

**STARVATION AND REACTIVATION OF  
PARTIAL NITRIFYING BACTERIA:  
COMPARISON BETWEEN FLOCCULAR AND GRANULAR BIOMASS**

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**UNIVERSITI SAINS MALAYSIA**

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**STARVATION AND REACTIVATION OF  
NITROGEN REMOVING BACTERIA:  
COMPARISON BETWEEN FLOCCULAR AND GRANULAR BIOMASS**

**by**

**TAN TEONG EE**

**Thesis submitted in fulfilment of the requirements  
for the degree of  
Master of Science**

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## **TABLES OF CONTENTS**

Acknowledgement	ii
Table of Contents	iii
List of Tables	vii
List of Figures	viii
List of Abbreviations	xii
List of Symbols	xiii
Abstrak	xiv
Abstract	xvi

### **CHAPTER 1 - INTRODUCTION**

1.1 Water	1
1.2 Water pollution in Malaysia	1
1.3 Problem Statement	6
1.4 Research Objectives	9
1.5 Scope of Study	10
1.6 Organisation of Thesis	11

### **CHAPTER 2 - LITERATURE REVIEW**

2.1 Nitrogenous Compounds in Water	12
2.2 Nitrogen Removal	15
2.2.1 Nitrification and Denitrification	15
2.2.2 Ammonia Oxidizing Bacteria and Nitrite Oxidizing Bacteria	16
2.2.3 Partial Nitrification	17

2.3	Factors affecting Biological Nitrogen Removal	19
2.3.1	Dissolved Oxygen	20
2.3.2	Temperature	20
2.3.3	pH and Inhibitions	21
2.3.4	Sequencing Batch Reactor	22
2.3.5	Starvation and Subsequence Reactivation	24
2.4	Granular Biomass	25
2.4.1	Settling Time	26
2.4.2	Height over Diameter Ratio	26
2.4.3	Sludge Retention Time	27
2.5	Summary	27

## CHAPTER 3 - MATERIALS AND METHODS

3.1	Ammonia Oxidizing Bacteria Enrichment	31
3.1.1	Sequencing Batch Reactor Operation	31
3.1.1(a)	Floccular Biomass Reactor	31
3.1.1(b)	Granular Biomass Reactor	32
3.1.2	Wastewater Preparation	35
3.1.3	Monitoring the Performance of Sequencing Batch Reactor	36
3.1.4	Respirometer Study	36
3.2	Starvation Study	37
3.2.1	Energy Starvation	38
3.2.1(a)	Feed Preparation	38
3.2.1(b)	Air Supply	39
3.2.2	Carbon Starvation	39

3.2.2(a)	Feed Preparation	39
3.2.2(b)	Air Supply	39
3.3	Recovery Study	40
3.3.1	Energy Starvation and Carbon Starvation	40
3.3.1(a)	Complete Feed Source (Energy and Carbon Source)	41
3.3.1(b)	Incomplete Feed Source (Only Energy Source)	41
3.4	Analytical Procedure	42
3.4.1	Ammonia Test	42
3.4.2	Nitrite Test	42
3.4.3	Nitrate Test	43
3.4.4	Total Organic Carbon	43
3.4.5	Oxygen Uptake Rate	43
3.4.6	Mixed Liquor Suspended Solid	44
3.4.7	Extracellular Polymeric Substances	44
3.4.8	Sludge Volume Index	46

## CHAPTER 4 - RESULTS AND DISCUSSIONS

4.1	Ammonia Oxidizing Bacteria Enrichment	47
4.1.1	Enrichment of Floccular Ammonia Oxidizing Bacteria	47
4.1.2	Performance of Floccular Sequencing Batch Reactor	48
4.1.3	Enrichment of Granular Ammonia Oxidizing Bacteria	53
4.2	Starvation Study	60
4.2.1	Floccular Biomass under Inorganic Carbon Starvation	60
4.2.2	Floccular Biomass under Energy Starvation	64
4.2.3	Granular Biomass during Inorganic Carbon Starvation	67

4.2.4	Granular Biomass during Energy Starvation	72
4.3	Recovery Study	76
4.3.1	Floccular Biomass of Ammonia Oxidizing Bacteria during Carbon Starvation	76
4.3.2	Floccular Biomass of Ammonia Oxidizing Bacteria during Energy Starvation	82
4.3.3	Granular Biomass of Ammonia Oxidizing Bacteria during Carbon Starvation	87
4.3.4	Granular Biomass of Ammonia Oxidizing Bacteria during Energy Starvation	90
4.4	Maintenance and Growth Energy Distribution Study	94
4.4.1	Maintenance and Growth Energy Distribution Study for Floccular Biomass	94
4.4.2	Maintenance and Growth Energy Distribution Study for Granular Biomass	99
 CHAPTER 5 - CONCLUSIONS AND RECOMMENDATIONS		
5.1	Conclusions	104
5.2	Recommendations	105
 REFERENCES		107

## LIST OF TABLES

	<b>Page</b>
Table 1.1(a) The polluted Rivers and Classes Based on BOD, AN and SS	4
Table 1.1(b) Department of Environment Water Quality Index Classification in Malaysia, 2013 (Source: Malaysia Environment Quality Report 2013. Department of Environment, Ministry of Natural Resources and Environment, Malaysia)	4
Table 1.2 Acceptable Conditions for Discharge of Industrial Effluent for Mixed Effluent of Standard A and B (Extracted from Environment Quality (Industrial Effluents) Regulation 2009 (PU (A) 434))	5
Table 4.1 Free Ammonia and Free Nitrous Acid in the wastewater during the feeding and decanting phase of the floccular in the Sequencing Batch Reactor	52
Table 4.2 Average Specific Oxygen Uptake Rate and maintenance energy distribution of floccular biomass during recovery and energy distribution study	99
Table 4.3 Average Specific Oxygen Uptake Rate and maintenance energy distribution of granular biomass during recovery and energy distribution study	103



## LIST OF FIGURES

		<b>Page</b>
Figure 1.1	River Water Quality Trend in Malaysia (2005-2013) (Source: Malaysia Environment Quality Report 2013. Department of Environment, Ministry of Natural Resources and Environment, Malaysia)	3
Figure 1.2	River Water Quality Trend based on AN sub-index in Malaysia (2005-2013) (Source: Malaysia Environment Quality Report 2013. Department of Environment, Ministry of Natural Resources and Environment, Malaysia)	5
Figure 2.1	Schematic diagram representing the marine nitrogen cycle (By Treachang - Own work, CC BY-SA 3.0, <a href="https://commons.wikimedia.org/w/index.php?curid=17731391">https://commons.wikimedia.org/w/index.php?curid=17731391</a> )	14
Figure 2.2	The overview of the nitrification and denitrification processes	19
Figure 3.1	Reactor setup for Ammonia Oxidizing Bacteria floccular biomass enrichment	33
Figure 3.2	Flowchart of overall experimental activities involved in this study	34
Figure 4.1(a)	Original seed sludge from Jelutong Wastewater Treatment Plant	47
Figure 4.1(b)	Enrichment Ammonia Oxidizing Bacteria biomass	47
Figure 4.2	Long term performance of floccular Ammonia Oxidizing Bacteria in the Sequencing Batch Reactor based on the effluent	49
Figure 4.3	Typical profiles of ammonium and nitrite during a cycle of floccular biomass in the Sequencing Batch Reactor	49
Figure 4.4	Typical profiles of dissolved oxygen concentration and pH during a cycle of floccular biomass in the Sequencing Batch Reactor	51
Figure 4.5	Ammonia Oxidizing Bacteria in different period (a=0, b=30, c=60 and d=365 days)	54
Figure 4.6	Typical profiles of ammonium and nitrite during a cycle of granular biomass in the Sequencing Batch Reactor	56
Figure 4.7	Long term performance of granular in Sequencing Batch Reactor based on SVI	57

Figure 4.8	Long term performance of granular in the Sequencing Batch Reactor based on the effluent	57
Figure 4.9	Typical profiles of dissolved oxygen concentration and pH during a cycle of granular biomass in the Sequencing Batch Reactor	58
Figure 4.10	Typical profile of Ammonium Concentration and Ammonium Uptake Rate during Inorganic Carbon Starvation for Floccular Biomass of Ammonia Oxidizing Bacteria	61
Figure 4.11	Typical Profile for Mixed Liquor Volatile Suspended Solid and Sludge Volume Index of Floccular Biomass of Ammonia Oxidizing Bacteria during Inorganic carbon Starvation	63
Figure 4.12	Typical Profile of Inorganic carbon Concentration during Energy Starvation for Floccular Biomass of Ammonia Oxidizing Bacteria	66
Figure 4.13	Typical Profile of Mixed Liquor Volatile Suspended Solid and Sludge Volume Index for Floccular Biomass of Ammonia Oxidizing Bacteria during Energy Starvation	66
Figure 4.14	Typical Profile of Ammonium Concentration and Ammonium Uptake Rate during Inorganic carbon Starvation for Granular Biomass of Ammonia Oxidizing Bacteria	68
Figure 4.15	Typical Profile for Mixed Liquor Volatile Suspended Solid, Sludge Volume Index, and Total Extracellular Polymeric Substance of Granular Biomass of Ammonia Oxidizing Bacteria during Inorganic carbon Starvation	69
Figure 4.16	Typical Profile of Bicarbonate Concentration during Energy Starvation for Granular Biomass of Ammonia Oxidizing Bacteria	73
Figure 4.17	Typical Profile for Mixed Liquor Volatile Suspended Solid and Sludge Volume Index of Granular Biomass of Ammonia Oxidizing Bacteria during Energy Starvation	74
Figure 4.18	Oxygen profile of floccular biomass of Ammonia Oxidizing Bacteria during recovery study for carbon starvation at 7th day	77
Figure 4.19	Ammonium profile of floccular biomass of Ammonia Oxidizing Bacteria during recovery study for carbon starvation at 7th day	78
Figure 4.20	Specific Oxygen Uptake Rate profile and ammonium uptake of floccular biomass of Ammonia Oxidizing Bacteria during recovery study for carbon starvation at 14th day	79

Figure 4.21	Specific Oxygen Uptake Rate profile and ammonium uptake of floccular biomass of Ammonia Oxidizing Bacteria during recovery study for carbon starvation at 21st day	80
Figure 4.22	Oxygen profile of floccular biomass of Ammonia Oxidizing Bacteria during recovery study for carbon starvation at 28th day	81
Figure 4.23	Average Specific Oxygen Uptake Rate and Specific Ammonium Uptake Rate of the floccular biomass of Ammonia Oxidizing Bacteria for the recovery studies throughout the carbon starvation	82
Figure 4.24	Oxygen profile of floccular biomass of Ammonia Oxidizing Bacteria during recovery study for energy starvation	83
Figure 4.25	Ammonium profile of floccular biomass of Ammonia Oxidizing Bacteria during recovery study for energy starvation	83
Figure 4.26	Ammonium profile of floccular biomass of Ammonia Oxidizing Bacteria for prolonged recovery	86
Figure 4.27	Oxygen profile of granular biomass of Ammonia Oxidizing Bacteria during recovery study for carbon starvation	87
Figure 4.28	Ammonium profile of granular biomass of Ammonia Oxidizing Bacteria during recovery study for carbon starvation	88
Figure 4.29	Reduction rate of average Specific Oxygen Uptake Rate during the recovery study for floccular and granular biomass during the carbon starvation as compared to 0 <sup>th</sup> day of carbon starvation	90
Figure 4.30	Oxygen and ammonium profiles of granular biomass of Ammonia Oxidizing Bacteria during recovery study for energy starvation	91
Figure 4.31	Ammonium profile of granular biomass of Ammonia Oxidizing Bacteria for prolonged recovery after 21st day of energy starvation	92
Figure 4.32	Specific Oxygen Uptake Rate and SOUR <sub>xc</sub> of Control Floccular Biomass of AOB during Energy Distribution Study	95
Figure 4.33	Specific Oxygen Uptake Rate and SOUR <sub>xc</sub> of Floccular Biomass of Ammonia Oxidizing Bacteria during Energy Distribution Study after 7 days of carbon starvation	96
Figure 4.34	Specific Oxygen Uptake Rate and SOUR <sub>xc</sub> of Floccular	98

Biomass of Ammonia Oxidizing Bacteria during Energy  
Distribution Study after 14 days of carbon starvation

Figure 4.35	Specific Oxygen Uptake Rate and $\text{SOUR}_{\text{xc}}$ of Floccular Biomass of Ammonia Oxidizing Bacteria during Energy Distribution Study after 21 days of carbon starvation	98
Figure 4.36	Specific Oxygen Uptake Rate and $\text{SOUR}_{\text{xc}}$ of Control Granular Biomass of Ammonia Oxidizing Bacteria during Energy Distribution Study	101
Figure 4.37	Specific Oxygen Uptake Rate and $\text{SOUR}_{\text{xc}}$ of Granular Biomass of Ammonia Oxidizing Bacteria during Energy Distribution Study after 14 days of carbon starvation	102
Figure 4.38	Specific Oxygen Uptake Rate and $\text{SOUR}_{\text{xc}}$ of Granular Biomass of Ammonia Oxidizing Bacteria during Energy Distribution Study after 21 days of carbon starvation	102
Figure 4.39	Specific Oxygen Uptake Rate and $\text{SOUR}_{\text{xc}}$ of Granular Biomass of Ammonia Oxidizing Bacteria during Energy Distribution Study after 28 days of carbon starvation	103

## **LIST OF ABBREVIATION**

AN	Ammonium Nitrogen
AOB	Ammonia Oxidizing Bacteria
ATP	Adenosine Triphosphate
BOD	Biological Oxygen Demand
DO	Dissolved Oxygen
DOE	Department of Environment
EPS	Extracellular Polymeric Substances
FA	Free Ammonia
FNA	Free Nitrous Acid
H/D	Height/Diameter Ratio
HRT	Hydraulic Retention Time
MLSS	Mixed Liquor Suspended Solid
MLVSS	Mixed Liquor Volatile Suspended Solid
NOB	Nitrite Oxidizing Bacteria
OUR	Oxygen Uptake Rate
SAUR	Specific Ammonium Uptake Rate
SBR	Sequencing Batch Reactor
SOUR	Specific Oxygen Uptake Rate
SRT	Sludge Retention Time
SS	Suspended Solid
SVI	Sludge Volume Index
TOC	Total Organic Carbon
VER	Volume Exchange Ratio

## LIST OF SYMBOLS

$\text{NaHCO}_3$	Sodium Bicarbonate
$\text{NH}_4\text{-N}$	Ammonium-Nitrogen
$\text{NH}_4\text{Cl}$	Ammonium Chloride
$\text{NH}_4\text{HCO}_3$	Ammonium Hydrogen Carbonate
$\text{SOUR}_{\text{xc}}$	Specific Oxygen Uptake Rate without carbon

**KEBULURAN DAN PENGAKTIFAN SEMULA**  
**BAKTERIA NITRIFIKASI SEPARA:**  
**PERBANDINGAN ANTARA BAKTERIA JENIS**  
**FLOKULUS DAN BERBUTIR**

**Abstrak**

Dalam kajian ini, nitrogen telah disingkarkan dari air sisa melalui penitritan separa dengan menggunakan kedua-dua bakteria flokulus dan berbutir. Tujuan kajian ini adalah untuk menyiasat akibat kebuluran terhadap bakteria penyingkiran nitrogen, penukaran bentuk bakteria ketika dalam keadaan kebuluran, dan kebolehan bakteria untuk pemulihan. Dua reaktor kelompok urutan (SBR) dengan kapasiti 8 L telah dijalankan untuk memperkayakan bakteria flokulus dan berbutir. Kedua-dua bakteria flokulus dan berbutir diperkayakan oleh air sisa sintetik yang mengandungi kandungan tinggi dalam SBR dengan keadaan yang sama kecuali nisbah tinggi/garis pusat (H/D) dan masa pengenapan. Nisbah H/D dalam reaktor kelompok urutan yang digunakan oleh bakteria jenis berbutir ialah 10, lebih tinggi berbanding dengan bakteria jenis flokulus (5). Masa pengenapan untuk bakteria berbutir dengan dikurangkan secara berperingkat dari 5 ke 1 minit untuk penambahan proses pembutiran. Bakteria berbutir yang dihasilkan mempunyai garis pusat purata 2 mm. Prestasi bakteria jenis flokulus dan berbutir jangka panjang adalah tekal dan keputusan menunjukan penitritan separa telah dicapai untuk kedua-dua bakteria jenis flokulus dan berbutir. Perbandingan antara bakteria jenis flokulus dan berbutir dalam kebuluran, pengaktifan semula, dan pengagihan tenaga. Kajian kebuluran (kebuluran karbon dan tenaga) telah dijalankan atas bakteria jenis flokulus dan berbutir. Kajian

kebuluran menunjukkan bahawa kebuluran karbon (ketiadaan sumber karbon, hanya membekal sumber tenaga) mempunyai kurang kesan terhadap aktiviti bakteria jenis flokulus dan berbutir jika dibandingkan dengan kebuluran tenaga (ketiadaan sumber tenaga, hanya membekal sumber karbon). Bakteria jenis berbutir mempunyai pemingkasan yang lebih baik terhadap kebuluran apabila dibandingkan dengan bakteria jenis flokulus disebabkan oleh pengurangan kadar pengambilan ammonium yang lebih rendah. Di samping itu, kajian pengaktifan semula terhadap bakteria jenis flokulus dan berbutir telah dijalankan selepas tempoh kebuluran yang tertentu (kebuluran karbon dan tenaga). Keputusan kajian pengaktifan semula menunjukkan bahawa bakteria yang mengalami kebuluran karbon lebih senang diaktifkan semula berbanding dengan bakteria yang mengalami kebuluran tenaga. Bakteria berbutir yang mengalami kebuluran karbon mempunyai responsif yang lebih baik terhadap pengaktifan semula berbanding dengan bakteria jenis flokulus. Pengaktifan semula berpanjangan adalah diperlukan untuk mengaktifkan semula bakteria yang mengalami kebuluran tenaga. Bakteria jenis berbutir yang mengalami kebuluran tenaga memerlukan tempoh yang lebih singkat untuk pengaktifan semula berbanding dengan bakteria jenis flokulus yang mengalami kebuluran tenaga. Kajian pengagihan tenaga penyaraan telah dijalankan terhadap kedua-dua bakteria jenis flokulus dan berbutir. Kajian menunjukkan bakteria jenis berbutir mempunyai tenaga penyaraan yang lebih tinggi (89%) berbanding dengan bakteria jenis flokulus ketika keadaan biasa. Pengagihan tenaga penyaraan untuk kedua-dua bakteria jenis flokulus dan berbutir tidak dijejaskan oleh kebuluran (70%). Namun demikian, pengagihan tenaga penyaraan yang serupa selepas kebuluran adalah dipercayai disebabkan pemecahan struktur berbutir selepas kebuluran yang mengakibatkan kelakuan yang serupa dengan bakteria jenis flokulus.



**STARVATION AND REACTIVATION OF  
PARTIAL NITRIFYING BACTERIA:  
COMPARISON BETWEEN FLOCCULAR AND GRANULAR BIOMASS**

**ABSTRACT**

In this study, nitrogen was removed from wastewater via partial nitrification using both floccular and granular biomass. The purpose of this study is to investigate the effect of starvation on nitrogen removing bacteria, changes of biomass in term of structure during starvation, and recovery ability of biomass. 2 Sequencing Batch Reactor (SBR) with working volume of 8L were operated to enrich floccular and granular biomass. Both floccular and granular biomass were cultivated using high strength synthetic wastewater in SBR operating under similar parameters except height/diameter (H/D) ratio and settling time. The height/diameter ratio of SBR used to cultivate granular biomass was 10 which was higher than floccular biomass (5). The settling period of granular biomass was stepwise decreased from 5 to 1 minute for the enhancement of granulation. The cultivated granular biomass has an average diameter of 2mm. The long term performance of floccular and granular biomass were consistent and the results showed that partial nitrification was achieved for both floccular and granular biomass. Comparison was made between floccular and granular biomass in starvation, reactivation and energy distribution. Starvation studies (carbon starvation and energy starvation) were carried out on both floccular and granular biomass. The starvation studies showed that carbon starvation (absence of carbon source, only energy source is provided) has lesser impacts in the activity of both floccular and granular biomass as compared to energy starvation (absence of energy source, only carbon source is provided). Granular biomass was found to be

more resilient to the starvation as compared to floccular biomass due to slower rate of reduction in ammonium uptake. Furthermore, recovery study was carried out on both floccular and granular biomass after certain period of starvations (both carbon and energy starvations). The recovery study results showed that carbon starved biomass were able to recovery easily as compared to energy starved biomass. Carbon starved granular biomass was found to be more responsive to the recovery as compared to carbon starved floccular biomass. Prolonged recovery was required for energy starved biomass to reactivate. Energy starved granular biomass required shorter period to recover as compared to energy starved floccular biomass. Maintenance energy distribution study was carried out on both carbon starved floccular biomass and granular biomass. It was found out that granular biomass has higher maintenance energy distribution (89%) during normal condition as compared to floccular biomass (69%). The maintenance energy distribution of both floccular and granular biomass were not affected by the starvation (70%). However, it was believed that the disintegration of the granular structure after starvation induced similar behaviour to the floccular biomass, which was probably the reason behind the similar maintenance energy distribution after starvation.

## **CHAPTER 1**

### **INTRODUCTION**

#### **1.1 Water**

Water is an important resource on Earth which none of us would survive more than 5 days without drinking, whereas one could survive more than a week without food. Thus, quality of the water have to be preserved as much as possible so that clean water could be recycled naturally rather than applying tons of chemicals just to purify the water to drinkable level. Although applying tons of chemicals could solve the clean water crisis problem, but it creates other problems which are pollutions, either soil, water, or air pollution.

Besides that, water also used by many industries to serve multiple purposes such as washing the media in electronic industries, blending in food industries, fertilizer industries, and pharmaceutical industries. Thus, preservative of clean and usable water, either in term of drinking or industrial usage, become imperative from time and time.

#### **1.2 Water pollution in Malaysia**

Division of Environment of Malaysia was established on 15 September 1975 under Ministry of Local Government & Environment. Division of Environment was then renamed to Department of Environment (DOE) at 1983 and it was placed under Ministry of Natural Resources and Environment on 27 March 2004. The main objectives of the establishment of DOE are to (i) provide a clean, safe, healthy and productive environment for present and future generation, (ii) conservation of the country's unique and diverse cultural and natural heritage with effective participation

by all sectors of society, and (iii) sustainable lifestyles and patterns of consumption and production (Department of Environment, 2014).

According to the Malaysia Environment Report 2013, among 473 rivers that were being monitored, 275 (58.1%) were found to be clean, 173 (36.6%) slightly polluted and 25 (5.3%) polluted as shown in Figure 1.1. The three major pollutants detected were Biochemical Oxygen Demand (BOD), Ammonium Nitrogen (AN), and Suspended Solid (SS). Among the 25 rivers that were classified as polluted rivers, 23 (92%) were polluted with AN. Table 1.1(a) shows the 25 rivers that are being polluted with AN and (b) shows the DOE water quality index classification (DOE, 2014).

Ammonium-Nitrogen ( $\text{NH}_4\text{-N}$ ) is a component of nitrogen which is adopted as an indicator to determine pollution by water. The main sources of  $\text{NH}_3\text{-N}$  are from domestic sewage, industrial wastewater, and livestock farming. Ammonium-Nitrogen is important to be removed from the rivers because excessive of it will cause eutrophication. Eutrophication is a phenomena whereby algae in the river is overgrown due to excessive Ammonium-Nitrogen being supplied. This will cause the oxygen level in the river decreased and will endanger other marine species in the river. Figure 1.2 shows a general trend of decrease in the number of clean river based on Ammonium-Nitrogen. Thus, it is important to reduce the concentration of Ammonium Nitrogen to the level that being enforced in the regulation as highlighted in Table 1.2 (DOE, 2014)

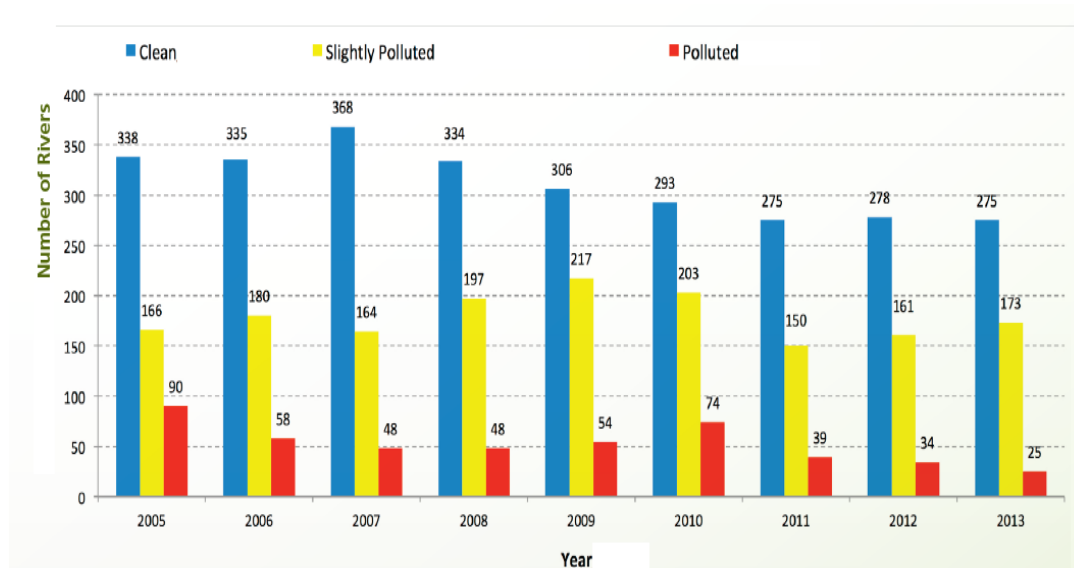


Figure 1.1 River Water Quality Trend in Malaysia (2005-2013)  
 (Source: Malaysia Environment Quality Report 2013. Department of Environment,  
 Ministry of Natural Resources and Environment, Malaysia)

Table 1.1(a) The Polluted Rivers and Classes Based on BOD, AN and SS and (b)  
DOE Water Quality Index Classification in Malaysia, 2013 (Source: Malaysia  
Environment Quality Report 2013. Department of Environment, Ministry of Natural  
Resources and Environment, Malaysia)

(a)

STATE/ NEGERI	RIVER BASIN/ LEMBANGAN SUNGAI	RIVER/ SUNGAI	STATUS 2013		CLASS BASED ON:/ KELAS BERDASARKAN:		
			WQI/ IKA	CLASS/ KELAS	BOD	AN	SS
SELANGOR/ WPKL	KLANG	SG. BUNOS	59	III	V	IV	I
SELANGOR/ WPKL	KLANG	SG. TOBA	59	III	V	V	II
JOHOR	RAMBAH	SG. RAMBAH	58	III	V	II	II
JOHOR	KAW. PASIR GUDANG	SG. PEREMBI	58	III	IV	IV	II
MELAKA	SERI MELAKA	SG. SERI MELAKA	57	III	IV	IV	I
P.PINANG	JURU	SG. RAMBAI	57	III	IV	IV	II
SARAWAK	MIRI	SG. DALAM	57	III	V	V	II
JOHOR	TEBRAU	SG. TAMPOI	57	III	V	IV	II
P.PINANG	JAWI	SG. JAWI	55	III	IV	IV	III
P.PINANG	JURU	SG. JURU	55	III	V	IV	II
JOHOR	KAW. PASIR GUDANG	SG. MASAI	54	III	IV	IV	I
P.PINANG/KEDAH	PERAI	SG. KEREH	52	IV	V	V	II
JOHOR	SEGGET	SG. SEGGET	52	IV	V	V	II
JOHOR	AIR BALOI	SG. AIR BALOI	49	IV	V	I	III
JOHOR	KEMPAS	SG. KEMPAS	49	IV	V	V	II
P.PINANG	PERAI	SG. PERTAMA	48	IV	V	IV	III
JOHOR	TEBRAU	SG. SEBULUNG	48	IV	V	IV	I
SELANGOR/ WPKL	KLANG	SG. UNTUT	48	IV	V	V	I
JOHOR	KAW. PASIR GUDANG	SG. BULUH	48	IV	V	IV	II
JOHOR	DANGA	SG. DANGA	47	IV	V	V	II
MELAKA	MERLIMAU	SG. MERLIMAU	45	IV	V	V	II
JOHOR	KAW. PASIR GUDANG	SG. TUKANG BATU	38	IV	V	V	II
JOHOR	TEBRAU	SG. SENGKUANG	37	IV	V	V	II
P.PINANG	PINANG	SG. JELUTONG	35	IV	V	V	II
JOHOR	PONTIAN BESAR	SG. AYER MERAH	32	IV	V	IV	I

(b)

PARAMETER	UNIT	CLASS				
		I	II	III	IV	V
Ammoniacal Nitrogen	mg/l	< 0.1	0.1 – 0.3	0.3 – 0.9	0.9 – 2.7	> 2.7
Biochemical Oxygen Demand	mg/l	< 1	1 – 3	3 – 6	6 – 12	> 12
Chemical Oxygen Demand	mg/l	< 10	10 – 25	25 – 50	50 – 100	> 100
Dissolved Oxygen	mg/l	> 7	5 – 7	3 – 5	1 – 3	< 1
pH	-	> 7.0	6.0 – 7.0	5.0 – 6.0	< 5.0	> 5.0
Total Suspended Solid	mg/l	< 25	25 – 50	50 – 150	150 – 300	> 300
Water Quality Index (WQI)		> 92.7	76.5 – 92.7	51.9 – 76.5	31.0 – 51.9	< 31.0

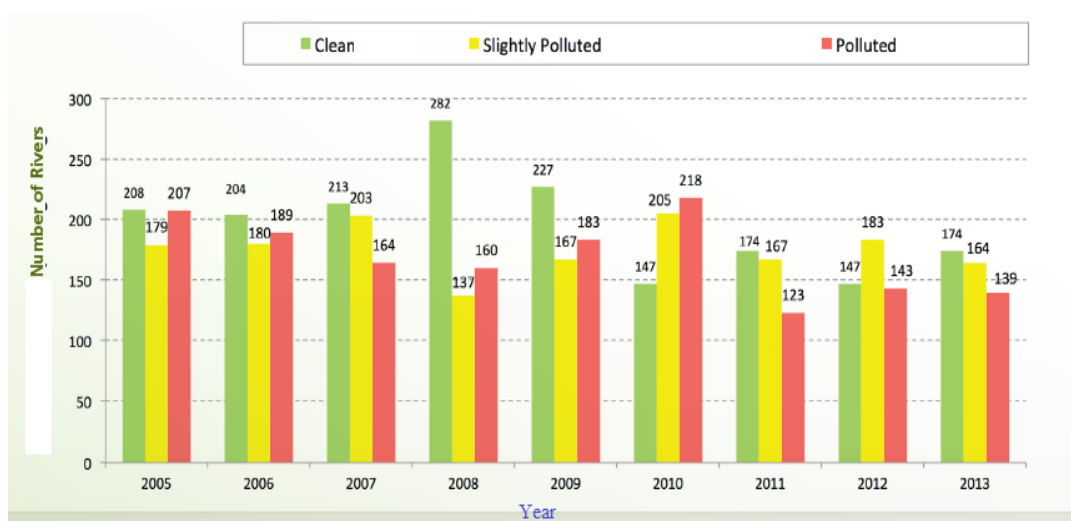


Figure 1.2 River Water Quality Trend based on AN sub-index in Malaysia (2005-2013)

(Source: Malaysia Environment Quality Report 2013. Department of Environment, Ministry of Natural Resources and Environment, Malaysia)

Table 1.2 Acceptable Conditions for Discharge of Industrial Effluent for Mixed Effluent of Standard A and B (Extracted from Environment Quality (Industrial Effluents) Regulation 2009 (PU (A) 434))

FIFTH SCHEDULE [Paragraph 11(1) (a)]				
ACCEPTABLE CONDITIONS FOR DISCHARGE OF INDUSTRIAL EFFLUENT FOR MIXED EFFLUENT OF STANDARDS A AND B				
	Parameter	Unit	Standard	
	(1)	(2)	A (3)	B (4)
(i)	Temperature	°C	40	40
(ii)	pH Value	-	6.0-9.0	5.5-9.0
(iii)	BOD <sub>5</sub> at 20°C	mg/L	20	40
(iv)	Suspended Solids	mg/L	50	100
(v)	Mercury	mg/L	0.005	0.05
(vi)	Cadmium	mg/L	0.01	0.02
(vii)	Chromium, Hexavalent	mg/L	0.05	0.05
(viii)	Chromium, Trivalent	mg/L	0.20	1.0
(ix)	Arsenic	mg/L	0.05	0.10
(x)	Cyanide	mg/L	0.05	0.10
(xi)	Lead	mg/L	0.10	0.5
(xii)	Copper	mg/L	0.20	1.0
(xiii)	Manganese	mg/L	0.20	1.0
(xiv)	Nickel	mg/L	0.20	1.0
(xv)	Tin	mg/L	0.20	1.0
(xvi)	Zinc	mg/L	2.0	2.0
(xvii)	Boron	mg/L	1.0	4.0
(xviii)	Iron (Fe)	mg/L	1.0	5.0
(xix)	Silver	mg/L	0.1	1.0
(xx)	Aluminium	mg/L	10	15
(xxi)	Selenium	mg/L	0.02	0.5
(xxii)	Barium	mg/L	1.0	2.0
(xxiii)	Fluoride	mg/L	2.0	5.0
(xxiv)	Formaldehyde	mg/L	1.0	2.0
(xxv)	Phenol	mg/L	0.001	1.0
(xxvi)	Free Chlorine	mg/L	1.0	2.0
(xxvii)	Sulphide	mg/L	0.50	0.50
(xxviii)	Oil and Grease	mg/L	1.0	10
(xxix)	Ammoniacal Nitrogen	mg/L	10	20
(xxx)	Colour	ADMI*	100	200

ADMI- American Dye Manufacturers Institute

### **1.3 Problem Statement**

In modern age, people lives with a luxury lifestyle and having expensive technology. On the positive side, it helps the economy to grow. On the negative side, industrial produce a lot of waste due to the increasing of production in order to cope with the demand in the market. There are three types of wastes from industrial area which are solid, liquid and gas. Gas waste required filtration system that can be installed in the chimney to remove any greenhouse gases and other toxic gases using suitable absorption materials before the gases are being released to the environment. Solid waste does not require a big space for temporarily storage and disposal of the solid waste can be arranged with certain agency that specialized in handling the solid waste. However, liquid waste requires a big reservoir for temporarily storage. Thus, compared to storage, it is more feasible to treat the wastewater as it requires smaller reservoir due to continuous process and it reduces the cost of operation (Wan et al., 2013).

On the other hand, the discharge quality of the effluent of the industrial wastewater treatment plant is bounded by regulation as listed in Table 1.2. Under normal situation, various production lines produce various type of wastewater and they are collected in an equalizer tank whereby various sources of wastewater are mixed before the treatment begins. Thus, a series of processes would normally be applied until final discharge can meet the requirements as amended in the regulation. The processes involve pre-treatment, primary treatment, and secondary treatment. Tertiary (or advance treatment) treatment is applied if necessary. Primary treatment is usually chemical treatment which apply chemical in order to extract certain ion and form a precipitate so that it can be settled and extracted from the wastewater easily. Secondary treatment is biological treatment which apply biomass (ammonia