg/L	120	200
(e)	Suspended Solids	mg/L	50	100
(f)	Oil and Grease	mg/L	5.0	10.0
(g)	Ammonical Nitrogen (enclosed water body)	mg/L	5.0	5.0
(h)	Ammonical Nitrogen (river)	mg/L	10.0	20.0
(i)	Nitrate – Nitrogen (river)	mg/L	20.0	50.0
(j)	Nitrate – Nitrogen (enclosed water body)	mg/L	10.0	10.0
(k)	Phosphorous (enclosed water body)	mg/L	5.0	10.0

The Secondary treatment plant also known as a biological treatment plant. The objective of biological treatment are (Metcalf and Eddy, 2003):

a) To transform dissolved and particulate biodegradable into the acceptable end product
b) Capture and incorporate suspended and non-settleable colloidal solids into a biological floc or biofilm
c) Transform or removed nutrients such as nitrogen and phosphorus.

Nitrogen being removed via nitrification, denitrification, anaerobic ammonium oxidation while phosphorus is removed by phosphorus removal process

Lastly, wastewater will undergo tertiary treatment also called advanced treatment process. The main concern of this treatment is to remove remaining suspended solid, organic contents, nitrogen and phosphorus from preceding stages. The processes involved are chemical coagulation followed by flocculation, sedimentation, filtration, and disinfection (Kirkpatrick and Takashi, 1986). The main disadvantage of this treatment is high capital cost and maintenance. Equipment such as membrane filter is expensive and need regular maintenance to ensure high efficiency of treatment.

Among all treatments, biological treatment is the main concern in order to save total cost in treating wastewater. This fact supported by biological treatment function itself. By increasing it removal efficiency, tertiary treatment plant can be eliminated. Hence, the total capital cost can be reduced.

In biological treatment, nitrification is a process where ammoniacal nitrogen is biologically oxidized to nitrite and then to nitrate with oxygen (O_2) as terminal electron acceptor. This process followed by denitrification where nitrate will be reduced to nitrite before further reduce into nitrogen gas (Cuidad et al., 2005). Nitrification is accomplished by the chemolitho-autotrophic microorganism which obtains energy from the oxidation of substrate whereas denitrification performs by the heterotrophic microorganism that obtains energy by using organic carbon or chemical oxygen demand (COD).

Unfortunately, in real wastewater the amount of COD usually low. Therefore, the addition of a source of organic carbon like methanol, ethanol, and molasses needed which lead to additional operating cost (Sinha and Annachhatre, 2007). In order to overcome this situation many research have been done which lead shortcut biological nitrogen process such as PN. PN based on fact that nitrite as an intermediate product instead of nitrate (Cuidad et al., 2005). This nitrogen removal pathway will reduce 40% COD requirement, save 25% O_2 consumption, reduce 300% biomass production and 20% carbon dioxide (CO_2) emission during the denitrification (Shijian et al., 2015).

1.2 Problem Statement

Currently, many researchers focusing on the development of granular bacteria. This is due to the benefit of granular compare to floccules and filamentous bacteria. The granulation process involved of feast and famine stage where aeration will be supplied continuously causing bacteria cell become hydrophobic and it will accelerate microbial aggregation (Yu and Joo-Hwa, 2004). The aeration supply contains dissolve oxygen (DO) where it is important as the electron acceptor. Theoretically, the O_2 is important in feast phase because it will be used to oxidize substrate whereas in famine phase microorganism undergoes starvation. The aeration during famine phase is crucial to provide shear force for the microbial transform itself to granule. Conventionally, aeration rate is maintained throughout both phases which lead to high cost. Thus, the study on the reduce aeration rate during famine period is vital to reduce additional cost on developing and maintaining aerobic granule. Besides that, this study will provide information on the ability of granule in PN process in reducing nitrogen in wastewater.

1.3 Research Objective

a) To study the effect of aeration pattern development of granular AOB

b) To evaluate the performance of granular AOB under reduce aeration

CHAPTER TWO

LITERATURE REVIEW

2.1 Nitrogen in Wastewater

Nitrogen that found in wastewater usually in form of ammonia which originated from urea, amino acid products, casein, corrosion inhibitors, process chemicals and raw materials or cleaning chemicals containing quaternary ammonium compounds. Discharging wastewater containing a high level of nitrogen in form of ammonium into freshwater can increase the existing amount of nutrient. It will lead to several problems to aquatic environment such eutrophication and algae bloom (Xian-Yang et al., 2010; Conley et al., 2009).

Eutrophication is a phenomenon where the high level of nutrients such as nitrogen in wastewater triggering the growth of algae (algae bloom) and higher plant. In freshwater, the major cause of this phenomena is anthropogenic inputs of nutrients. This results in dominance by cyanobacteria and dinoflagellate (Shaw et al., 2009).

Algae or algal blooms is an overgrowth of algae in freshwater. The algae involve are cyanobacteria including genera *Anabaena, Aphanizomenon, Lyngbya, Microcystis, Nodularia* and *Oscillatoria* (Hans et al., 2001). Algae bloom will create dead zones in the water, produce extremely dangerous toxins that can sicken or kill people and animals and will increase treatment cost for drinking water.

Therefore, it is very important to remove nitrogen in wastewater before discharge into the environment. This can be achieved through biological nitrogen removal which consists of nitrification, denitrification and anaerobic ammonium oxidation.

Figure 2.1: Algae bloom phenomena

2.2 Nitrification and denitrification

Nitrification is a process where the ammonium oxidized into nitrite over hydroxylamine by AOB. This is an endothermic reaction involving membrane bound ammonia mono-oxygenase (AMO) and hydroxylamine oxidoreductase (HAO). Based on equation 2.1, ammonia initially oxidized into hydroxylamine before further converted into nitrite (equation 2.2) (Sinha and Annachhatre, 2007). By using oxygen supply through aeration as terminal electron acceptor the resulting reaction pathway is shown in equation 2.3 (Hopper, 1989).

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

$$NH_2OH + H_2O \longrightarrow NO_2 + 5\,H^+ + 4\,e^- \qquad (2.2)$$

$$NH_3 + 1.5\,O_2 \longrightarrow NO_2^- + H^+\ \ C \qquad (2.3)$$

Subsequently, nitrite will be oxidized to nitrate by making used of membrane-bound nitrite oxidoreductase (NOR) by nitrite oxidizing bacteria (NOB). This reaction is shown in equation 2.4. The overall energy generation of nitrification is given in equation 2.5. Nitrification is a rate-limiting step in biological nitrogen removal process. This fact supported by the slow growth of slow growth rates and poor yields of the organisms involved (Holben et al., 1998).

$$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^- \qquad (2.4)$$

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \qquad (2.5)$$

In order to discharge nitrogen gas into the atmosphere, nitrate will undergo denitrification where nitrate will be converted into nitrite. This process will continue with the conversion of nitrite into nitrogen gas (Ruiz et al., 2003). Denitrification process catalysed by heterotrophic which use COD as their carbon source and energy. However, denitrification always limited by low COD in wastewater (Loosdrecht et al., 2004). In order to counter this problem, addition COD such as methanol are needed to achieve high removal of nitrate in the treatment plant. Thus it will to addition cost in biological removal (Loosdrecht and Jetten, 1998).

2.3 Microorganism

Nitrification process involves two type of chemolithotrophic bacteria which are AOB and NOB. Chemolithotrophic microbial obtain carbon from the oxidation of inorganic carbon (Holben et al., 1998). Nitrosomonas and Nitrosospira are a type of AOB which have been used widely in many research regarding biological nitrogen removal (Harms et al., 2003). However, there are approximately 25 cultured species which have a different salt requirement and substrate affinity. Type of NOB which usually is Nitrospira which specialised in nitrite oxidiser. Member Nitrospira prefers relatively low nitrite concentration and is found as the most abundant nitrite oxidizer in wastewater treatment systems (Shijian et al., 2015).

2.4 Partial nitrification

In order to overcome problem exists in conventional nitrogen removal such as require additional COD due to low COD contents in real wastewater, the researcher found a new pathway to remove nitrogen effectively. PN is a shortcut biological nitrogen removal which nitrite as an intermediate product. In this pathway, ammonium will be converted into nitrite without further oxidised to nitrate (Sinha and Annachhatre, 2007). Generally, PN is a couple with ANAMMOX process. Dosta, et al., (2015) reported that PN effluent was used as feed ANAMMOX reactor. ANAMMOX feed comprises of ammonium and nitrite with a molar ratio of 1:1. To achieve that molar ratio, the inlet molar ratio of inorganic carbon to the substrate used was 1: 2. Consequently, only 50 % of ammonium will be oxidised to nitrite due to lack of inorganic carbon.

PN is favorable compare to conventional nitrification/denitrification due to several advantages. Firstly, it can save energy and COD consumed during denitrification. The energy is in term of aeration. Aeration used during PN is lesser compared to the conventional process (Loosdrecht et al., 2004), (Sinha and Annachhatre, 2007). Based on the stoichiometric equation, for 1 mole of ammonia, AOB use 1.5 moles of oxygen and NOB use 0.5 mole of oxygen. Complete nitrification requires 2 moles of oxygen per mole of nitrogen to be nitrified. This means that PN to nitrite will only require 1.5 mol of oxygen per mole of nitrogen, implying a 25% less oxygen demand for PN compared to complete nitrification (Ruiz et al, 2003).

Strategy to achieve PN is by ensuring the NOB completely washout from the system or adjusting system condition that incompatible to NOB growth. NOB can be inhibited by regulating system parameter such as DO, pH level, temperature, free nitrous acid (FNA) and free ammonia (FA) (Yongzhen and Guibing, 2006).

2.4.1 Dissolved Oxygen

In PN, O_2 is important as terminal electron acceptor. O_2 has been supplied to the system through aeration. The O_2 half saturation constant for AOB is lower that NOB which indicate that AOB has high oxygen O_2 affinity toward DO compared with NOB (Picioreanu et al, 1997). Therefore, the inhibition of NOB can be performed at low DO level since NOB prefer high DO level which leads to washout of NOB.

Cuidad et al., (2005) reported at DO level of 4 mg/ L there is no nitrite accumulate in effluent which indicates NOB was not inhibited. At 1.4 mg/L, 95% of ammonia removal achieve with 75% of nitrite accumulation. When DO level further reduce to 1.0 mg/L maximum nitrite accumulation was achieve but the ammonia removal was affected. Thus, the DO level for the reactor was increased to 1.4 mg/L in order to increase ammonia removal. Yong et al., (2009) treated domestic water through PN which can be achieved at DO level 0.4 – 0.7 mg/L. The nitrite pathway was failed when DO level increased to 2- 3 mg/ L. Therefore, it can be concluded that optimum DO level which can inhibit NOB is between 0.4 – 1 mg/L.

2.4.2 pH

Oxidation of ammonium to nitrite cause hydrogen ion in wastewater to increase. pH affect PN process through the equilibrium between nitrite and FNA and ammonium with FA (anthonisen et al., 1976). The optimum pH for pH is slightly alkaline, which will inhibit NOB activity in wastewater (Surmacz-Gorska et al., 1997; Suthersan and Ganczarczyk, 1986). Wookeun et al (2002) reported that high nitrite accumulation achieve at pH 8 to 9. At pH 10 ammonia utilisation rate was drop to a minimum. Tokutomi (2004) also reported the optimum pH for PN is at 8.5. However, complete inhibition of AOB and NOB take place at ph lower than 6.45 and higher than 8.95 (Ruiz et al., 2003).

2.4.3 Temperature

The growth rate of microorganism affected by temperature since heat will be used as activation energy for growth. AOB and NOB have a different sensitivity toward temperature. Alleviating temperature will promote microorganism growth rate and will expand the difference specific growth rate between AOB and NOB (Jianhua, et al., 2010). Hellinga et al. (1998) reported that at temperature 25 ˚C AOB will out compete NOB because AOB growth rate is higher at high temperature. By decreasing temperature from 25 to 15 ˚C the ammonia oxidation rate decreased by 1.5 times. However, microorganism starting to adapt at low temperature. This is because s at longer period nitrite accumulation achieve is 90 %.

2.4.4 Free Nitrous Acid and Free Ammonia

The protonated form of nitrite in wastewater is FNA and FA for protonated form of ammonia. AOB is more affected by FNA compared to FA. The amount of FNA and FA can be calculated by using equation 2.6 and 2.7. (Anthonisen et al., 1976).

$$\mathrm{FA}(mg/\mathrm{L}) = \frac{17}{14} \times \frac{[NH_4{\cdot}N] \times 10^{pH}}{\exp[6,334/(273+T)] + 10^{pH}} \tag{2.6}$$

Where;

$NH_4{\cdot}N$ is ammonium concentration.

T is temperature of system in ˚C

$$FNA\ (mg/\mathrm{L}) = \frac{46}{14} \times \frac{\left[NO_2^{-}{\cdot}N\right]}{\exp[-2,300/(273+T)] \times 10^{pH}} \tag{2.7}$$

Where;

$NO_2^{-}{\cdot}$ N is nitrite concentration

T is temperature of system in ˚C

Based on given equation, the value of FNA and FA directly depend on temperature, pH and substrate concentration. As nitrite is produced, pH decreases due to the release of hydrogen ions. It have concluded that the inhibition on nitrification is related to the concentration of unionized nitrous acid (FNA) rather than the nitrite anion concentration, and the inhibition on nitrification will be initiated at an FNA concentration of 0.22-2.8 mg HNO_2-N/L (Wookeun et al., 2002).

FA inhibition to AOB starts from 10 to 150 mg/l and FA inhibition to NOB start from 0.1 to 1.0 mg/l. The inhibition of nitrifying organisms was initiated at concentrations of FNA between 0.22 and 2.8 mg/l (Anthonisen et al., 1976). Based on Vadivelu et al. (2006), FA concentration up to 16 mg/L does not inhibit AOB. However, at FNA concentration of 0.5 – 0.63 mg/L AOB initiate to inhibit and at 0.4 mg/L the biosynthesis of AOB completely stop which means no more AOB growth in SBR. Zhou et al. (2011) reported continuos feeding will reduce FNA inhibitions effect compares to dump feeding. 50% of AOB activity reduced when FNA concentration is between 0.42 to 1.72 mg/L.The inhibition of NOB start at 0.011 to 0.07 mg/L HNO2-N/L and the complete inhibition occur at FNA concentration of 0.026 to 0.22 mg/L HNO2-N/L.

The different between the threshold level for inhibition of AOB and NOB may cause by the different species used in each study. However, based on the range of inhibition of AOB and NOB mentioned before. It can be concluded that NOB is more sensitive towards FNA compared to AOB. AOB also can tolerate with the higher amount of FA compared to NOB (Wookeun et al., 2002). Therefore, inhibiting NOB is more feasible instead of AOB. This is because NOB is more sensitive toward FNA and FA concentration.

2.5 Granulation process

There are two type of granulation process which is anaerobic granulation and aerobic granulation. Anaerobic granulation was performed under anaerobic condition while aerobic granulation was performed under aerobic condition. Aerobic granule favorable compared to anaerobic granule because it has several drawbacks such as long start-up period, high operation temperature and no application for low-strength organic wastewater is a process (Qin et al., 2004). Aerobic granule also has excellent physical properties such as more compact and tightly structure (Li et al., 2008) which lead to better settling properties. Aerobic granular sludge systems have a higher mixed liquor suspended solid (MLSS) concentration and longer solid retention times (SRT) than conventional activated sludge systems. Longer SRT important for the growth of the microorganism (Zhang et al., 2011). Besides that, it also has greater ability to withstand shock loadings (Liu et al, 2009).

Initially, aerobic granule was developed in continuous up flow aerobic sludge blanket bioreactor by Mishima and Nakamura (1991). Later SBR become popular in aerobic granulation because it can provide a shear force which important in the formation of the granule (Liu and Tay, 2004). The mechanism of aerobic granulation starts with a physical movement which initiates bacterium-to bacterium contact. The movement will initiate attractive physical forces such as chemical forces, and biochemical forces between cells leading to stabilization of the multicellular. Next, the cell will become aggregated mature cell will form through the production of extracellular polymer, the growth of cellular clusters, metabolic change, environment-induced genetic effects that facilitate the cell–cell interaction and result in a highly organized microbial structure. Lastly, a three-dimensional structure of microbial aggregate will form (Yu et al., 2007).

There is still lack of detail explanation on granulation process because different species of bacteria have different physiochemical properties. Thus, each species have their own agglomerate abilities. For the cultivation aerobic granule number of factors have been identified as influencing the bio aggregation processes, including settling time, volume exchange rate and aeration rate (Bao at al., 2009)

2.5.1 Settling time

Settling time is defined as the given time for bacteria to settle to the bottom of SBR before decanting phase. Settling time play an important role as a major hydraulic selection pressure on sludge particle (Liu and Tay, 2004). Very weak selection pressure did not favor aerobic granulation, and relatively strong selection pressure was essential for the development of aerobic granules in SBR. Shorter settling time is desired to washout poor settling particle from SBR and retained particle that can settle within allocated time.

Wang et al.(2011) stated that shorter settling time reactor develops granule faster. By decreasing settling time granule form successfully. Based on Qin et al. (2004), four reactors operated a 20, 15, 10 and 5 minutes settling time where only reactor that has 5 minutes settling time have dominated by aerobic granule. There is no granule form in another reactor. However, when settling time was reduce from 20 to 5 minutes, 15 to 2 min, 5 to 1 minutes, the biofloc completely replaced by aerobic granule.

2.5.2 Hydrodynamic shear force

The formation of excellent granules is as a result of high shear force. The shear force applied is in term of aeration rate or superficial velocity. Aeration will be supplied continuously through the reaction period which consists of two phase. Firstly, bacteria will undergo degradation process where the substrate is oxidised to extensively. After that, aerobic starvation takes place due to depletion of external substrate. As a result, bacteria became more hydrophobic (Tay et al., 2001).

During the starvation phase, the cell will immobilise itself and attach to one another. Micro-organisms are able to change their surface characteristics when faced with starvation and such changes can contribute to their ability to aggregate (Tay et al., 2001). Thermodynamically, when hydrophobicity of cell surface increase, it will lead to decrease in the excess Gibbs energy. As a result, connecting fibrils start to form and the cell-to-cell interaction strength will be high making it structure denser and stable (Varon and Choder, 2000).

Extracellular polysaccharides act as building block and maintaining aerobic granule structure. It usually contain protein, carbohydrate, and humid substance. As the shear force such as aeration rate increase, the protein contents will also increase. Aeration will cause microbial cell to experience shear force. This situation will stimulated bacteria to secrete more extracellular polysaccharide to maintain their shape (Ohashi and Harada, 1994). Density of aerobic granule also related with shear force. Dense and compact granules are obtained during high aeration rate. (Tay et al., 2001) Stated that at aeration rate of 3.01 L/min will form compact and rounder granule. Based on Tsuneda et al., (2003) too low shear stress will cause no development of granule. There are no granule form at aeration volume of 0.036 L/min/L-bed. Adav et al., (2007) reported that, aeration rate of 1 L/min cause sludge flocs to compact but it unable to aggregate into larger granule. Granule successfully develop at aeration rate of 2 L/min and 3 L/min. However, reactor failure occur at rate of 2 L/min causing by overgrowth of filamentous

CHAPTER THREE

MATERIAL AND METHOD

3.1 Synthetic wastewater

The synthetic wastewater used for experiment was adapted from Kuai and Verstraete (1998) compromised per liter : 0.1 g of ammonium bicarbonate, $NH_4(HCO_3)$, 0.1 g of potassium dihydrogen phospate (KH_2PO_4), and 0.1 ml of micronutrient solution (4.93 g $NaNO_2$, 0.4 g $NaHCO_3$, 1g K_2HPO_4, 1.25 g EDTA, 0.55 g $ZnSO_4.7H_2O$, 0.4 g $CoCl_2$, 1.275 g $MnCl_2$, 0.4 g $CuSO_4.5H_2O$, 0.05 g $NaMoO_4.2H_2O$, 1.375 g $CaCl_2.H_2O$, 1.25 g $FeCl_3.6H_2O$ and 44.4 g $MgSO_4.7H_2O$) which give 4.43 mg of NH_4^+-N.

3.2 Sequencing batch reactor

Three different SBR is labeled Reactor 1, Reactor 2 and Reactor 3 were operated at different aeration rate. Each reactor has 2 L working volume with height to diameter ratio of 10 and 25 % exchange ratio. The reactors cycle time was 8 hour. Reactor 1 was inoculated with enrich floccule AOB taken from parent reactor in Universiti Sains Malaysia. This used to study the effect of aeration pattern on granule development and performance of AOB. During aerobic aeration, the aeration rate for the first 150 minutes was 1 L/min and the aeration was reduced to 0.5 L/min. Settling time for Reactor 1 was summarized in Table 3.1.

Table 3.1 Settling time of Reactor 1

Days	Settling time (minutes)
0-6	5
7-15	3
16-25	2
26-87	1

Reactor 2 and Reactor 3 were used to evaluate the AOB performance under reducing aeration. Both reactors were inoculated with enriched granular AOB taken from parent reactor in Universiti Sains Malaysia. Samples were collected from each reactor to evaluate its development and performance. The cycle time used for both reactor comprising of 2 minutes of feeding, 1 minutes of settling, 2 minutes of effluent withdraw and 475 minutes of aerobic reaction. The aeration rate for Reactor 2 was 1 L/min for first 150 minutes and at 151th to 480th minutes the reaction rate was reduced to 0.5 L/min. For reactor 3 the aeration rate was 1 L/min for first 150 minutes and at 151th to 480th minutes the reaction rate was reduced to 0 L/min.

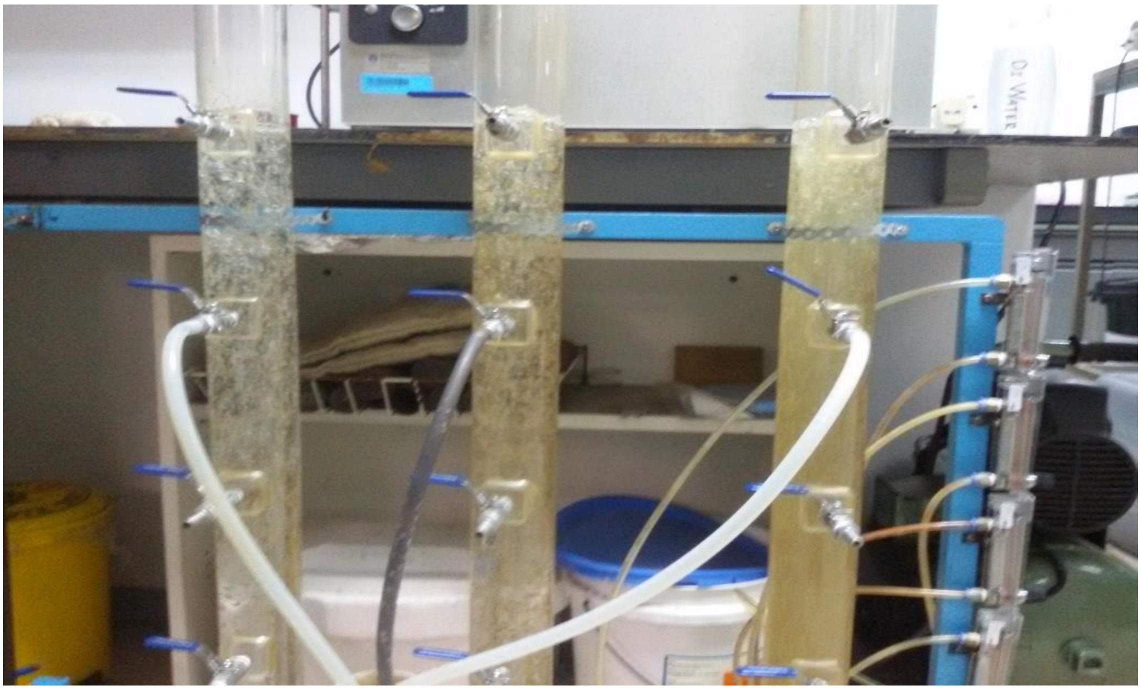


Figure 3.1: SBR (from right, Reactor1, 2 and 3)

3.3 Analytical method

3.3.1 Ammonium and Nitrite Measurement

Firstly, the sample was prepared before analytical analysis. For measurement of ammonium concentration, the mixed liquor sample taken from SBR was diluted with 20 times dilution. The sample was diluted by mixing 0.1 ml of mixed liquor with 1.9 ml deionized water into a test tube. Later, 0.1 ml mixed sample was put into high range ammonia test N tube reagent (Hach Company). Ammonia salicylate (Hach Company) and ammonia cyanurate reagent (Hach Company) were mixed together and shaken. The sample was left for 20 minutes for the reaction to take place.

For measurement of nitrite concentration, the mixed liquor sample was diluted with 2 times of dilution. 5 ml of mixed liquor with 5 ml of deionized water were mixed in screw cap test tube. Nitriver 2 nitrite reagent was poured into the diluted sample and shaken. The sample was left for 5 minutes for the reaction to take place.

Both prepared samples were measured by using DR 2800 Spectrometer. Ammonium and nitrite measurement for SBR's effluent were performed weekly for Reactor 1, Reactor 2, and Reactor 3.

3.3.2 MLSS and mixed liquor volatile suspended solid (MLVSS) measurement

Firstly, the weight of Whatman GF/A filter paper were measured by using weighing balance and recorded as (A) mg. Next 10 ml of mixed liquor was taken using a pipette and was filtered through the Whatman GF/A filter paper. Later, the residue remained on the filter paper was dried in the oven with the temperature of 105 ˚C for 24 hours. The weight dried filter paper with residue was measure and recorded as (B) mg. Lastly, the dried filter paper with residue was burned in a furnace to burn microorganism on the filter paper by using temperature of 550 ˚C for 30 minutes. After that, the weight of the filter paper was measured and recorded a (C) mg. All procedure were repeated for 2 times to obtain average weight. MLSS and MLVSS were calculated by using equation 3.1 and 3.2 respectively (Metcalf and Eddy, 2003). All procedure were repeated for 2 times to obtain an average value of MLSS and MLVSS. The MLSS and MLVSS measurement were performed weekly to monitor biomass growth in all reactor

$$\text{MLSS (mg/L)} = \frac{(A-B)\times 1000}{sample\ volume\ (ml)} \qquad (3.1)$$

$$\text{MVLSS (mg/L)} = \frac{(B-C)\times 1000}{sample\ volume\ (ml)} \qquad (3.2)$$

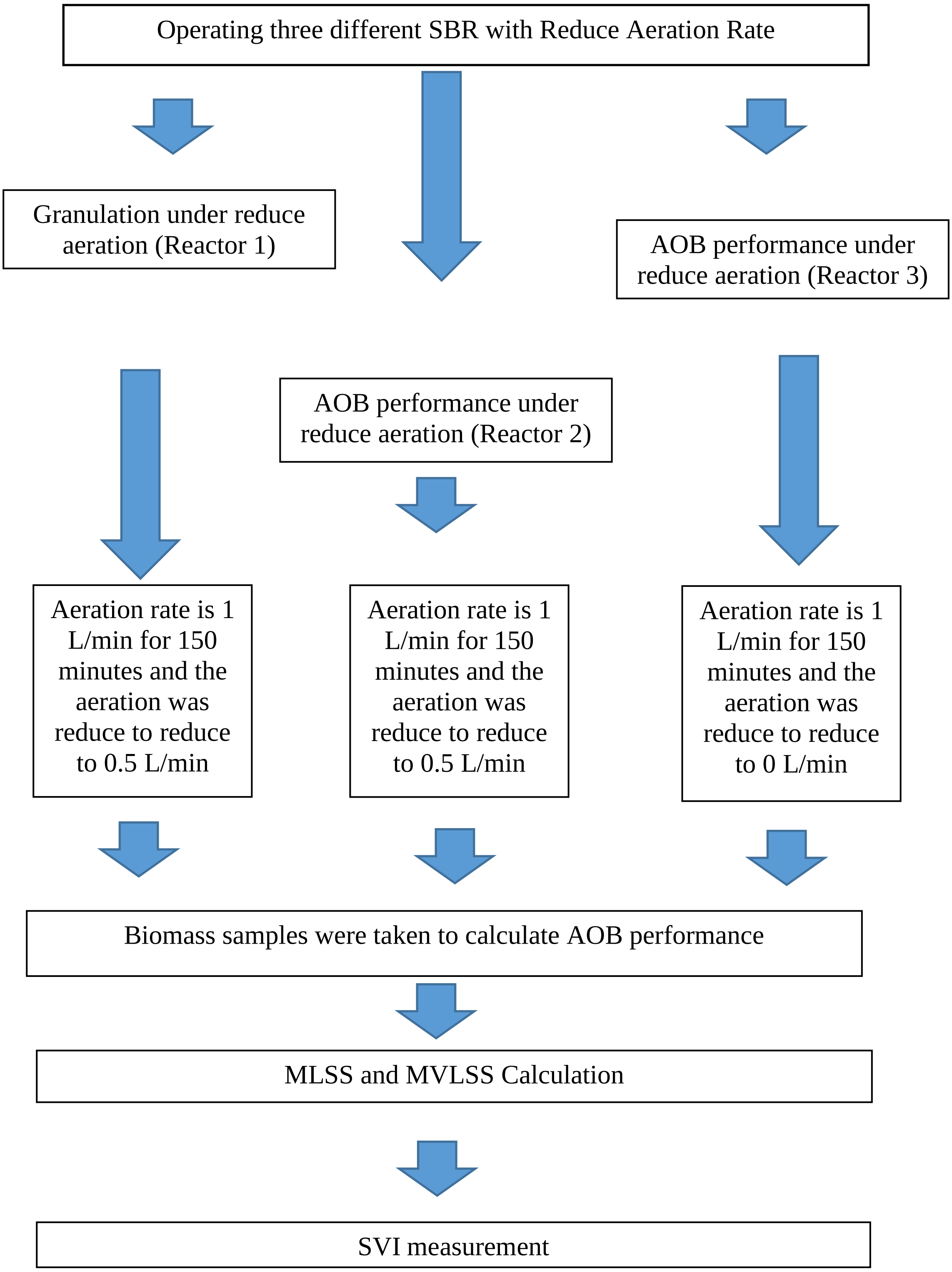
3.3.3 Setling velocity measurement

Several granule of AOB were selected randomly and was put into 1000 ml measuring cylinder. The time taken for each granule to settle was taken. The settling velocity was calculated by using formula 3.2.

$$\text{V (m/s)} = \frac{height\ of\ measuring\ cylinder\ (metre)}{settling\ time\ (second)} \qquad (3.2)$$

3.4 Experimental analysis

The overall experimental activities carried out in this study are presented in the following schematic flow diagram:



CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Performance of granular AOB

4.1.1 Reactor 1

Reactor 1 was used to develop granular ammonia oxidising bacteria (AOB) from floccular AOB originated from parent reactor. The aeration rate of this reactor is 1 L/min for 150 min and later the aeration rate was reduced to 0.5 L/min. The feed concentration is shown in Table 4.1. For phase 1 inlet feed contain 1000 mg NH_4^+-N /L .The 9th and 16th day of ammonium and nitrite concentration are shown in Figure 4.1. Based on Figure 4.1, the removal of ammonium on the day 9 and 16 achieved were 53 % and 55 % respectively and the nitrite concentration in the effluent is 785 mg/L and 775 mg/L respectively. However, due to a technical problem the feed to Reactor 1 was not supplied for a week starting from day 22 to day 28.

Table 4.1: Feed Concentration of Reactor 1

Phase	Day	Feed Concentration (mg NH_4^+-N /L)
1	1 to 21	1000
2	22 to 28	No feed
3	30 to 37	500
4	38 to 87	460

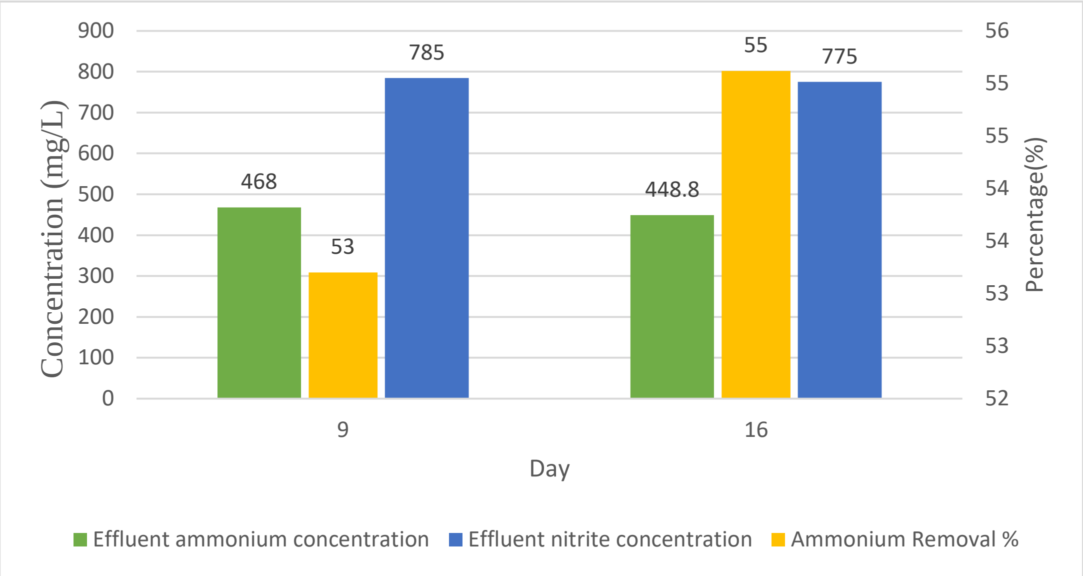


Figure 4.1: Reactor performance during phase 1

Based on Table 4.1, during phase 3 the feed for Reactor 1 was changed to 500 mg NH_4^+-N /L for 1 week. Based on Figure 4.2 the effluent ammonium concentration on day 35 is 358 mg/L and the effluent ammonium concentration increase to 436 mg/L at day 37 with ammonium removal of 28.4 % and 12.8 % respectively. Meanwhile, there is very low nitrite concentration in the effluent in phase 3. The low nitrite concentration obtains at the effluent indicate that the NOB presence in the reactor. The ammonium converted to nitrite further oxidised to nitrate.

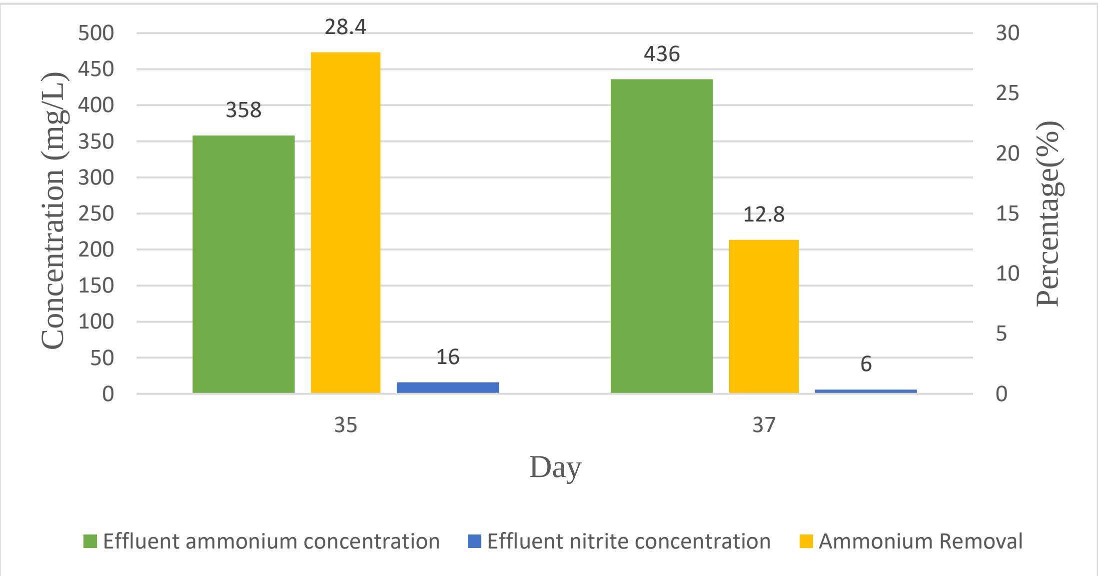


Figure 4.2: Reactor performance during phase 3

Due to the decreasing of ammonia removal and low nitrite accumulation, the feed was reduce to 460 mg NH_4^+-N /L in phase 4. The reactor performance is shown in Table 4.2 and Figure 4.3. Based on Figure 4.3, the performance of Reactor 1 is increasing since the effluent of ammonia concentration is decline while the effluent nitrite concentration rises up with time. On 44th day, the effluent ammonium concentration is 374 mg/L while the nitrite concentration only 8 mg/L. This point out that only 8 mg/L of ammonium are converted into nitrite. This fact is because the relationship between ammonia remove and nitrite accumulate is directly proportional. Based on this relationship, the reduction concentration of ammonium is effluent is directly converted into nitrite.

The relationship is achieved on day 63 and 64 when the removal of ammonia and the nitrite in effluent are have the same concentration which are 146 mg/L on day 63 and 186 mg/L on day 64. The highest removal is obtained on day 64 where the ammonium removal achieved is 40 %. From Figure 4.3, the increasing of ammonia removal is due to acclimation of biomass in the reactor. In parent reactor, the ammonium is continuously feed for a certain period while in Reactor 1 the dump feeding approach are used. Reduce in ammonium concentration in feed from phase 1 to phase 3 will reduce the shock loading.

Table 4.2: Reactor performance during phase 4

Day	Effluent ammonium concentration (mg/L)	Effluent nitrite concentration (mg/L)	Ammonium removal (%)
44	374	8	13
49	322	22	30
60	334	50	27
63	314	146	32
64	274	186	40

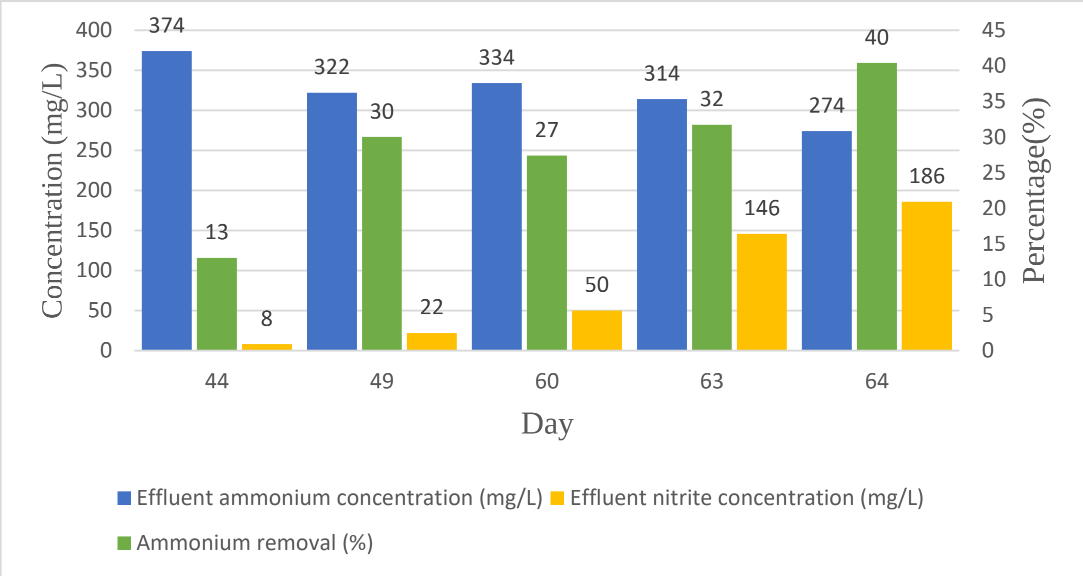


Figure 4.3: Reactor performance during phase 4

Table 4.3 and Figure 4.4 show the cycle study carried out on day 87. This study was conducted to monitor the ammonium removal within the system. Based on the graph given, the concentration of ammonium reduces significantly from tenth minutes to 2.5^{th} hour. This is due to feast phase undergoes by the biomass. During this period the conversion of ammonium by AOB is at the high rate. When the aeration rate were reduced to 0.5 L/min, the dissolved O_2 in reactor also decreases causing reduce in electron acceptor needed by AOB. Hence, the ammonium removal rate decreases greatly after 2.5^{th} hour. The trend for nitrite production increase as time increase as depicted in Figure 4.4.