

**DEVELOPMENT AND UTILIZATION OF AEROBIC GRANULES FOR
TREATING LOW STRENGTH DOMESTIC WASTEWATER**

by

PEYONG YET NEE

Thesis submitted in fulfillment of requirements

for the degree of

Master of Science

March 2012

ACKNOWLEDGEMENT

First of all, I would like to thank my supervisor, Dr. Vel Murugan Vadivelu, for his ideas and suggestions. I would also like to thank my supervisor for his patient and valuable guidance, support, useful comments, which have been a great help in achieving the objectives of this study. Dr Vel Murugan also shares his knowledge and professional experience to solve the problems that happened throughout the study period. Special thanks are extended to Assoc. Prof. Dr. Ahmad Zuhairi Abdullah as my co-supervisor for his suggestions and advices.

My greatest gratitude goes to USM for providing fellowship and RU-PRGS (1001/PJKIMIA/8033034) grant for providing fund that made me able to pursue the master program at USM.

Not to forget, words of thanks must be also conveyed to the personnel from Jelutong Sewage Treatment Plant and Taman Sempadan Sewage Treatment Plant for helping me to obtain the domestic sewage samples.

Next, I would also like to thank my research mates, Gobi Kanadasan, G. Sivarajah Ganesan, Nor Azyati who also work with biological treatment. I will not forget the friendship we built while carrying out our laboratory works. My deepest appreciation also goes to the lab assistant and technician for helping and assisting me throughout my project period.

Finally, to my family and friends, my deepest sincere thanks to them for the continuous full support, encouragement and motivation.

Peyong Yet Nee

November 2011

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LIST OF ABBREVIATIONS

DOE	Department of Environmental
EQR	Environmental Quality Report
SBR	Sequencing Batch Reactor
AOB	Ammonia Oxidizing Bacteria
NOB	Nitrite Oxidizing Bacteria
PAO	Polyphosphate Accumulating Organisms
GAO	Glycogen Accumulating Organisms
OLR	Organic Loading Rate
MLSS	Mixed Liquor Suspended Solid
SVI	Sludge Volume Index
COD	Chemical Oxygen Demand
BOD	Biochemical Oxygen Demand
IWK	Indah Water Konsortium
UASB	Upflow Anaerobic Sludge Blanket (UASB)
NTA	Nitrilotriacetic Acid
EPS	Extracellular Polymeric Substance
DO	Dissolved Oxygen
FA	Free Ammonia
FNA	Free Nitrous Acid
VER	Volume Exchange Ratio
HRT	Hydraulic Retention Time
WAS	Waste Activated Sludge
SEM	Scanning Electron Microscope

OUR	Oxygen Uptake Rate
SOUR	Specific Oxygen Uptake Rate
A/V	Area to Volume Ratio

LIST OF SYMBOL

A	Weight of empty filter paper and dried residue	(mg)
B	Weight of empty filter paper	(mg)
K_b	Ionization constant of ammonia equilibrium equation	
K_w	Ionization constant of water	
K_a	Ionization constants of the nitrous acid equilibrium equation	

PENGHASILAN DAN PENGGUNAAN BIOJISIM BUTIRAN AEROBIK DALAM RAWATAN AIR SISA DOMESTIK

ABSTRAK

Butiran aerobik telah dihasilkan dengan menggunakan air sisa yang berkekuatan rendah dalam reaktor penjujukan kelompok. Perubahan morfologi dan struktur mikrob butiran aerobik di bawah kadar bebanan organik yang berbeza dan toleransinya terhadap ammonia bebas dan asid nitrus bebas telah dikaji. Penghasilan butiran aerobik telah dijalankan dalam tiga peringkat. Di peringkat pertama, butiran aerobik telah dikulturkan dengan menggunakan air sisa sintetik berkekuatan rendah dan kadar bebanan organik sebanyak $1.2 \text{ kg/m}^3\text{hari}$. Butiran aerobik yang stabil terbentuk selepas 60 hari dengan saiz purata butiran sebanyak 2.2 mm. Kepekatan campuran pepejal terampai butiran aerobik ialah 1.2 g/L dengan indeks isipadu enapcemar bernilai 78 mL/g dan halaju pemendapan minimum sebanyak 22.5 m/jam. Di peringkat kedua, air sisa domestik yang mempunyai berkekuatan rendah dengan kadar bebanan organik sebanyak $0.13 \text{ kg/m}^3\text{hari}$ telah dimasukkan ke dalam reaktor. Setelah beroperasi selama 14 hari dengan air sisa domestik, satu campuran butiran aerobik termampat dan serpihan kecil akibat daripada perpecahan butiran telah diperhatikan. Kepekatan biojisim dan keupayaan untuk memendap telah berkurang kepada 0.17 g/L dan 165 mL/g, masing-masing. Di peringkat ketiga, sumber karbon luar telah ditambah kepada air sisa domestik untuk meningkatkan kadar bebanan organik kepada $0.6 \text{ kg/m}^3\text{hari}$. Di bawah keadaan baru ini, lebih banyak butiran aerobik dengan pemendapan yang baik telah terhasil dengan saiz purata 2.0 mm,

indeks isipadu enapcemar sebanyak 88 mL/g dan halaju pemendapan maksimum sebanyak 110 m/jam. Imej mikroskopik menunjukkan bahawa morfologi dan struktur mikrob yang berbeza terdapat dalam butiran aerobik di tiga peringkat yang berbeza. Kajian perencatan ammonia bebas terhadap aktiviti butiran aerobik menunjukkan bahawa ammonia bebas pada kepekatan 36.9 mg NH₃-N/L tidak merencat kadar pengambilan oksigen butiran aerobik. Walau bagaimanapun, FNA mula merencat kadar pengambilan oksigen butiran aerobik pada 0.015 mg HNO₂-N/L dan merencat sepenuhnya pada sekitar 0.134 mg HNO₂-N/L. Perencatan FNA terhadap butiran aerobik adalah berbalik.

DEVELOPMENT AND UTILIZATION OF AEROBIC GRANULES FOR TREATING LOW STRENGTH DOMESTIC WASTEWATER

ABSTRACT

Aerobic granules were developed using low strength wastewater in sequencing batch reactor (SBR). The changes in microbial morphologies and structures of aerobic granules under different organic loading rates (OLR) and their tolerance towards free ammonia (FA) and free nitrous acid (FNA) were studied. The development of aerobic granules was carried out in 3 stages. In the first stage, aerobic granules were cultivated using low strength synthetic domestic wastewater with OLR of 1.2 kg/m³day. Stable aerobic granules were formed after 60 days operation with an average granule size of 2.2 mm. The mixed liquor suspended solid (MLSS) concentration of aerobic granules was 1.2 g/L with the sludge volume index (SVI) value of 78 mL/g and minimal settling velocity of 22.5 m/h. Subsequently, in the second stage, low strength real domestic wastewater with OLR of 0.13 kg/m³day was fed to the reactor. After 14 days operation, a mixture of compacted aerobic granules and small debris resulted from the breakage of granules was observed. Biomass concentration and settle ability were reduced to 0.17 g/L and 165 mL/g, respectively. In the third stage, external carbon source was added to the domestic wastewater in order to increase the OLR by 0.6 kg/m³day. Under this new condition, more aerobic granules with excellent settle ability were formed with an average size of 2.0 mm, SVI of 88 mL/g and maximum settling velocity of 110 m/h. Microscopic images showed that different microbial morphologies and structures were found in aerobic

granules at the three different stages. The inhibitory studies of FA on the activity of aerobic granules reveals that FA at a concentration up to 36.9 mg NH₃-N/L did not inhibit the specific oxygen uptake rate (SOUR) of aerobic granules. However, FNA started to inhibit the SOUR at 0.015 mg HNO₂-N/L and completely stopped the oxygen consumption at around 0.134 mg/L HNO₂-N/L. FNA inhibition of aerobic granules was found to be reversible.

CHAPTER 1

INTRODUCTION

1.1 Water Pollution in Malaysia

Department of Environment (DOE) of Malaysia was set up in the year 1975 as an agency to enforce the legislation, Environmental Quality Act, 1974. Each year, an Environmental Quality Report (EQR) will be generated based on their monitoring on selected rivers in Malaysia (Table 1.1) to find out the sources of pollution. According to the finding, the sources of water pollution can be divided into two, point sources and non point sources. Point sources refer to the discharge from domestic wastewater treatment plant, manufacturing plant and industrial area. Non point sources apply to surface runoffs and agricultural activities. The EQR for river water quality from year 2005 to 2009 were summarized in Figure 1.1. It shows that the numbers of clean basin rivers were dropping dramatically since 2007. Meanwhile the slightly polluted and polluted basin rivers were increasing. Water pollution is getting worse and more serious even though enforcement has been launched strictly in year 2007 to control the pollution due to point sources. The 20702 polluted point sources as recorded in year 2009 consists of 47.2 % from manufacturing industries, 46.7 % from domestic wastewater treatment plant, 3.7 % from animal farm, and 2.4 % from agro based industries. Literature shows that most of researches related to wastewater treatment are dealing with industrial wastewater (Su and Yu, 2005, Cassidy and Belia, 2005, Wang et al., 2007a) with only few focusing on issues related to domestic wastewater. However, EQR report on water pollution sources clearly indicates that domestic wastewater is equally contributing (as industrial wastewater) to the pollution problem.

Due to the significant contribution of the discharge from domestic wastewater treatment plant to the water pollution, a new regulation has been added to Environmental Quality Act 1974. Under the new regulation, the acceptable discharge limit for new and existing domestic wastewater treatment plant become stringent and the limit of ammonium-nitrogen, nitrate-nitrogen and phosphorus concentrations were added in the new regulation (Table 1.2, 1.3, 1.4). The stringent limit for ammonium-nitrogen as well as the addition of nitrate-nitrogen in the new regulation (Table 1.2) is hoped to reduce rivers pollution due to nitrogen compounds (ammonia, nitrate, and nitrite). As can be seen in Figure 1.2, the data revealed by DOE on river water quality based on nitrogen compounds, clearly shows that lately the content of the nitrogen compounds are increasing in Malaysia' rivers.

The presence of high concentration of nitrogen compounds in water bodies can be undesirable for several reasons. High ammonium-nitrogen in the water will end up with eutrophication. Eutrophication is referring to excessive growth of undesirable algae and aquatic weeds due to high nutrients loading in water bodies. Growth of excessive algae and plants will utilize the oxygen in the water bodies (Sharpley et al., 1999). Consequently, it will cease the growth of other aquatic species due to insufficient of oxygen. Besides that, it has been found that toxic and volatile chemical produced by some algae species could bring serious health hazard to animals and humans. Thus, it is crucial to reduce the amount of nitrogen compounds in the effluent of wastewater treatment plants to below regulation limits.

Table 1.1 River Basin for Water Quality Monitoring.

State	River Basin	No. stations
Perlis	Perlis	1
Kedah	Kisap, Kuah, Ulu Melaka, Kedah, Merbok	5
Kedah/ P.Pinang	Muda	1
P.Pinang	Juru, Kluang, Perai, Bayan Lepas, Jawi, Pinang	6
P.Pinang / Perak	Kerian	1
Perak	Kurau, Sepetang, Bruas, Perak, Raja Hitam, Wangi, Tengi, Buloh	8
Perak / Selangor	Bernam	1
Selangor / WPKL	Klang, Langat, Sepang	3
Selangor	Selangor	1
Melaka	Duyong, Tuang, Seri Melaka, Melaka, Kesang, Merlimau	6
N. Sembilan	Lukut, Linggi,	2
Johor / N. Sembilan	Muar	1
Johor	Johor, Selidi Besar, Paloi, Mersing, Jemaluang, Endau, Batu Pahat, Benut, Pontian Besar, Pontian Kecil, Skudai, Kempas, Sanglang, Pulai, Kim-Kim, Air Baloi, Segget, Tebrau, Danga, Rambah, Kaw. Pasir Gudang,	21
Pahang	Anak Endau, Rompin, Merchong, Pahang, Bebar, Kuantan, Balok, Cherating, Tonggok	9
Terengganu	Kemaman, Kertih, Paka, Dungun, Marang, Terengganue, Setiu, Besut, Kluang, Chukai, Ibai, Merchang, Merang	13
Kelantan	Kemasin, Kelantan, Golok, Pengkalan chepa, Penkalan Datu,	5
Sarawak	Kayan, Semunsam, Sarawak, Balingian, Similajau, Niah, Limbang, Trusan, Sadong, Lupar, Saribas, Kerian, Rajang, Oya, Mukah, Tatau, Kemena, Suai, Sibuti, Miri, Baram,	21
Sabah	Lawas, Menggalong, Lakutan, Lingkungan, Padas, Membakut, Kimanis, Bongawan, Papar, Moyog, Tuaran, Kedamaian, Tenghilan, Bingkongan, Bengkoka, Paitan, Sugut Labok, Sapi, Mounad, Segama, Tungku, Silabukan, Tingkayu, Tawau, Apas, Balung, Merotai, Umas-umas, Brantian, Kalabakan, Sembulan, Likas, Telipok, Segaliud, Kinabatangan, Kalumpang	36

* Source : Malaysia Environment Quality Report, Department of Environmental, Ministry of Natural Resources and Environment, Malaysia

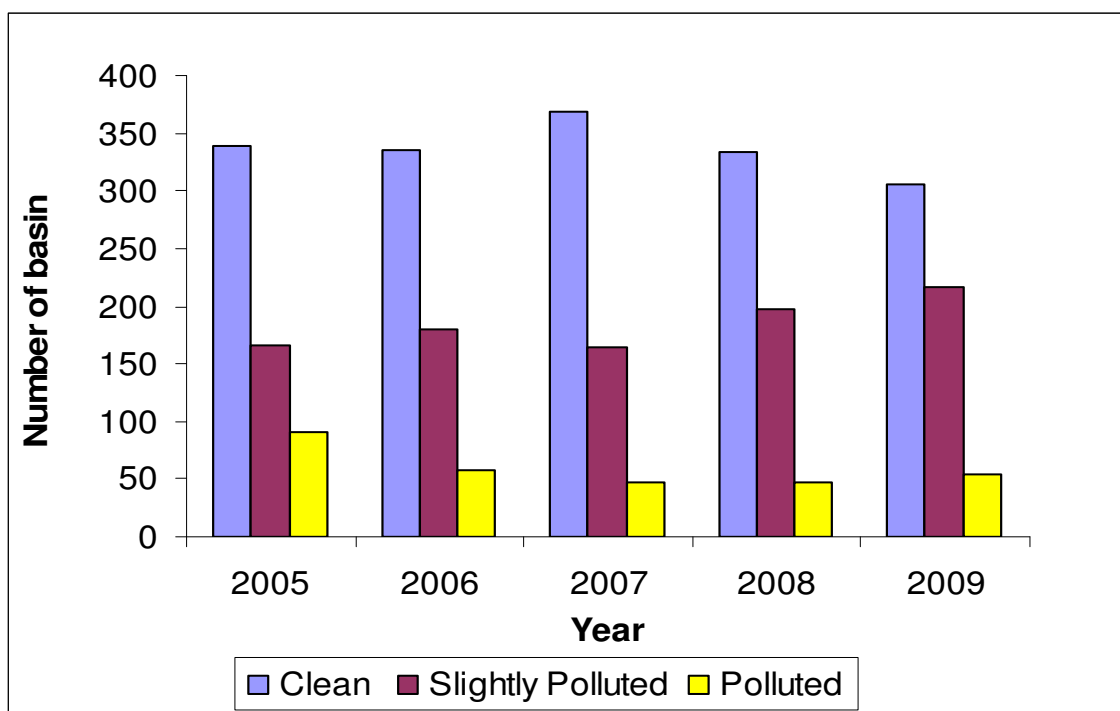


Figure 1.1 River water quality trend in Malaysia.

(Source: Figure 3-1 Malaysia: River Water Quality Trend (2005 - 2009), Malaysia Environment Quality Report, 2009. Department of Environmental, Ministry of Natural Resources and Environment, Malaysia)

Table 1.2 Environmental Quality Act 1974, Environmental Quality (Sewage), Regulations 2009, Second Schedule (Regulation 7), Acceptable Conditions of Sewage Discharge of Standards A and B, (i) New Sewage Treatment System.

Parameter	Unit	Standard	
		A	B
Temperature	°C	40	40
pH Value	-	6.0-9.0	5.5-9.0
BOD ₅ at 20°C	mg/L	20	50
COD	mg/L	120	200
Suspended Solids	mg/L	50	100
Oil and Grease	mg/L	5.0	10.0
Ammoniacal Nitrogen (Enclosed water body)	mg/L	5.0	5.0
Ammoniacal Nitrogen (River)	mg/L	10.0	20.0
Nitrate- Nitrogen (River)	mg/L	20.0	50.0
Nitrate- Nitrogen (Enclosed water body)	mg/L	10.0	10.0
Phosphorous (Enclosed water body)	mg/L	5.0	10.0

Table 1.3 Environmental Quality Act 1974, Environmental Quality (Sewage) Regulations 2009, Second Schedule (Regulation 7), Acceptable Conditions of Sewage Discharge of Standards A and B, (ii) Existing Sewage Treatment System (approved before January 1999).

		Type of Sewage Treatment System									
		Communal Septic Tank		Imhoff Tank		Aerated Lagoon		Oxidation Pond		Mechanical System	
Parameter	Unit	A	B	A	B	A	B	A	B	A	B
BOD ₅ at 20°C	mg/L	200	200	175	175	100	100	120	120	60	60
COD	mg/L	-	-	-	-	300	300	360	360	180	240
Suspended Solids	mg/L	180	180	150	150	120	120	150	150	100	120
Oil and Grease	mg/L	-	-	-	-	-	-	-	-	20	20
Ammoniacal Nitrogen	mg/L	-	-	100	100	80	80	70	70	60	60

Table 1.4 Environmental Quality Act 1974, Environmental Quality (Sewage), Regulations 2009, Second Schedule (Regulation 7), Acceptable Conditions of Sewage Discharge of Standards A and B, (iii) Existing Sewage Treatment System (approved after January 1999).

Parameter	Unit	Standard	
		A	B
BOD ₅ at 20°C	mg/L	20	50
COD	mg/L	120	200
Suspended Solids	mg/L	50	100
Oil and Grease	mg/L	20	20
Ammoniacal Nitrogen	mg/L	50	50

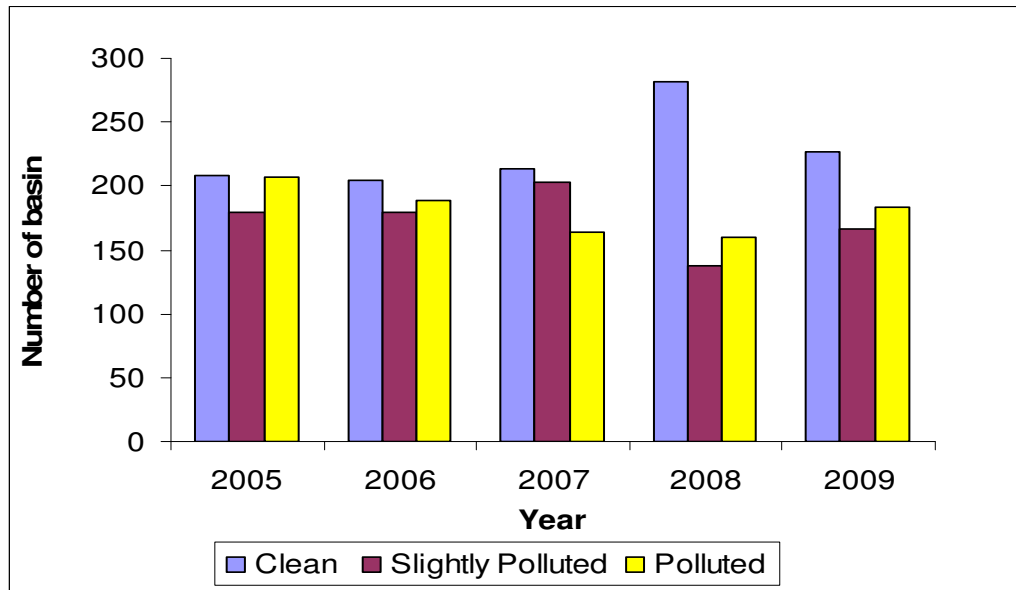


Figure 1.2 River water quality trend based on ammonium-nitrogen sub-index in Malaysia.

(Source: Figure 3-7 Malaysia: River Water Quality Trend based on AN sub-index (2005- 2009), Malaysia Environment Quality Report, 2009. Department of Environmental, Ministry of Natural Resources and Environment, Malaysia)

1.2 Problem Statement

As our lifestyle is improving in conjunction with economic growth, water pollution is getting worse. As a result, the development of new treatment with more cost effective, higher efficiency and simple operation is needed. Sequencing batch reactor (SBR) technology for treating wastewater seems to be superior over others as it required small footprint, able to be used in conventional activated sludge treatment plant without much modification. Beside that, it is easy to operate and able to withstand shock organic loading. Another advantage of SBR is the possibility to form aerobic granules which improve the sludge settleability and increase the treatment capacity (Morgenroth et al., 1997, Peng et al., 1999, Arrojo et al., 2004). Aerobic granules refer to a group of self-immobilization microorganism which having a denser, stronger microbial structure and settle significantly faster than

floccular activated sludge. Floccular biomass refers to fine particle with loose and fluffy structure.

Development of aerobic granules in SBR is attracting more and more attention nowadays. SBR was favorable for granulation process as it retains high biomass in the column for a certain period and encourage the slow growing bacteria to grow. The flexibility of SBR process in terms of settling time could work as sludge selection chamber where only the compact and denser sludge could retain in the reactor while lighter sludge will be washed out. Implementation of aerobic granules to replace the conventional treatment could be done with less modification and able to reduce the problems, such as long settling time and poor sludge settle ability that facing in current treatment plants. Further, different techniques for the cultivation of aerobic granules have been studied by Li et al, 2011 and showed that start-up time of treatment process (aerobic granulation) can be significantly shorten. This makes the aerobic granulation process become advance and superior compared to other treatment process.

In recent years, many researches have been carried out to study the formation and development of aerobic granules (Morgenroth et al., 1997, Beun et al., 1999, Peng et al., 1999, Tay et al., 2002a, Liu et al., 2005b). Aerobic granules has been described as regular, smooth, round in shape and having good settle ability, which lead to good liquid-solid separation (Morgenroth et al., 1997). Compare with conventional activated sludge, the aerobic granules is much denser and having stronger microbial structure, which improved the sludge settle ability and ability to withstand high organic loading and toxicity [Hulshoff et al., 2004, Lettinga, 1995].

Having the aerobic granules in the system will thus increase the number of microorganism in it.

Previous studies showed that aerobic granules could be formed with both synthetic wastewater and industrial wastewater (Hu et al., 2005, Liu et al., 2005a, Cassidy and Belia, 2005). However, past studies were mainly focused on formation of aerobic granules by using synthetic wastewater where the influent concentration and loading could be easily controlled (Morgenroth et al., 1997, Muda et al., 2010). The development of aerobic granules in SBR with synthetic wastewater, such as acetate, ethanol and glucose has shown satisfactory result (Beun et al., 1999, Hu et al., 2005a, Liu et al., 2005a). However, previous studies were mainly focused on high strength wastewater (Liu et al., 2005a, Muda et al., 2010). Although there are many studies show that aerobic granules are capable in treating high strength wastewater, the application of aerobic granules in low strength real domestic wastewater is still scarce especially for equatorial climate countries.

Nitrogen compounds, such as ammonia and nitrite have been reported to show adverse effect on the formation of aerobic granules through its inhibition to the energy metabolism of biomass (Yang et al., 2004). Wide range of biomass, such as ammonia-oxidizing bacteria (AOB), nitrite oxidizing bacteria (NOB), denitrifiers, polyphosphate accumulating organisms (PAO) and glycogen accumulating organisms (GAO) have been studied previously and found to be inhibited by nitrogen compounds present in wastewater (Anthonisen et al., 1976, Meinhold et al., 1998, Weon et al., 2002, Vadivelu et al., 2007a, Ye et al., 2010).

The purpose of this study is to investigate the formation of aerobic granules in SBR and its application for treating low strength domestic wastewater for equatorial climate countries. As the concentration of nitrogen compounds in domestic wastewater keep on increasing, this study is also focused on the effect of nitrogen compounds on the aerobic granules. The application of aerobic granules in domestic sewage treatment will results in good solid/liquid separation allowing separation of granules and treated effluent takes place in a short period and enables more compact reactor design, thus reduces the space requirement.

1.3 Research Objectives

In this project, the development and application of aerobic granules for treating low strength domestic wastewater in equatorial climate countries will be carried out using SBR. The objectives of this study are:

- i) To study the development of aerobic granules in SBR using synthetic wastewater for treating low strength domestic wastewater.
- ii) To study the effect of different organic loading rate (OLR) on the morphology and performance of aerobic granules.
- iii) To investigate the effect of nitrogen compounds, namely ammonium and nitrite on the activities of aerobic granules.

1.4 Scope of Study

In the first part of this research, aerobic granules was developed using low strength synthetic wastewater. Activated sludge taken from a local domestic SBR treatment

plant was used as seed sludge and inoculated with low strength wastewater. Operational conditions were decided based on previous studies on aerobic granulation (Beun et al, 1999, Qin et al., 2004, Ni et al., 2009). Analytical parameters such as mixed liquor suspended solid (MLSS), sludge volume index (SVI), particle size analysis, microscopic test were used to monitor the transformation of the seed sludge from floccular sludge to aerobic granules. The ability of the biomass to remove the chemical oxygen demand (COD) also was monitored throughout the experimental period.

In the second part of this research, the adaptation and effectiveness of aerobic granules for treating low strength domestic wastewater was studied. The reactor was fed with low strength domestic wastewater that is taken from local domestic wastewater treatment plant. The stability of aerobic granules in terms of size, structure, COD removal and SVI was recorded.

In the third part of this research, the re-grow ability of aerobic granules after starvation and shock loading was investigated. All the operating condition remained as previous studies except the organic loading rate. External carbon source was added to the domestic wastewater to increase the COD in the feed (thus, increased the OLR). Microscopic examination, SVI and MLSS test were conducted to determine the recovery ability of aerobic granules after starvation and shock loading.

Lastly, the effect of nitrogen compounds (ammonium and nitrite) on the activities of aerobic granules was studied. Nitrogen compounds have been reported as second pollution indicator that triggers the water quality in Malaysia. In these

studies, the inhibitory effect of ammonium and nitrite on aerobic granules were measured in term of their oxygen uptake rate. The recovery ability of aerobic granules from nitrite inhibition was also studied.

1.5 Organisation of Thesis

This thesis contains five chapters. An overall review of each chapter is as below:

Chapter 1 introduces the water pollution in Malaysia with its main cause. This chapter also covers the problem statement, research objectives, scopes of study and organization of thesis.

Chapter 2 presents the characteristics of wastewater in Malaysia and SBR operation. It also summarizes the current technologies used in Malaysia for domestic wastewater treatment; the past research works in the field related to aerobic granules and parameters that affects the granulation process.

Chapter 3 presents the materials, chemicals and experimental procedures used in this research. This chapter also covers the analytical method to determine the efficiency of reactor and measurement of sludge stability. Flowchart of the overall experimental activities is also presented.

Chapter 4 covers the results and discussions. Results obtained from the experiment will be elaborated and discussed.

Chapter 5 concludes all the findings obtained from this study.
Recommendations for future research were given.

CHAPTER 2

LITERATURE REVIEW

2.1 Characteristic of Wastewater

The main sources of domestic wastewater are the discharge from residential and commercial areas. The origin of wastewater will have serious influences on the characteristics of wastewater in the treatment plant. As an example, wastewater from restaurant or hawker centre will have more oil and grease, and also detergents content while wastewater from hospital will contains antibiotics, antibacterial soaps and cleansers.

The primary components of the domestic wastewater are the oxidisable organic matter and the nutrients (typically nitrogen and phosphorus compounds). Organic matters, which mainly composed of proteins, carbohydrates and fats, are the most polluting constituent of domestic wastewater. It is usually measured in terms of biochemical oxygen demand (BOD) and/or chemical oxygen demand (COD). The discharge of untreated wastewater containing organic matters will leads to depletion of oxygen in the receiving water bodies as biological stabilization occurs.

The nutrients such as nitrogen compounds (ammonium and nitrite) and phosphorus are also very important polluting constituents of wastewater. The nutrients will leads to algae growth and eutrophication of water bodies. Nitrogen is present in fresh domestic wastewater in the form of proteinaceous matter urea (i.e.

organic nitrogen) while phosphorus is usually present in orthophosphate, polyphosphate and organic phosphate forms (Metcalf & Eddy, 2004).

Knowing the characterization of domestic wastewater is very useful to ensure that the design capacity as well as the operation and maintenance of the treatment plant are fulfilled. Table 2.1 shows the typical composition of untreated domestic wastewater in Malaysia.

The characteristics of wastewater are being influenced not only by the sources but also the weather or climate. Volumetric flow of wastewater can be divided into normal season and peak season. Normal season will have an average COD, no more than 400 mg/L, which was considered as low strength domestic wastewater, Table 2.2 (Mara, 1976). During the peak season (rainy season), the rain water will diluted the domestic wastewater into a huge number with low organic content.

2.2 Current Technologies for Wastewater Treatment in Malaysia

In Malaysia, most of the public wastewater treatment plants were managed by Indah Water Konsortium (IWK) Sdn Bhd. IWK is responsible to plan and design the public wastewater treatment plants for most of the urban area. This is to reduce the numbers of individual treatment plant, such as septic tank that currently are being used at urban area. 38% of public treatment plants in Malaysia are mechanical plants which utilized mechanical equipment to accelerate the breakdown of wastewater. Example of the mechanical plants includes conventional activated sludge, extended aeration ,

oxidation ditch, aerated lagoons, rotating biological contactors, trickling filter and sequencing batch reactors. The description of process, advantages and disadvantages of those treatments plants are summarized in Table 2.3. Table 2.4 shows the different types of wastewater treatment plant in Malaysia with its quantity and capacity. As shown in Table 2.4, mechanical plants take the major role in wastewater treatment in Malaysia.

Table 2.1 Typical Composition of Untreated Domestic Sewage.

Constituent	Concentration (mg/l)		
	Strong	Medium	Weak
Solids, total	1,200	720	350
Dissolved, total	850	500	250
Fixed	525	300	145
Volatile	325	200	105
Suspended, total	350	220	100
Fixed	75	55	20
Volatile	275	165	80
Settleable solids, mg/L	20*	10*	5*
Biochemical oxygen demand, 5-day, 20°C (BOD ₅ , 20°C)	400	250	110
Total Organic Carbon (TOC)	290	160	80
Chemical oxygen demand (COD)	1,000	500	250
Nitrogen (total as N)	85	40	20
Organic	35	15	8
Free Ammonia	50	25	12
Nitrites	0	0	0
Nitrates	0	0	0
Phosphorus (total as P)	15	8	4
Organic	5	3	1
Inorganic	10	5	3
Chlorides	100	50	30
Alkalinity (as CaCO ₃)	200	100	50
Grease	150	100	50

* All values except settleable solids are expressed in mg/l.

(Source: Table A.3 - Typical Composition of Untreated Domestic Sewage, Guidelines for Developers, Sewage Treatment Plants, Second Edition, Volume IV, 1998. Ministry of Housing and Local Government, Sewerage Services Department. Malaysia.)

Table 2.2 Wastewater Strength in terms of BOD₅ and COD (Mara, 1976).

Strength	BOD ₅ (mg/L)	COD (mg/L)
Weak	< 200	< 400
Medium	350	700
Strong	500	1000
Very strong	>750	>1500

Table 2.3 Process, Advantages and Disadvantages of Wastewater Treatment Plant in Malaysia (Metcalf & Eddy, 2004, Al-Rekabi et al., 2007, Mace and Mata-Alvarez, 2002).

Treatment Plants	Process description	Advantages	Disadvantages
Communal Septic Tank	Combination of sedimentation and digestion tank. When the wastewater flow through the tank, solid matter decomposed by bacteria and settled. Settled sludge will further undergo anaerobic decomposition process.	<ul style="list-style-type: none"> • Easy constructed • Cheap in operating cost • Low sludge volume 	<ul style="list-style-type: none"> • Occupied large area needed • Effluent with high BOD and fouling smell. • Leakage of gases causes bad smell and air pollution. • Required periodic cleaning.
Imhoff Tank	Works as septic tank with special funnel-shaped baffles design inside the tank to prevent the turbulences in the flow and the mix-up of sludge particle with effluent.	<ul style="list-style-type: none"> • Economical in operation. • Easy sludge removal and less sludge volume. 	<ul style="list-style-type: none"> • Low BOD removal, 30-40% • Bad odour.
Oxidation Ponds	Soluble organic is metabolized by bacteria and produced carbon dioxide, ammonium and nitrate ions and phosphate ions that support algae growth. Photosynthetic by autotrophic algae will generate oxygen which needed for oxidation of organic matter by heterotrophic bacteria. By using the oxygen generated by algae and wind action, the pond can be maintained at aerobic conditions.	<ul style="list-style-type: none"> • Economical system if land is inexpensive. • No mechanical aerated needed. 	<ul style="list-style-type: none"> • Effluent contains bacteria, algae, soluble organic and inorganic compound. • Large land needed

Table 2.3 Continued.

Treatment Plants	Process description	Advantages	Disadvantages
Conventional Activated Sludge	Aggregation of heterotrophic microorganisms, such as bacteria, protozoa, fungi and suspended in the reactor during mixing. Microorganism utilized the oxygen supplied by diffuser or mechanical aerated and decompose the organic matter in the influent. Waste activated sludge will be returned to the reactor in order to maintain high biomass concentration in the reactor.	<ul style="list-style-type: none"> • Effluent can be control by vary the process parameter • Shorter time for cleaner effluent. 	<ul style="list-style-type: none"> • High energy consumption • Large land needed
Aerated Lagoon	The size of the basin is shallow, 8 to 10 ft which serve as reactor. Aeration provided by diffuser or mechanical aerator. Aeration in the reactor also provides mixing to disperse the air with organic matter.	<ul style="list-style-type: none"> • Less care and maintenance needed 	<ul style="list-style-type: none"> • Large land area needed • Low efficient than conventional treatment due to poor mixing.
Extended aeration	Same flow scheme with conventional process, but the wastewater is aerated for 24 hours in a complex mix flow regime. The system operated in endogenous respiration phase of bacterial growth cycle which microorganism was under starvation phase when BOD in the system was low. Partial oxidation process will occurred where the microorganism utilize their own cell structure for food.	<ul style="list-style-type: none"> • Low sludge production. • Highly treated effluent. 	<ul style="list-style-type: none"> • High Operating cost as large oxygen needed • Large tank volume.
Oxidation Ditch	Wastewater is feed along the circular channel and aerated by mechanical paddles along both sides of channel. Having long retention time (24 hours) and high solid retention time (10 to 59 days)	<ul style="list-style-type: none"> • High degree of nitrification. • Low sensitive to shock organic loading 	<ul style="list-style-type: none"> • Large land needed. • High energy consumption.

Table 2.3 Continued.

Treatment Plants	Process description	Advantages	Disadvantages
Trickling Filter	Aerobic attached growth biological process where organic matter will be adsorbed and oxidized when passing through the permeable media which microorganisms attached on it.	<ul style="list-style-type: none"> • Nitrification process might exist after BOD finished. • Short hydraulic retention time. • Effectively removed BOD 	<ul style="list-style-type: none"> • Primary treatment needed to remove settleable solid and secondary sedimentation is needed to separate microbial solids from effluent. • Regular maintenance needed as media will be clogged by inhibitory compound. • Consistent loading is needed for the growth of microorganism.
Rotating Biological Contactors	The build up of microbial film on partly submerged support medium which rotates slowly in horizontal axis in the tank and allow the microbial film access to the nutrient and air.	<ul style="list-style-type: none"> • Less land needed compare to trickling filter. • Short hydraulic retention time and good resistance to sudden changes in operating condition. • High treatment efficiency. • Simple operation. 	<ul style="list-style-type: none"> • High installation and operational costs.
Sequencing Batch Reactors	Modification of activated sludge process where all the unit process is completed within a single tank in a sequence of time. Basically consists of five processes, Fill, React, Settle, Decant and Idle.	<ul style="list-style-type: none"> • Low land usage and can be used in present conventional activated sludge process without much modification. • Simple operation. • Flexibility in process. • Insensitive to shock organic loading 	<ul style="list-style-type: none"> • High level of maintenance.

Table 2.4 Types of Wastewater Treatment Plant in Malaysia.

No.	Type of wastewater treatment plant	Quantity (As at Dec 2008)	Population Equivalent (PE)	Flow rate (m ³ /day)
1	Imhoff Tank	760	557,752	125,494.2
2	Oxidation Ponds	436	1,824,403	410,490.7
3	Mechanical Plants	4,026	15,099,139	3,397,306.3
4	Network Pump Stations	668	3,558,108	800,574.3
Total		5,890	21,039,402	4,733,865.5
Communal Septic Tank		3,635	433,573	97,553.9

(Source: Table 1 – Public Sewage Treatment Plants in Malaysia. Available from Indah Water Konsortium Sdn. Bhd. <http://www.iwk.com.my/f-sewerage-fact.htm>)

2.3 Aerobic Granules Technology

Granules were first found in anaerobic reactors and have been widely used in upflow anaerobic sludge blanket (UASB) reactors (Lettinga et al., 1984). Compare with conventional activated sludge, the anaerobic granules is much denser, having stronger microbial structure (Lettinga, 1995, Hulsoff Pol et al., 2004). Having the granules in the system will thus increase the microorganism in the system and with its stronger microbial structure; it could sustain shock loading and high strength wastewater. Previous studies also showed that anaerobic granules improved the sludge settleability in the system (Schmidt et al., 1996). Further, the anaerobic granules formed in the reactor will be more compact and having good liquid-solid separation (Morgenroth et al., 1997). However, anaerobic granules system still facing some disadvantages, such as long set-up period, a relatively high operating temperature, slow biodegradation rate, low quality of effluent, sensitivity to toxicant and unsuitability for low strength organic wastewater (Liu at al., 2004, Adav et al., 2008).

Since the development of anaerobic granules was proved to have advantages in treating wastewater, researchers moved their focus to explore the benefits of aerobic granules. After the first application of aerobic granules has been published by Mishima et al. (1991), many researches (Morgenroth et al., 1997, Beun et al., 1999, Peng et al., 1999, Liu et al., 2004, Adav et al., 2008) were carried out to further investigate on aerobic granules and found that aerobic granules showing superior efficient over anaerobic granules. The most significant advantage of aerobic granules was its rapid formation rate which shortens the setup period of reactors (Liu et al., 2005a). Adav et al. (2008) has summarized the characteristic of aerobic granules as regular, smooth, nearly round in shape and having good settleability. Beside that, aerobic granules are having denser and stronger microbial structure which enables it to withstand high organic loading and toxicity.

Previous studies showed that aerobic granules could be formed with both synthetic and real wastewater (Hu et al., 2005, Liu et al., 2005a, Muller et al., 1995). Table 2.5 shows the researches related to aerobic granules in both synthetic and real wastewater. However, most of the studies were mainly focused on high strength wastewater which having high COD. The study of aerobic granules formation on low strength wastewater (such as domestic wastewater) is still scarce.

Further, most of the aerobic granules studies were carried out in the laboratory with room temperature, ranging from 20-25°C (Li et al., 2008, Carucci et al., 2009). These studies are only applicable for treating wastewater which operates under the shelf shield conditions, while not applicable for the treatment which operates under the direct sunlight at hot climate countries. It is believed that the

temperature in the real treatment plant in the equatorial climate countries (countries which are located near to Equator and having hot temperature, around 26°C throughout the year) will be raised up to 30-35°C under the effect of direct sunlight (Zhou et al., 2011). However, there were only limited studies on the development of aerobic granules being carried out in the temperature ranging from 30-35°C (Johnson et al., 2009, Muda et al., 2010).

Table 2.5 Comparison of Aerobic Granulation with Various Substrates.

Sources	Substrate	Influent COD (mgL ⁻¹)	SVI (mgL ⁻¹)	Settling Velocity (mh ⁻¹)	Diameter (mm)	Temperature (° C)
Synthetic Wastewater						
Morgenroth et al., 1997	Molasses	400	-	33.1-38.8	2.35	-
Peng et al., 1999	Synthetic wastewater	600	80-100	-	0.3-0.5	-
Beun et al., 1999	Ethanol	830	-	>24	4.6	20 ± 2
Hu et al., 2005a	Synthetic wastewater	500	30-40	20-60	0.5-4.0	25 ± 2
Hu et al., 2005b	Synthetic organic chemicals	500	98	38.3	1.2-5.0	21 ± 2
Liu et al., 2005a	Glucose	1000-7000	40-130	24.2-36.4	1.2-1.9	20 ± 5
Muda et al., 2010	Textile dyeing wastewater	1270	69	72-88	1.3-3.3	-
Real Wastewater						
Arrojo et al., 2004	Diary wastewater	500-3000	60	20	0.25-4.0	15 - 20
Su and Yu, 2005	Soybean processing wastewater	2000	30.8	36.6	1.24	25 ± 1
Cassidy and Belia, 2005	Abattoir wastewater	7685	22	51	1.4-2.0	20
Wang et al., 2007a	Brewery wastewater	1300-2300	32	91-157	2.0-7.0	25 ± 2
Ni et al., 2009	Low strength wastewater	<200	35	18-40	0.2-0.8	-

2.4 Parameters That Affect The Formation of Aerobic Granules

2.4.1 Organic Loading Rate (OLR)

Organic loading rate plays a crucial role in the formation of aerobic granules. Aerobic granules formation and its stability have been studied widely with the OLR ranging from as low as 1.68 kg/m³day (Wang et al., 2008a) up to 15 kg/m³day (Chen et al., 2008). However, studies showed that aerobic granules that formed with high OLR is smoother, denser and having more compact structure than those formed in low OLR (Tay et al., 2004, Chen et al., 2008). High OLR exerts the microbial selection pressure and improved the stability of the aerobic granules by selection and enriching of bacterial species (Moy et al., 2002). Aerobic granules, which formed with high OLR was dominated by rod-shaped bacterial and only a few web-like filamentous fungi was found surrounding the bacterial (Li et al., 2010). Filamentous bacteria were found to dominate when the aerobic granules were developed using low OLR (Ni et al., 2009).

COD/N/P ratio was important for aerobic granulation as deficiency in nutrient supply (N/P) will results in sludge disintegrated and causing bulking of sludge (Metcalf & Eddy, 2004). It is commonly accepted that the ratio of influent ammonium-nitrogen to biodegradable organic carbon (N/COD) should be around 5/100 in activated sludge processes. A minimum of 1.5 mg/L NH₃-N has been recommended in the effluent for selection of floc forming microorganism growth (Liu and Liu, 2006). The ratio of COD/N has been proved to have significant effect on simultaneous nitrification and denitrification (SND) process (Wang et al., 2009).

SND was favorable with the increased of COD/N ratio and high biomass retention in the reactor.

2.4.2 Type of Substrate

Various substrates were used to cultivate aerobic granules in SBR including synthetic wastewater, such as sodium acetate, glucose and ethanol; real wastewater such as dairy and soybean processing wastewater (Adav, 2009, Xiao et al., 2008, Jiang et al., 2002, Wang et al., 2008a, Morgenroth et al., 1997) (Table 2.5). These studies show that filamentous fungi was dominant in the glucose fed reactor while rod-shaped bacteria was dominant in the acetate fed reactor. This could be explained as acetate is hard to degrade compare with glucose and it creates the selection of slow growing bacteria. Previous studied shows that the selections of slow growing bacteria will forms granule with good stability (Liu et al., 2004). However, the formation of aerobic granules with particulate substrate as sole feed was showed unsatisfactory result due to the slower process of hydrolysis and caused high concentration gradient throughout the granules.

Recent research focuses on additional of co-substrate, such as chelating agent, NaHCO_3 , and divalent ion, such as Ca^{2+} , Fe^{2+} and Mg^{2+} to enhance the formation of aerobic granules (Nancharaiah et al., 2008, Jiang et al, 2003). These co-substrate will be bind with negatively charged cell to form a microbial nuclei and accelerate the formation of aerobic granules. Nancharaiah et al, (2008) uses synthetic chelating agent, nitrilotriacetic acid (NTA) as co-substrate and found that the granules formed in the presence of NTA were denser, smoother and having better settleability than

those formed in the acetate alone. Li et al., (2009) investigated the effect of Mg^{2+} augmentation on aerobic granulation process and discovered that the addition of Mg^{2+} could accelerated the granulation process and lead to the formation of larger sludge through physico-chemical function. Comparison of Mg^{2+} and Ca^{2+} for aerobic granulation was done by Liu et al., 2010 and found that both divalent metal ions showed different characteristics of mature granules. Ca- rich granules showed better physical characteristic (fast forming granules with denser and compact structure) while Mg-rich granules play main role on the biological properties of granules (higher production yield of EPS and faster substrate biodegradation).

2.4.3 Settling Time

Settling time is another decisive parameter in aerobic granulation process. Previous studies showed that short settling time could enhance the aerobic granulation process due to the selective discharge of slow settling sludge floc (Adav et al., 2009, Li and Li, 2009, Qin et al., 2004). At the settling stage, only the sludge that can be settled within a given time will be remained in the reactor, other flocs that suspended in the water will be discharged. Beun et al., (2002) suggested that a short settling time must be implemented for the selection of biomass with minimal settling velocity of 10 m/h. Qin et al., 2004 investigated the effect of settling time on aerobic granulation and found that the short settling time was not only influence the granulation process, but also affect the cell surface hydrophobicity, extracellular polymeric substance (EPS) and calcium content in the granules.

EPS consisted protein, polysaccharide, humic acids, lipids, nucleic acid, DNA and RNA (Zhang et al., 2007, Wingender et al., 1999). These biopolymer acts as adhesive and binds bioflocs together through specific protein-polysaccharide interaction, hydrophobic interactions, ionic interaction and hydrogen bonding (Zhang et al., 2007). It is believed that EPS plays an important role in aerobic sludge granulation and maintained the stability of sludge. Increased in the protein contents from EPS will leads to the increased of cell surface hydrophobicity, while increased in the polysaccharide will increased the hydrophilicity nature (Raszka et al, 2006). Hydrophobicity of cell surface had generally been considered to play an important role in the self-immobilization and attachment of cells to a surface, i.e. cell-to-cell attachment (Tay et al., 2002b). Increasing of surface hydrophobicity would promote the interaction between cell to cell, which will induce cell force for aggregating out of liquid phase, further favorable for aerobic granulation. Protein consisted of more negatively charge amino acids than polysaccharide and involved in the electrostatic bonds with multivalent cations, such as Zn^{2+} , Co^{2+} , Ca^{2+} , Mg^{2+} to form a high stability aggregates (Sun et al., 2009). The increased in protein will lesser the surface negative charge of bacterial and reduce the repulsive force between cells, thus favorable for aerobic granulation (Zhang et al., 2007).

2.4.4 SBR Operation

Normal SBR operation for aerobic granulation system consisted of 4 stages, fill, react, settle and decant. In the react stages, it actually can be divided into two phase, (1) degradation of organic substance and (2) periodic starvation (occurred when the external substrate was low/no longer available). Previous studies showed that