NITROGEN REMOVAL EFFICIENCY IN SLAUGHTERHOUSE WASTEWATER BY USING UPFLOW MICROAEROBIC SLUDGE REACTOR

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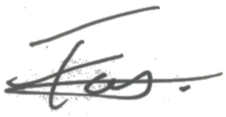

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Thesis submitted in partial fulfillment of the requirements
for the award of the
B. Eng (Hons.) Civil Engineering

Faculty of Civil Engineering & Earth Resources
UNIVERSITI MALAYSIA PAHANG

MAY 2019

ACKNOWLEDGEMENTS

Foremost, I would like to express my sincere gratitude to my supervisor Dr. Ngien Su Kong for his patience, motivation, enthusiasm, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better supervisor and mentor for my degree study. Besides my supervisor, I would like to thank Dr Nurul Nadrah Aqilah binti Tukimat, for her guidance along the research period.

Next, thank to my fellow technician assistants of Environmental Laboratory, Faculty of Civil Engineering and Earth Resources, University Malaysia Pahang (UMP) for their unfailing support and assistance. Also, I thanks to the workers from BoxDesign Sdn. Bhd. who give me the guidance in designing and constructing the prototype. They were shared their experience and taught me the technique to build up the prototype without extra charges.

I am grateful to my sibling and parents, who have provided me through moral and emotional support in my life. I am also grateful to my other family members and friends who have supported me along the way.

And finally, last but by no means least, also to everyone in the impact hub… it was great sharing laboratory with all of you during last four years.

Thanks for all your encouragement!

ABSTRAK

Kajian terdahulu telah membuktikan bahawa upflow microaerobic sludge reactor (UMSR) mempunyai keputusan yang baik dalam mengubati air piggeri dengan kepekatan ammonium (NH_4^+-N) yang tinggi dan nisbah yang rendah bagi permintaan oksigen kimia (COD) kepada nitrogen total (TN). Dalam kajian ini, satu prototaip UMSR telah dibina untuk merawat air sisa sembelih dengan kepekatan NH_4^+-N yang tinggi dan nisbah COD/TN yang tinggi. Kecekapan penyingkiran nitrogen untuk UMSR kemudiaan dibandingkan dengan loji rawatan air sisa penyembelihan yang sedia ada. Proses aklimatisasi untuk prototaip adalah selama 60 hari. Reaktor dikawal dalam keadaan mikroaerobik yang mana dilarutkan oksigen (DO) adalah dalam lingkungan 0.3 mg/L hingga 1.0 mg/L dan nilai pH adalah dalam julat 7.0-8.0. Sampel influen dan sampel efluen pada hari ke-61, hari ke-65 dan hari ke-70 telah diambil dari prototaip untuk analisis. Sampel influen dan sampel efluen telah diambil dari loji rawatan air sisa Rumah Penyembelihan Kuantan, Pahang untuk berbanding dengan kecekapan penyingkiran nitrogen dengan paramter yang sama. Kadar penyingkiran TN, NH_4^+-N, COD dan permintaan oksigen biokimia (BOD) untuk prototaip adalah 78.28%, 83.57%, 80.59% dan 25.30% masing-masing. Kadar penyingkiran TN, NH_4^+-N, COD dan BOD untuk loji rawatan sedia ada adalah 19.05%, 18.96%, 82.55% dan 9.08% masing-masing. Kesimpulannya, kadar penyingkiran nitrogen untuk UMSR dalam merawat air sisa dengan nisbah COD/TN yang tinggi adalah sangat bagus dan mempunyai prestasi yang lebih baik daripada loji rawatan yang sedia ada.

ABSTRACT

Rapid growth of the livestock industry has led to the increase number of slaughterhouses in Malaysia. The wastewater generated from slaughterhouse will contained a large amount of organic and inorganic pollutants which are harmful to the environment. Previous studies had been proved that upflow microaerobic sludge reactor (UMSR) had excellent results in treating piggery water with high ammonium (NH_4^+-N) and low chemical oxygen demand (COD) to total nitrogen (TN) ratio. In this study, a prototype of UMSR was constructed to treat the slaughterhouse wastewater with high NH_4^+-N concentration and high COD/TN ratio and to compare its nitrogen removal efficiency with the existing slaughterhouse's wastewater treatment plant. The acclimation stage of the prototype was 60 days. The reactor was controlled in the microaerobic condition which the dissolved oxygen (DO) was maintained within the range of 0.3 mg/L to 1.0 mg/L and pH value within the range of 7.0-8.0. Influent samples and effluent samples on Day 61, Day 65 and Day 70 were taken from the prototype for analysis. Influent sample and effluent sample were taken from the wastewater treatment plant of Slaughterhouse Kuantan for the comparison. The average percentage removal rate of TN, NH_4^+-N, COD and biochemical oxygen demand (BOD) of the prototype were 78.28%, 83.57% ,80.59% and 25.30%, respectively. The removal rate of TN, NH_4^+-N, COD and BOD for existing treatment plant were 19.05% ,18.96%, 82.55% and 9.08%, respectively. In conclusion, the UMSR can perform well in treating high COD/TN ratio wastewater and even had better performance than existing treatment plant.

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LIST OF SYMBOLS

RR'NH	Amines
NH_3-N	Ammonia
NH_4^+-N	Ammonium
CO_2	Carbon Dioxide
N_2	Dinitrogen
H_2	Hydrogen
NO_3^--N	Nitrate
NO_2^--N	Nitrite
N_2	Nitrogen Gas
RR'N-NO	Nitrosamines
C_{org}	Organic Carbon

LIST OF ABBREVIATIONS

AerAOB	Aerobic Ammonium-Oxidizing Bacteria
AOB	Ammonia-Oxidizing Bacteria
BOD	Biochemical Oxygen Demand
BNR	Biological Nitrogen Removal
COD	Chemical Oxygen Demand
CMU	Combined Manure with Urine
DO	Dissolved Oxygen
FKASA	Faculty of Civil Engineering & Earth Resources
GDP	Gross Domestic Product
HRT	Hydraulic Retention Time
NOB	Nitrite-Oxidizing Bacteria
STP	Sewage Treatment Plant
SMU	Soaked Manure with Urine
TN	Total Nitrogen
TSS	Total Suspended Solid
UMP	Universiti Malaysia Pahang
UFM	Urine-Free Manure

CHAPTER 1

INTRODUCTION

1.1 Background of Study

In Malaysia, the agriculture sector contributed RM89.5 billion or 8.1% to the Gross Domestic Product (GDP) in 2016. Livestock industry is one of the important sectors which contribute 11.6 % to the GDP of the agriculture sector. (Dept. of Statistics Malaysia, 2017). The growth of the livestock industry was followed by the increase in the number of slaughterhouses. Large volumes of highly polluted wastewater were generated by the slaughterhouses. The wastewater generated from slaughterhouse contains large amounts of organic and inorganic pollutants which are harmful to the environment (Anijiofor and Nik Daud, 2018). One of the common elements in these wastewater is Ammonium (NH_4^+-N), which if discharged without proper treatment can lead to the eutrophication of water bodies (Zhang *et al.*, 2016; Meng, Li, Li, Astals, *et al.*, 2018). Therefore, nitrogen removal has become one of the notable environmental issues in slaughterhouse wastewater treatment.

Generally, biological nitrogen removal (BNR) process is known as the combined process of nitrification and denitrification. Nitrification is known as a microbial process to oxidize the nitrogen compounds by the nitrifiers from the form of NH_4^+-N to nitrite (NO_2^--N) and nitrate (NO_3^--N). The nitrification process is accomplished by two groups of autotrophic nitrifying bacteria which are ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). Autotrophic bacteria are defined as a type of bacteria that can build organic molecules by using energy obtained from inorganic sources. During the nitrification process, AOB plays its role to oxidize the NH_4^+-N to NO_2^--N. Then NOB will further oxidize NO_2^--N to NO_3^--N (American Water Works Association, 2002). Besides that, another key process in BNR is denitrification. Throughout the denitrification process, the oxidized forms of nitrogen will be converted to dinitrogen

(N_2) or its lesser extent, nitrogen gas (N_2) (Ni *et al.*, 2017). Nitrification is an aerobic process while denitrification is an anaerobic process. One of the common engineered treatment technologies for treating wastewater is the anaerobic-aerobic combined process but it required high treatment cost and low treatment efficiency where the process is required to be carried out in two different reactors (Meng, Li, Li, Sun, *et al.*, 2016).

Microaerobic treatment process has been introduced for treating wastewater in recent decades. It has the advantages in producing low amount of excess sludge, lesser treatment cost, effective chemical oxygen demand (COD) removal rate and occupying lesser space (Zheng and Cui, 2012). Microaerobic is defined as a transitional state between aerobic and anaerobic conditions. It's dissolved oxygen (DO) concentrations are ranging from 0.3 mg/L to 1.0 mg/L, aerobic and anaerobic bacteria are allowed to coexist in the same activated sludge reactor (Zitomer, 1998; Zhang *et al.*, 2018). In the sludge flocs, the aerobic, anaerobic and facultative bacteria can live in different depths according to their needs towards oxygen (Zheng and Cui, 2012). Aerobic bacteria such as AOB, NOB and heterotrophic aerobic bacteria crave oxygen, therefore they live in the outer layer of the activated sludge. The outer layer of the sludge floc has a relative abundance of DO concentration while DO concentration deep in the sludge floc is to the contrary. The oxygen consumption of the aerobic bacteria cause DO concentration deep in the sludge floc to become relatively low or nearly zero. However, anaerobic microbes such as denitrifiers and anaerobic fermentation bacteria are suitable to live in this condition (Meng, Li, Li, Sun, *et al.*, 2016). In previous studies, microaerobic treatment process has proven that the nitrogen removal efficiency for domestic wastewater with low COD/TN ratio is as high as 80% (Zheng and Cui, 2012; Zhang *et al.*, 2018)

In the present study, an upflow microaerobic granular sludge reactor (UMSR) with falling water and reflux prototype is constructed to investigate nitrogen removal efficiency of the microaerobic treatment process towards slaughterhouse wastewater with high COD/TN ratio. The comfortable conditions for denitrification has been suggested as the COD/TN ratio of 6.0-8.0 (Obaja *et al.*, 2003). However, there were no studies on the microaerobic treatment process towards wastewater with high COD/TN ratio. 6 major parameters will be considered in the analysis which are TN, NH_4^+-N concentration, NO_2^--N concentration, NO_3^--N concentration, biochemical oxygen demand (BOD) and COD. pH and DO concentration will be observed every day during the acclimation stage of the inoculum sludge and controlled within the suitable range. Nitrogen removal efficiency of the prototype will be deduced from all these 6 parameters. It is believed that the microaerobic treatment process is favourable for the slaughterhouse wastewater treatment. The nitrogen removal efficiency of the UMSR will be compared with that of the existing treatment plant using the same parameters.

1.2 Problem Statement

Large volumes of wastewater will be discharged to the water bodies due to the development of the agricultural sector and rapid industrialization in Malaysia. Wastewater produced from slaughterhouses is characterized by high organic content such as nitrogen and phosphorus (Anijiofor and Nik Daud, 2018). Nitrogen exists in various forms in the wastewater. It can be in organic form such as NH_4^+-N or inorganic forms which included NO_2^--N and NO_3^--N (Yaakob et al., 2018). Nitrogen components are one of the common contaminants in wastewater which can cause eutrophication to occur on the surface of water bodies (Zhang et al., 2016; Meng, Li, Li, Astals, et al., 2018). Proper treatment process should be implanted before the slaughterhouse wastewater is discharged to the water bodies. In fact, there are no standards available for ammoniacal nitrogen discharge in Malaysia. Removal of ammoniacal nitrogen is not a design criterion for the wastewater treatment plants in Malaysia (Indah Water, 2018).

Two major branches of treatment processes have been introduced for treating the wastewater with high concentration of ammoniacal nitrogen which are biological treatment process and chemical treatment process. In chemical treatment process, air-stripping, breakpoint chlorination and ion exchange are the common method to be used. Air-stripping is the volatilization of ammonia gas. Breakpoint chlorination is the addition of chlorine into the wastewater to oxidize the ammonia. Lastly, ion exchange is using a type of clay-clinoptilolite to remove the ammonia. However, these processes will bring negative environmental issues and require high operation cost. Air stripping can cause odour problems, addition of chlorine will need other treatment process to dilute the excess chlorine and ion exchange will be a high operation cost treatment process. In the biological treatment process, aerobic treatment, anaerobic treatment and aerobic-anaerobic combined treatment are the common treatment methods to be used for removal of ammoniacal nitrogen. By comparing to the chemical treatment process, biological treatment process will be more economic, environmentally friendly and has higher removal efficiency.

Microaerobic treatment process has been introduced for nitrogen removal in recent decades. Nitrogen removal efficiency in slaughterhouse wastewater by using UMSR has been studied by a lot of researchers in China. Based on the results, UMSR has been proven to remove around 90% nitrogen from slaughterhouse wastewater with low COD/TN ratio (Meng, Li, Li, Sun, et al., 2016; Meng, Li, Li, Wang, et al., 2016; Meng, Li, Li, Antwi, et al., 2018). However, the slaughterhouse wastewater in Malaysia do not contain low COD/TN ratio due to different operation and management procedure between the two countries (Anijiofor and Nik Daud, 2018). Therefore, a prototype of UMSR has been constructed to investigate the nitrogen removal efficiency in slaughterhouse wastewater with high COD/TN by using the microaerobic treatment. Since there is no standard for ammoniacal nitrogen removal in Malaysia, the study will compare the nitrogen removal efficiency of the UMSR with the efficiency of the existing slaughterhouse wastewater treatment.

1.3 Research Objectives

The objectives of the research are as follows:

- To investigate the nitrogen removal efficiency in slaughterhouse wastewater by using the UMSR.
- To compare the UMSR with the existing wastewater treatment reactor in terms of nitrogen removal efficiency.

1.4 Scope of Study

The main focus of the study is to investigate the nitrogen removal efficiency in slaughterhouse wastewater by using UMSR and compare its efficiency with the existing treatment plant. A UMSR prototype has been constructed to determine the efficiency of the microaerobic treatment process. The slaughterhouse wastewater samples were collected from the aeration pond in Slaughterhouse Kuantan. 100 to 125 L of wastewater samples were collected in every collection of wastewaters. Collection of wastewaters from Slaughterhouse Kuantan were carried out every two weeks. The seed sludge used for the acclimation process was the aerobic activated sludge collected from the aerobic tank of sewage treatment plant (STP) of Kolej Kediaman 2, Universiti Malaysia Pahang (UMP). The prototype was undergone acclimation process for 60 days and continued with the actual treatment process for 10 days. Influent samples and effluent samples on day 61, 65 and 70 were collected from the reactor of UMSR for the analysis. 500 mL of samples were taken from both influents and effluents. TN, NH_4^+-N concentration, BOD and COD were the parameters using to determine the nitrogen removal efficiency of the UMSR. 500 mL of samples were taken from both influents and effluents of the aeration pond in Slaughterhouse Kuantan in order to compare the nitrogen removal efficiency with the UMSR by using the same parameters. The research was just studied the nitrogen removal efficiency in the slaughterhouse wastewater with high COD/TN ratio.

1.5 Significance of Study

Rapid growth of livestock industry in recent decades have generated large volume of highly polluted wastewater. The wastewater generated from slaughterhouse will contain a large amount of organic and inorganic pollutants which are harmful to the environment (Anijiofor and Nik Daud, 2018). Organic pollutants such as nitrogen and phosphorous were harmful to the environment and human defects. Discharged of the high organic pollutants slaughterhouse wastewater without proper treatment could lead to the negative impacts to the environment and living things. Studies on UMSR or microaerobic treatment process had been conducted by a lot of researchers in China. Based on the result, microaerobic treatment had been proved that can remove ammoniacal nitrogen from slaughterhouse wastewater with low COD/TN ratio in around 90% (Meng, Li, Li, Sun, *et al.*, 2016; Meng, Li, Li, Wang, *et al.*, 2016; Meng, Li, Li, Antwi, *et al.*, 2018). However, wastewater discharged from slaughterhouse in Malaysia were majority high COD/TN ratio. Therefore, the present research was conducted to study the nitrogen removal efficiency in slaughterhouse wastewater with high COD/TN by using UMSR or microaerobic treatment process. The present research can indicate the suitability of using UMSR in Malaysia for the treatment of nitrogen. It is an important issue since there is no standard for the ammoniacal nitrogen removal and the STPs in Malaysia are not designed for the ammoniacal nitrogen removal (Indah Water, 2018). Besides that, the operation cost and construction cost for STP with nitrogen removal design are normally relatively higher since it requires both aerobic and anaerobic treatment process. By using UMSR, it can reduce the cost for the nitrogen treatment process. This is because the UMSR can provide the microaerobic conditions which both aerobic and anaerobic bacteria can coexist in the same reactor. Implementation of microaerobic treatment process in the STP of slaughterhouse can help to ensure the nitrogen removal of the discharged wastewater are not harmful to the environment and living things.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Wastewater is defined as the water that has been influenced by human activities. All effluent from commercial establishments and institutions, residential, industries, medical institution and others can be defined as wastewater. Stormwater and urban runoff are also considered as wastewater although they are caused by natural phenomena. Wastewater has become one of the sources of pollutant that lead to water pollution. Slaughterhouse wastewater is one type of wastewater with high pollutants contents. Rapid growth of the livestock industry has led to the increase in the number of slaughterhouses in Malaysia. The wastewater produced from slaughterhouse contain large number of nutrients such as nitrogen and phosphorus. Therefore, nutrient removal is one of the notable issues in slaughterhouse wastewater. Eutrophication or water borne diseases can be caused by the direct discharge of the nutrients to the water body. A proper and efficient wastewater treatment system should be implanted to prevent or reduce the flow of pollutants to the streams.

2.2 Wastewater Treatment in Malaysia

In the past fifty years, different methods of wastewater treatment processes have been developed in Malaysia. The aim of the development is to meet the requirement to protect the environment and public health. Treatment systems in urban areas have evolved to produce high-quality effluent due to the concentrated population and environmental issues caused by the wastewater. However, simpler treatment systems are still used in small communities even though they cannot meet the environment standard that has increased in recent decades (Indah Water, 2018). Table 2.1 and Table 2.2 show the sewage treatment systems that are used in urban area and small communities in Malaysia.

Table 2.1: Major Biological Sewage Treatment Processes

Aerobic Processes	**Suspended Growth**	**Activated Sludge**
		-plug flow -complete mix -sequencing batch reactor -extended aeration* -Oxidation ditch* -deep shaft* -Aerated Lagoons*
	Attached Growth	**Trickling Filters**
		-low rate -high rate* -Rotating Biological Contactors* -Submerged Biological Contactors*
	Combines	**Biofilter Activated Sludge**
		* Trickling Filter Activated Sludge
Anaerobic Process	**Suspended Growth** **Attached Growth**	Anaerobic Contact Anaerobic Filter Expanded Bed
Pond Processes		Aerobic Stabilization (Oxidation) Facultative Anaerobic

* Systems used in Malaysia.

Source: Indah Water, 2018

Table 2.2: Commonly Used Treatment Systems for Small Communities

Primary Treatment	-Individual Septic Tanks -Communal Septic Tanks -Imhoff Tanks
Secondary Treatment	**Package (pre-fabricated) Plants -** activated sludge systems - sequencing batch reactors - contact stabilization - rotating biological contactors
	Individually Designed Plants - activated sludge systems - oxidation ponds - sequencing batch reactors - rotating biological contactors - trickling filter - facultation lagoons - aerated lagoons

Source: Indah Water, 2018

2.3 Slaughterhouse Wastewater

2.3.1 Growth of Livestock Industry

Livestock industry is one of the important factors that drive the development of agricultural sector in the recent decades. It increases the employment opportunities, supplies the milk, meat and dairy products to coping the large demand of population. In Malaysia, agriculture sector stood was contribute RM89.5 billion or 8.1% to the Gross Domestic Product (GDP) in 2016. Livestock industry is one of the importance sectors which contribute 11.6 % for the GDP of agriculture sector. (Dept. of Statistics Malaysia, 2017). The demand for the meat and its product will increase, stated by Malaysian National Agro-food Policy 2011-2020 (NAP). From 2010 to 2020, the demand will increase from 1.4 million MT to 1.8 million MT with the growth rate of 2.4% per annum. On the other hand, the meat production is expected to increase from 1.6 million MT to 2.1 million MT with a growth rate of 2.7% per annum within the same decade. However, the rapid growth of the livestock industry also results in rising of the environmental issues.

2.3.2 Environmental Issues Brought by Growth of Livestock Industry

Slaughterhouse wastewater pollution is one of the notable environmental issue that brought by the rapid development of the livestock industry. Large volume of the wastewater has been produced by the development of agricultural sector and rapid industrialization. Slaughterhouse wastewater had been characterizing as highly polluted wastewater due to high BOD, COD, total suspended solid (TSS), blood and nutrient (nitrogen and phosphorus) from slaughtering and cleaning activities (Yaakob *et al.*, 2018). The wastewater must be treated properly before it discharged to the water bodies or any drainage system due to its high pollutant concentration. However, the treatment processes are not being done before the discharged to the water bodies in most of the slaughterhouse around the world. The issue is more critical in the in some developing or under-developed countries (Anijiofor and Nik Daud, 2018). A proper wastewater treatment is needed before been discharged into the water body, to avoid environmental degradation such as eutrophication and spreading of water borne diseases (Yaakob *et al.*, 2018).

2.3.3 Nitrogen Components in Wastewater

Nitrogen is one of the important nutrients that affect the survival of all living things. It can be found in many biomolecules such as proteins, chlorophyll, deoxyribonucleic acid (DNA), and so on (Bernhard, 2010). Nitrogen can be found in the wastewater; it exists in different forms which are organic form and inorganic form. In most of the wastewater, NH_4^+-N, NO_2^--N, NO_3^--N are the common forms of inorganic nitrogen while amino acid and nucleic acid are the examples of organic nitrogen. By the assist of the microbes, nitrogen can be transformed frequently in the wastewater ecosystem. It can be changing from one form to another, and convert back to the original form. Main mechanisms in the transformation of nitrogen are nitrification, denitrification, anammox, nitrogen fixation, ammonification, nitrite oxidation and ammonia oxidation (Bernhard, 2010) . Figure 2.1 shows the major mechanisms in the nitrogen transformation cycle.

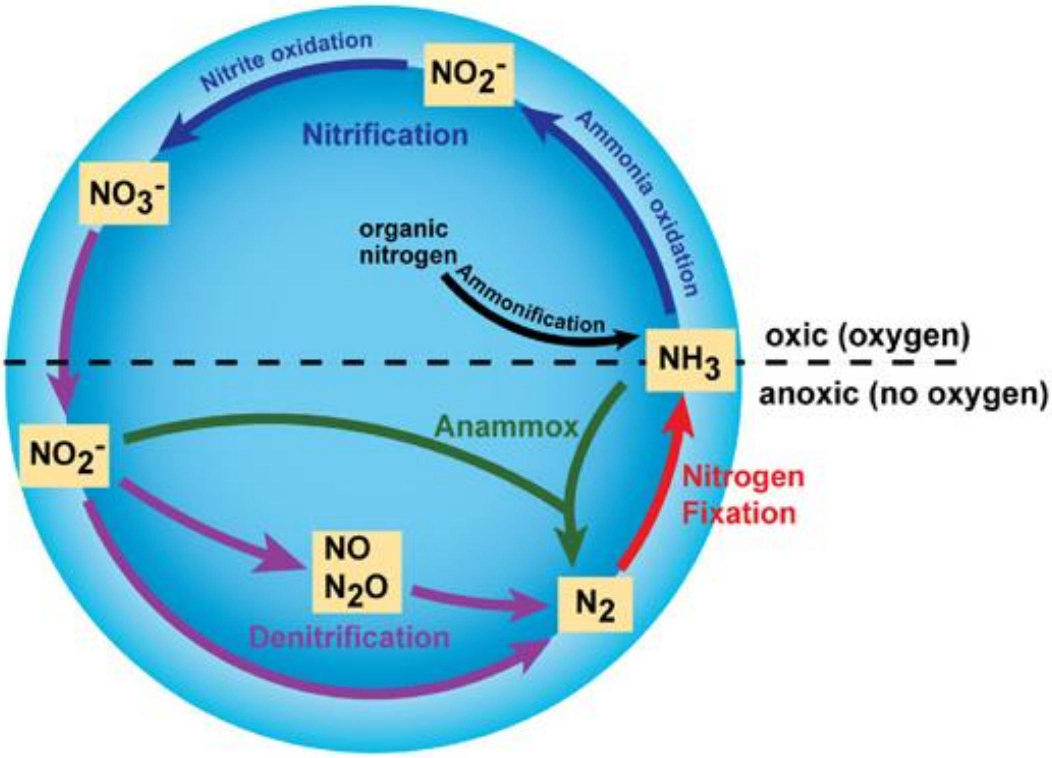


Figure 2.1: The Major Mechanisms in Nitrogen Transformation Cycle

2.3.4 Importance of Nitrogen Removal in Slaughterhouse Wastewater

The main pollutants in the slaughterhouse wastewater are ammoniacal nitrogen, discharged without proper treatment will lead to some undesirable environmental issues such as eutrophication and algal blooms (Kundu, Debsarkar and Mukherjee, 2013). Besides that, nitrate poisoning will be happened when human infants consumed water with 10mg/L or greater nitrate concentrations during the first few months. The disease is known as blue-baby syndrome or methemoglobinemia (Hey, 2001). Therefore, an efficient nitrogen removal system should be implanted to reduce the impacts that brought by nitrogen pollutants. However, the ammoniacal nitrogen concentration discharged does not restrict to any standard in Malaysia and even STPs in Malaysia are not designed for the ammoniacal nitrogen removal.

2.4 Physical-Chemistry Method in Nitrogen Removal Process

Generally, two major branches of treatment methods have been introduced for treating the wastewater with high concentration of ammoniacal nitrogen which are biological methods and physical-chemistry method. For physical-chemistry methods, air-stripping, breakpoint chlorination and ion exchange are the common methods to be used. Air-stripping is the volatilization of ammonia gas. Breakpoint chlorination will add chlorine into the wastewater to oxidize the ammonia. Lastly, ion exchange is using a type of clay-clinoptilolite to remove the ammonia (Indah Water, 2018). However, these methods also bring some negative effects to the population and environment. Air stripping is a technique to make the collisions to be occurred in between the air and wastewater. In the end, the volatile compounds in wastewater will be released into the air. Odour pollution will be one of the disadvantages by using this method (EMIS, 2015). On the other hand, the major negative impact that brought by breakpoint chlorination is the possibility of generating the by-products. Organic material in the wastewater can combine with the excess chlorine in the wastewater to form some dangerous substances such as Trihalomethanes. It is harmful to the liver, kidney and nervous system and may cause the increase risk of the cancer. Therefore, addition process needed to be implanted to remove the chlorine and it may cause the increase of operation cost. Iron scaling, calcium sulfate scaling, organic contamination, organic matter adsorption, chlorine contamination and bacterial contamination are the common impacts that brought by the ion exchange process. Therefore, high operation cost will be needed to reduce the negative impacts and increase the efficiency (Tripathi, 2017).

2.5 Biological Nitrogen Removal Process

Biological nitrogen removal (BNR) treatment method will be the main mechanism used in the present study. In the BNR treatment process, aerobic treatment, anaerobic treatment and aerobic-anaerobic combined treatment are the common treatment method to be used. Generally, BNR is the combined treatment process of nitrification under aerobic conditions and denitrification under anaerobic conditions. In the nitrification process, NH_4^+-N is firstly oxidized to NO_2^--N by the AOB and after that NOB will oxidized NO_2^--N to NO_3^--N. In the denitrification process, NO_2^--N and NO_3^--N will be then denitrified to N_2 or N_2 gas with the exists of organic carbon source (Wang *et al.*, 2015).

2.5.1 Nitrification

Nitrification is the microbial process to oxidize the nitrogen compound from NH_4^+-N to NO_2^--N and NO_3^--N sequentially by the assists of microbes. The nitrification process is majorly accomplished by the two groups of autotrophic nitrifying bacteria which are AOB and NOB. Autotrophic bacteria are defined as a type of bacteria that can build organic molecules by using energy obtained from inorganic sources. First step of the nitrification is the oxidation of ammonia (NH_3) to NO_2^--N by the AOB according to the equation 2.1 (American Water Works Association, 2002). *Nitrosomonas* is known as the most frequently identified bacteria to be found in this step. *Nitrosococcus*, *Nitrosospira*, *Nitrosolobus* and *Nitrosovibrio* are the different type of autotrophic bacteria that can be found in the oxidation of ammonia (Watson, Valois and Waterbury, 1981).

$$\mathrm{NH_3} + O_2 \rightarrow NO_2^- + 3H^+ + 2e \qquad 2.1$$

During the second step of nitrification, NOB take the roles to oxidize NO_2^--N to NO_3^--N according to equation 2.2. In this step, *Nitrobacter* is known as the most frequently identified bacteria. Others autotrophic bacteria such as *Nitrospina*, *Nitrococcus* and *Nitrospira* can also be found in this step (Watson, Valois and Waterbury, 1981).

$$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e \qquad 2.2$$

2.5.2 Denitrification

Another key process in the BNR of wastewater is denitrification. Based on (Ni *et al.*, 2017), denitrification is defined as the only biotransformation process that can remove nitrogen compounds from ecosystems and it is essentially irreversible. During the denitrification process, nitrogen compounds that oxidized in the nitrification such as NO_2^--N and NO_3^--N are converted to N_2 and N_2 gas by the assists of denitrifying bacteria. Denitrification is an anaerobic process to convert NO_2^--N and NO_3^--N to N_2 according to equation 2.3. As mentioned above, nitrogen cannot be reconverted to a biologically available form after it has been converted to N_2.It is because N_2 is a gas and it will lost to the atmosphere rapidly (Ni *et al.*, 2017).

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad 2.3$$

2.6 Microaerobic Treatment

Microaerobic treatment process has been introduced for treating wastewater in recent decades. It has the advantages in producing low amount of excess sludge, lesser treatment cost, effective COD removal rate and occupying lesser space (Zheng and Cui, 2012). Microaerobic is defined as a transitional state between aerobic and anaerobic conditions. It's DO concentrations are ranging from 0.3 mg/L to 1.0 mg/L, aerobic and anaerobic bacteria are allowed to coexist in the same activated sludge reactor (Zitomer, 1998; Zhang *et al.*, 2018). In the sludge flocs, the aerobic, anaerobic and facultative aerobic bacteria can live in different depths according to their needs towards oxygen (Zheng and Cui, 2012). Aerobic bacteria such as AOB, NOB and heterotrophic aerobic bacteria are craving for the oxygen, therefore they can live in the outer layer of the activated sludge. The outer layer of the sludge floc is relatively abundance of DO concentration while DO concentration in the deep of the sludge floc is on the contrary. The oxygen consumption of the aerobic bacteria cause DO concentration in the deep of the sludge floc become relatively low or nearly zero. However, anaerobic microbes such as denitrifiers and anaerobic fermentation bacteria are suitable to live in this condition

(Meng, Li, Li, Sun, *et al.*, 2016). In the previous studies, microaerobic treatment process has been proved that the nitrogen removal efficiency for domestic wastewater with low chemical oxygen demand (COD) to total nitrogen (TN)ratio is as high as 80% (Zheng and Cui, 2012; Zhang *et al.*, 2018)

2.7 Microbial Community

Based on (Meng *et al.*, 2015), there are four mechanisms of nitrogen transformations that may occurred during the biological treatment process are nitrogen assimilation, traditional nitrification and denitrification, short-cut nitrification and denitrification and anammox (AMX). Nitrogen assimilation is the biological process that required organic and inorganic carbon sources. Traditional nitrification and denitrification are the process to remove 1g of nitrogen by using out 2.86g of COD without assimilation. Short-cut nitrification and denitrification are the process to remove 1g of nitrogen by using out 1.72g of COD without assimilation. Lastly, AMX is the autotrophic nitrogen removal process that require non-organic carbon as the energy sources. Different nitrogen removal mechanisms will lead to the different microbial community in the activated sludge.

Granular sludges will be formed during the microaerobic treatment process. AOB, NOB AMX, autotrophic denitrifying bacteria and heterotrophic denitrifying bacteria can be found in the different depth level of the sludge floc in the reactor (Meng *et al.*, 2015; Meng, Li, Li, Wang, *et al.*, 2016; Zhang *et al.*, 2018). BNR treatment process such as denitrification and nitrification will be undergone in the sludge core and on the its surface based on the requirement to the DO concentration. Figure 2 shows the schematic overview of the nitrogen conversions in the UMSR. On the surfaces of the granular sludge, heterotrophic bacteria oxidize the organic carbon (C_{org}) to carbon dioxide (CO_2) and then autotrophic bacteria in the system will fix it. AOB will firstly oxidize NH_4^+-N to NO_2^--N and subsequently NO_2^--N will be further oxidized to NO_3^--N by NOB. Anaerobic fermentation of organic carbon will produce hydrogen (H_2). Heterotrophic or autotrophic denitrifying bacteria reduce NO_3^--N to NO_2^--N by using the molecular hydrogen or organic carbon found in the sludge core as the energy sources. NO_2^--N is then reduced to the nitric oxide, autotrophic denitrifying bacteria will further convert it to N_2 gas or it will combine with NH_4^+-N by the anammox metabolism (Meng, Li, Li, Astals, *et al.*, 2018). In the AMX metabolism, ammonium is oxidized by using the NO_2^- -N as the electron receiver under anoxic conditions.

Sphingomonas and *Nitrosomonas* are the common species of the AOB. *Spartobacteria* also another type of AOB that can be found in the agricultural soil (Meng, Li, Li, Sun, *et al.*, 2016). In the oxygen limiting condition, *Proteobacteria* is the dominant aerobic ammonium-oxidizing bacteria (AerAOB) can be found in the BNR treatment process. Heterotrophic denitrifying bacteria is the most important bacteria for the process of nitrogen removal because the proportion is the highest in the microbial community (Zhang *et al.*, 2018). *Thermomonas*, *Thermogutta*, *Denitratisoma* are some of the species of heterotrophic denitrifying bacteria that can found in the microaerobic treatment process (Zhang *et al.*, 2018). Besides that, it was found that nitrite oxidation process cannot be undergone in the condition which pH value over 8.0. NOB can be inhibited in this condition. Based on research of (Meng, Li, Li, Sun, *et al.*, 2016), no NOB can be found in the sludge floc when the pH in the reactor over 8.0. Autotrophic denitrifying bacteria such as Thiobacuillus can be observed in the reactor with a low proportion. Autotrophic denitrifying bacteria and Anammox are the denitrification approach with no carbon source needed.

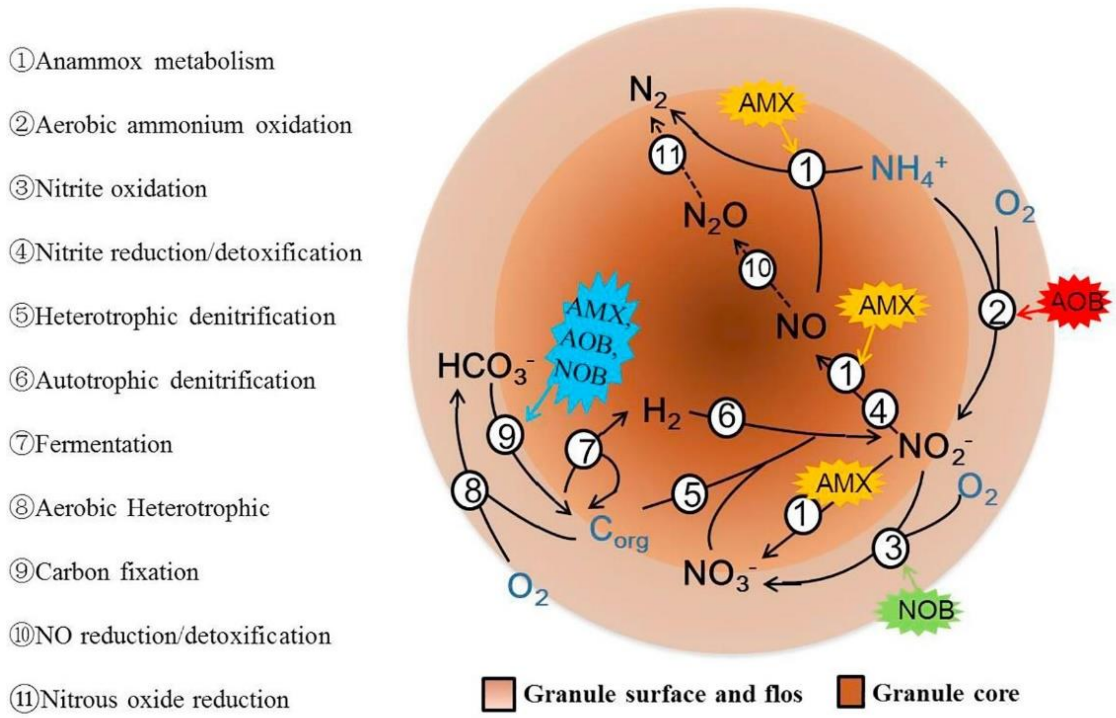


Figure 2.2: Schematic Overview of Nitrogen Conversions in UMSR.

2.8 Acclimation of Inoculated Sludge

Acclimation can be defined as a process of adapting to a new environment. The activated sludge to be used in biological treatment process must be acclimated to the particular activated sludge before the data can be obtained. The process involves many biochemical actions to be reviewed. Generally, acclimation can be defined as a procedure involving a new environment which acts as the stimulant on the organisms (microbes) to produce a new system of microbial community. These microbes or so call enzymes are act as the catalysts to hydrolyse, transport and degrade the organics. An activated sludge can be said to be acclimated to reduce organic waste when enough bacteria have been grown to produce a desired system of enzymatic reactions (Stephens, 1970).

In biochemical waste treatment, acclimation is the microbial community must adjust to the new food (organic and inorganic wastes) environment. Biodegradation is one of the processes involved in the adjustment. It can be defined as the ability to reduce the complexity of a chemical compound by splitting off one or more groups or larger components parts. A period of time is needed for the activated sludge microbial community to degrade the wastes to final non-biodegradable end products. The period of acclimation has been documented by some researchers (Stephens, 1970).

In the municipal wastewater treatment, the bacteria originate from human feces and earth bacteria. Bacteria utilizing industrial wastes can be obtained from the soil around ground spillage areas or from river beds receiving the untreated waste for some time period. Usually a fill and draw method has been employed to build up the concentrations of bacteria. In a municipal waste the bacteria originate from human feces and earth bacteria. The procedure follows:

(1) Feed waste and aerate 24 hours in vessel

(2) Remove 1/3 volume while mixing

(3) Settle remainder, remove 1/2 remaining volume as supernatant

(4) Replace 2/3 volume vessel with new waste.

This procedure can be repeated until a sludge mass is grown to a concentration similar to a value derived in the full-scale plant or until enough bacteria per unit volume are present to degrade the waste to a desired level. This method will develop bacteria which will treat the particular waste but, in general, a long time period will be required. Normally, it will be taking two to six months to develop large masses of activated sludge. Specific microbial community have been found necessary to develop a mixed culture to treat a specific wastes (Stephens, 1970).

Second most commonly procedure to be used in the design is obtaining the microbial community from an existing municipal or industrial activated sludge plant. Laboratory study procedures have been outlined to prove it workability. Batch studies follow a program similar to the one listed by Gaudy for a period of acclimation and then the removal rate can be obtained with a final batch run. If a continuous reactor setup was employed, as the authors state, it would give more consistent results for design. Once an acclimated state was obtained. a batch run was then performed to obtain the removal rate. No matter which system has been employed to get an acclimated sludge or which unit for feeding or aeration has been employed it is important to interpret data remembering the limitations of either the procedure or apparatus. It must be noted that acclimated microbial community must be subjected to similar hydraulic and food loadings required in actual plant operation (Stephens, 1970).

2.9 Parameters of Study

2.9.1 Total Nitrogen (TN)

TN is the combination of organic nitrogen, NH_3-N /NH_4^+-N, NO_2^--N and NO_3^--N. Organic nitrogen is defined as the nitrogen that organically bounded together in the tri-negative oxidation state. NH_3-N/NH_4^+-N can be found naturally in the surface of wastewaters. Oxidative digestion of all digestible nitrogen forms to NO_3^--N and continued with quantitation of NO_3^--N can be used to determine TN. Two major methods can be used which are persulfate/ultraviolet (UV) digestion and persulfate digestion. By using persulfate/UV digestion method, nitrogenous compounds are digested and oxidized in-line to nitrate by use of heated alkaline persulfate and UV radiation. Persulfate method determines TN by oxidizing all the nitrogenous compounds to NO_3^--N. These procedures can give good results for TN but molecular nitrogen is not determined (American Public Health Association, American Water Works Association and Federation, 1999).

2.9.2 Ammonia/Ammonium Concentration (NH_3-N/ NH_4^+-N)

NH_3-N/ NH_4^+-N can be found naturally in the surface of the wastewaters. Its concentration in groundwaters is generally low. It will adsorb to clays and soil particles and cannot be leached readily from soils. Hydrolysis of urea and deamination of organic nitrogen-containing compound are the main sources of NH_3-N/ NH_4^+-N. NH_3-N/ NH_4^+-N is added to some wastewater treatment plants to react with chlorine to form combined chlorine residual. NH_3-N/ NH_4^+-N concentration in natural surface and groundwaters is less than 10 µg/L while it can be more than 30mg/L in wastewater (American Public Health Association, American Water Works Association and Federation, 1999).

2.9.3 Nitrite Concentration (NO_2^--N)

NO_2^--N is an intermediate state in oxidation of NH_3-N/ NH_4^+-N to NO_3^--N and in the reduction of NO_3^--N. It can be found in the wastewater treatment plants, natural waters and water distribution systems. It can be added to the industrial process water to use as a corrosion inhibitor. NO_2^--N has been determined as the actual pathogen of methemoglobinemia (American Public Health Association, American Water Works Association and Federation, 1999). NO_2^--N in acidic solution is known as nitrous acid, it can react with secondary amines (RR′NH) to form carcinogens or known to be nitrosamines (RR′N-NO). Toxicologic significance of the reactions in natural environment and vivo is a notable issues that should have more attention and concern. (American Public Health Association, American Water Works Association and Federation, 1999).

2.9.4 Nitrite Concentration (NO_3^--N)

NO_3^--N generally high concentration in groundwaters while just small amount in surface waters. Excessive amounts of NO_3^-N will lead to methemoglobinemia in infants. Drinking water with NO_3^--N concentration more than 10 mg/L contributes to the disease. NO_3^--N is generally low concentration in domestic wastewater but may have high concentration in the effluent of nitrifying biological treatment plants. It has been identified as a growth-limiting nutrient and it is the essential nutrient for most of the photosynthetic autotrophs (American Public Health Association, American Water Works Association and Federation, 1999).

2.9.5 Biochemical Oxygen Demand (BOD)

BOD determination is a practical test used to determine the relative oxygen requirements in wastewater, effluents, and polluted waters by using standardized laboratory procedures. It can be used in evaluating BOD removal efficiency of water or wastewater treatment system. Besides that, it has the application in determine the waste loadings to treatment plants. BOD is determined by measuring the oxygen utilized for the biodegradation of organic substances and oxygen used for the oxidation of inorganic substances during a specific period. It will measure the oxygen used for the oxidation of reduced forms of nitrogen if the oxidation is not prevented by an inhibitor. pH of the water samples should be controlled within the range of 6.5 to 7.5 by dilution before BOD is tested (American Public Health Association, American Water Works Association and Federation, 1999).

2.9.6 Chemical Oxygen Demand (COD)

COD is determined by measuring the specific oxidant that required to react with the water samples under controlled conditions. Although oxidation is occurred in both organic and inorganic compounds, but organic compounds is the dominant and have greater interest in COD determination. Additions method should be taken to distinguish either organic and inorganic substances from each other in order to measure the COD alone. Digestion time, reagent strength, and COD concentration in water sample will affect the oxidation of the samples and hence influence the COD result (American Public Health Association, American Water Works Association and Federation, 1999). COD is often used to measure the pollutants in natural water bodies and treatment plants.

CHAPTER 3

METHODOLOGY

3.1 Introduction

The main objective of the present study is to investigate the nitrogen removal efficiency in the slaughterhouse wastewater by using the UMSR or microaerobic condition. The nitrogen removal efficiency of the UMSR were compared with the existing wastewater treatment reactor. The research methodology is actually to achieve the objectives above. The whole research was taken around 80 days including the set-up period and acclimation for the seed sludge. Influent and effluent of the wastewater in USMR on day 61, 65 and 70 were sampled and tested with 6 parameters which are TN, NH_4^+- N concentration, NO_2^--N concentration, NO_3^--N concentration, BOD and COD. TN, NH_4^{+-}N concentration, BOD and COD were then used to determine the nitrogen removal efficiency of the USMR. A sample of influent and effluent were taken from the aeration pond of wastewater treatment plant in Slaughterhouse Kuantan. Same parameters were tested and its efficiency were compared with the UMSR.

3.2 Flow Chart of the Methodology Study

The whole duration for the research was around 80 days which including the set-up of prototype and acclimation of inoculum sludge. The research started with the preparation stage which including the set-up of the prototype and preparation of the research venue. It took around 10 days to ensure the prototype could be functioned smoothly and would not bring any inconveniences to others. Activated sludge and wastewater were then taken from the sewage treatment plant of KK2, UMP and Slaughterhouse Kuantan respectively. Research was continued with the acclimation of inoculum sludge for around 60 days. The starting day of the acclimation was defined as the Day 1. The acclimation stage was divided into three phases according to the changed of the conditions. Phase I was started from Day 1 to Day 14, then phase II was continued until Day 33 and phase III was continued until the end of acclimation stage. During the acclimation stage, DO concentrations were controlled to be stable in the range of 0.3 mg/L to 1.0 mg/L. After the acclimation stage, the treatment process was continued for 10 days. Influent and effluent samples were taken on day 61, 65 and 70 for the analysis. Conclusion and recommendations were done after the analysis. Figure 3.1 shows the flow chart of the research and Figure 3.2 shows the actual timeline of works using Prototype.

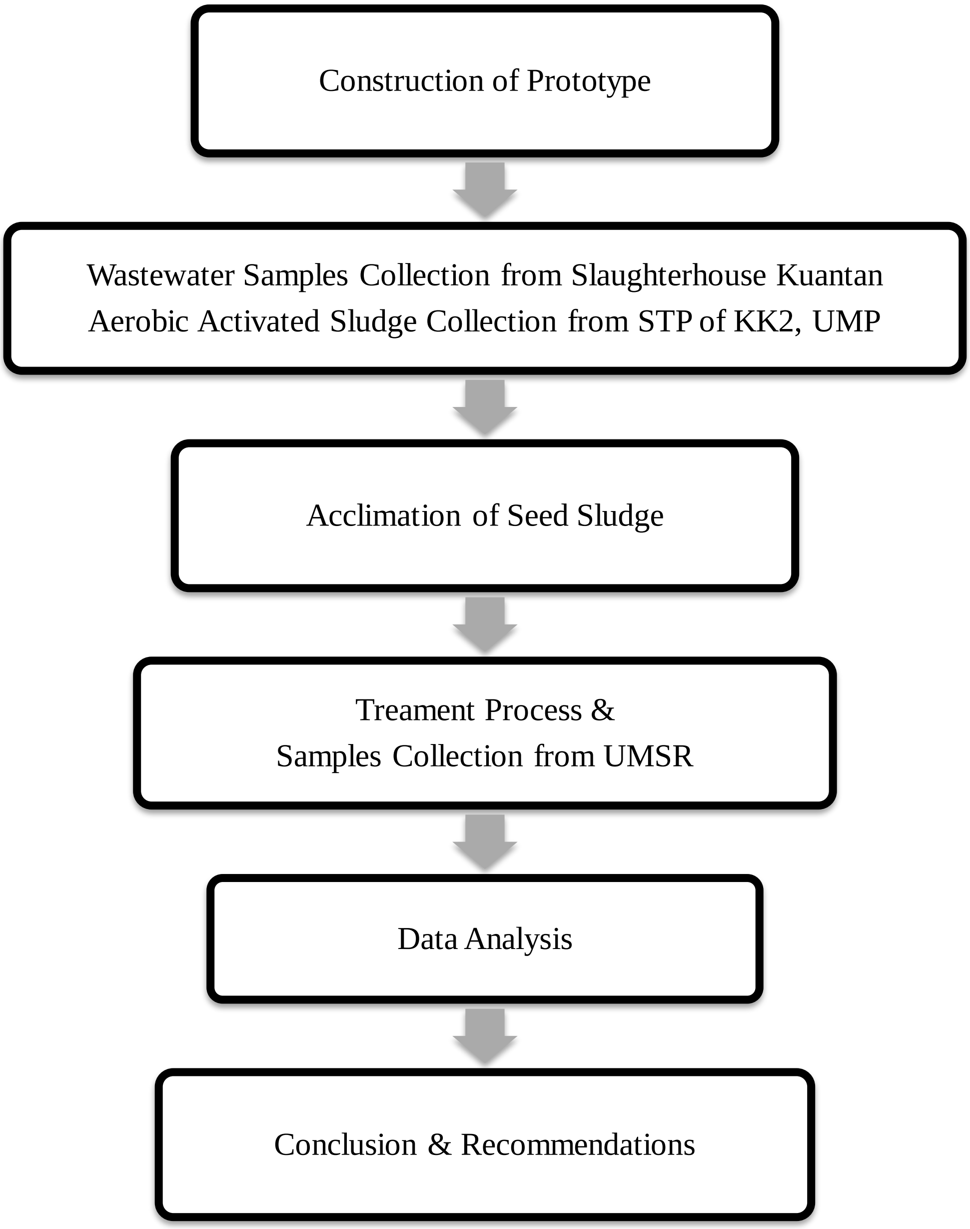


Figure 3.1: Research Flow Chart

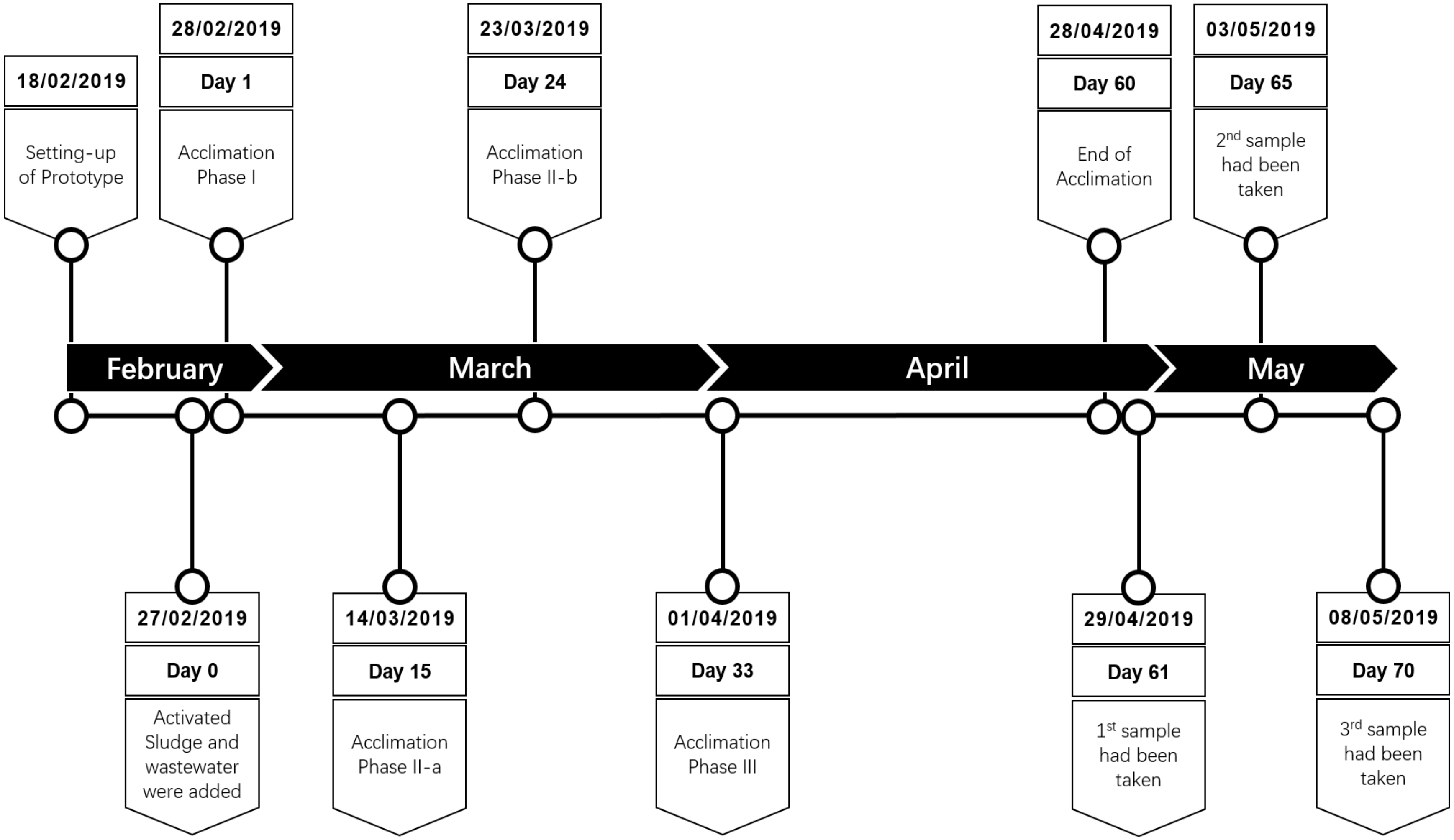


Figure 3.2: Actual Timeline of Works using Prototype

3.3 Prototype of UMSR

A prototype of the UMSR was constructed as shown in Figure 3.3. The UMSR prototype consisted of 5 major elements which are the slaughterhouse wastewater tank, influent tank, reoxygenation tank, aeration tank and reactor. A 0.3m high acrylic column with an effective volume of 5L was designated as the reactor and attached to a 0.5m x 0.5m x 0.1m acrylic cuboid. The acrylic cuboid was designed as the aeration tank with a volume of approximately 25L. The reactor and aeration tank were placed lower than other tanks with head loss approximately 1m. Two 5L containers with valves were used as the influent tank and reoxygenation tank. A 40L basin was designed as tank to store the slaughterhouse wastewater. DO concentration was controlled within the range of 0.3mg/L to 1.0mg/L. The temperature varied with the room temperature. The prototype was placed behind the Environmental Lab of the Faculty of Civil Engineering & Earth Resources (FKASA), Universiti Malaysia Pahang (UMP). The reactor and aeration tank were covered with black plastic started from phase II of acclimation stage to avoid penetration of sunlight in order to prevent eutrophication occurring on the surface of the wastewater. Figure 3.4 shows the actual prototype of UMSR.

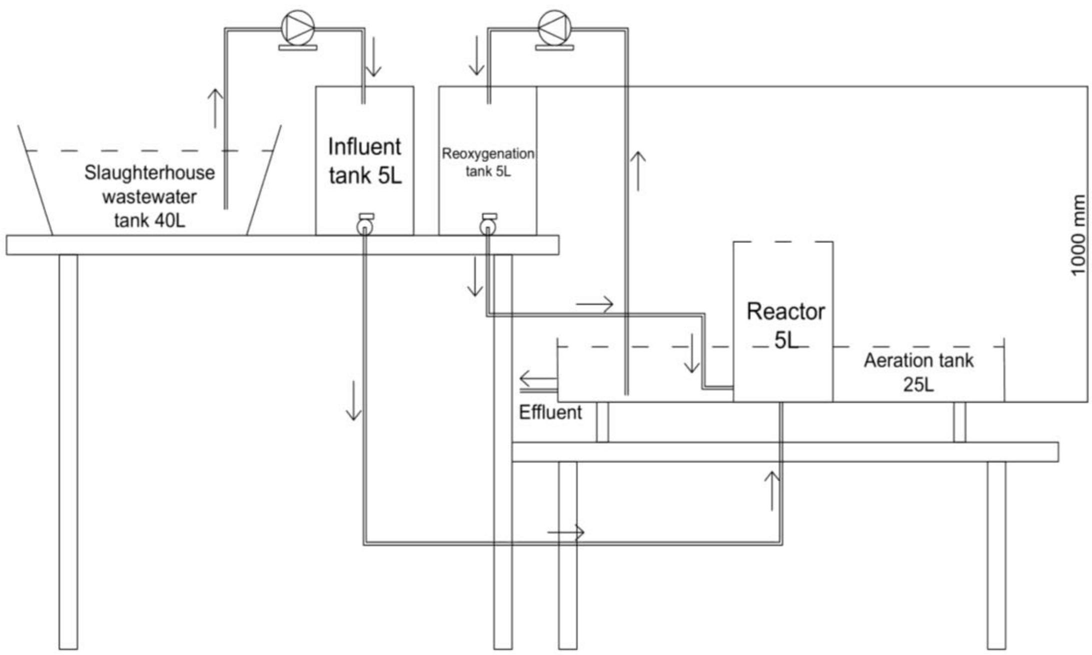


Figure 3.3: Schematic Drawing of UMSR Prototype.

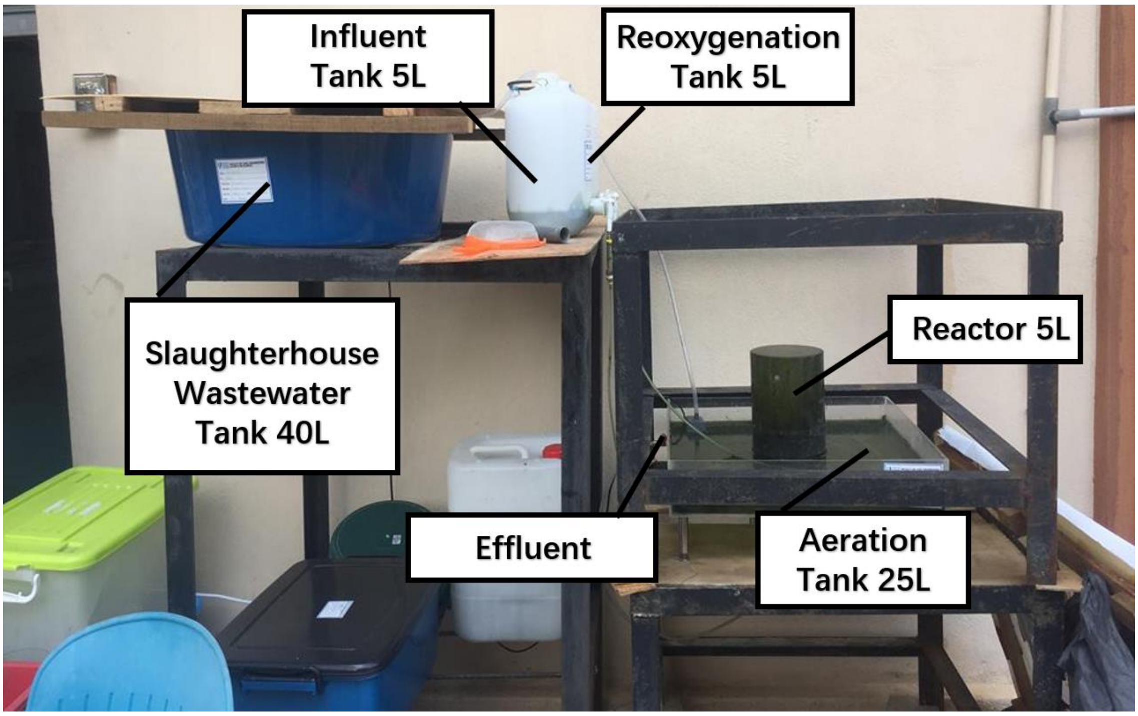


Figure 3.4: Actual Prototype of UMSR

In every treatment cycle, around 5L of slaughterhouse wastewater was pumped from the 40L tank to the influent tank at a pump rate of 100L/h which then flow to the reactor by gravitational force. The aquarium submersible pump was controlled by a digital timer. Hydraulic retention time (HRT) was set at the start of 8 hours, for 12 hours and then adjusted to 24 hours as the final HRT. The top of the reactor column was open and the water would overflow from the opening and then fall into the aeration tank. Reoxygenation process was done by the falling water. Reoxygenated water would be pumped to the reoxygenation tank at a pump rate of 100L/h and flow back to the reactor with the controlled flow rate of 25L/h. The flow rate was controlled by a valve to maintain the DO concentration. The reoxygenation cycle was controlled by a digital timer. At the beginning, each treatment process was set to have 3 cycles of reoxygenation which was adjusted to no reoxygenation cycle in the final adjustment. 5L of water from aeration tank would release as the effluent in the end of each treatment process.

3.4 Sample Collection

Raw slaughterhouse wastewater was collected from Slaughterhouse Kuantan, Malaysia. The wastewater was collected from the aeration pond of the wastewater treatment plant in Slaughterhouse Kuantan and sent to the Environmental Lab of FKASA, UMP immediately. The whole treatment process for the study was around 70 days including the acclimation of inoculum sludge. A large volume of wastewater was required throughout the treatment process. TN, NH_4^+-N concentration, NO_2^--N concentration, NO_3^--N concentration, BOD and COD for the wastewater were ranging from 27.0-96.0, 9.43-88.4, 0.02-0.055, 0.50-1.03, 10.55-26.75 and 580-3300 mg/L, respectively. The wastewater was preserved in order to maintain the existing compounds and to avoid degradation by the activities of the microbial communities. The wastewater was stored in the cold room in the Environmental Lab of FKASA, UMP at a temperature below 4^{o}C. Figure 3.5 shows the wastewater samples that collected from Slaughterhouse Kuantan.

Figure 3.5: Wastewater Samples Collected from Slaughterhouse Kuantan

Seed sludge was the aerobic sludge collected from the aeration tank of a sewage treatment plant (STP) in Kolej Kediaman 2, UMP. The seed sludge was then sent to the Environmental Lab of FKASA, UMP and stored in the cold room at a temperature below 4oC to avoid the degradation of the nutrient by the activities of the microbial communities.

Figure 3.6: Activated Sludge Collected from STP KK2, UMP

3.5 Acclimation of Seed Sludge

Before the actual treatment process of the UMSR, the inoculum sludge was acclimated for 60 days fed with the slaughterhouse wastewater continuously. 500g of aerobic activated sludge was placed in the reactor as the seed sludge. DO and pH were controlled to within the suitable range during the acclimation stage. pH was ranging from 7.2 to 7.8 during the whole acclimation process which was considered as acceptable. DO was controlled by adjusting the reoxygenation cycle and the HRT, which reduced from 12.05mg/L to the acceptable range between 0.3mg/L to 1.0mg/L. The readings of pH and DO were taken by using Handheld Multiparameter. The acclimation stage was divided into three major phases according to the changes of the conditions. Further explanations on the changes will be discussed in the discussion part.

3.5.1 Apparatus and Materials in Acclimation Stage

Table 3.1: List of Apparatus and Materials in Acclimation Stage

Apparatus	Description	Materials	Description
	Handheld Multiparameter -To observe the readings of pH and DO concentration		Raw Wastewater Samples
	UMSR Prototype		500g of activated sludge

3.6 Wastewater Treatment Process

After the acclimation of inoculum sludge, the UMSR continued to run for 10 days for the treatment process. The UMSR was continuously fed with the slaughterhouse wastewater collected on the slaughterhouse operation day. It is believed that the wastewater collected during operation will have the highest nutrient content. The treatment process was run with a constant HRT of 24 hours. Influent and effluent on day 61, 65 and 70 were taken for the analysis. DO concentration and pH were observed along the treatment process to ensure the prototype was run in the microaerobic conditions.

3.6.1 Apparatus and Materials in Treatment Process

Table 3.2: List of Apparatus and Materials in Treating Process

Apparatus	Description	Materials	Description
	Handheld Multiparameter -To observe the readings of pH and DO concentration		Raw Wastewater Samples
	UMSR Prototype		

3.7 Water Samples for Comparison

Since there is no standard for the ammoniacal nitrogen removal in Malaysia (Indah Water, 2018), therefore the nitrogen removal of the UMSR was compared to the existing wastewater treatment plants in Malaysia. The chosen location was the wastewater treatment plant in Slaughterhouse Kuantan, Malaysia. Slaughterhouse Kuantan was the nearest slaughterhouse around UMP. It took around 15 minutes to travel from the slaughterhouse to UMP, therefore no preservation method was needed before the wastewater sending back to UMP. The wastewater treatment system in the Slaughterhouse Kuantan is using the aeration pond method. It consists of two sandy drying compartments, a filtration tank, an aeration pond and a sedimentation pond. Influent and effluent samples were collected from the aeration pond of the treatment plant for analysis. Figure 3.7 shows the wastewater samples collected from Slaughterhouse Kuantan.

Figure 3.7: Influent and Effluent Samples Taken from Slaughterhouse Kuantan

3.8 Analytical Methods

TN, NH_4^+-N concentration, NO_2^--N concentration, NO_3^--N concentration, BOD and COD were analyzed to determine the nitrogen removal efficiency of the UMSR. DO and pH were controlling variables throughout the treatment process. Tests for NH_4^+-N concentration, NO_2^--N concentration, NO_3^--N concentration and COD were conducted in the UMP FKASA Environmental Lab using Method 8155, Method 8507, Method 8192 and Method 8000 respectively with HACH DR5000 and HACH DR3900. BOD testing was carried out in the same lab with the standard method based on ADHA 5210B (21st Edition). Filtration was done before analysis were carried out. Samples were sent to Central Laboratory, UMP for testing of TN. DO and pH were observed by the Handheld Multiparameter. Removal efficiencies were determined by the equation 3.1.

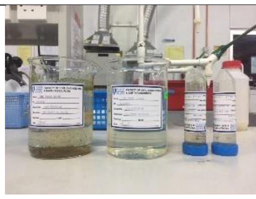
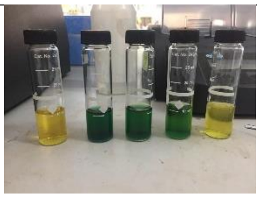
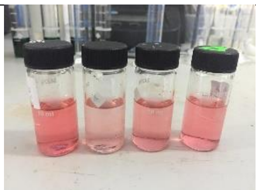
$$\text{Removal Efficiency } = \frac{\text{A} - \text{B}}{\text{A}} \times 100\% \quad (3.1)$$

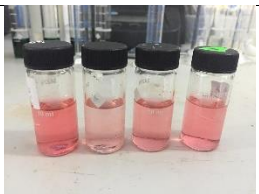
where A = Influent and

B = Effluent.

3.8.1 Apparatus and Materials in Analysis

Table 3.3: List of Apparatus and Materials in Analysis

General			
Apparatus	**Description**	**Materials**	**Description**
	Handheld Multiparameter -To observe the readings of pH and DO concentration		Raw Wastewater Samples
	UMSR Prototype		Wastewater Samples for analysis
	Filtration Set -Filter Funnels -500mL Volumetric Flask -Filter Papers		
NH_4^+-N concentration (Method 8155)			
Apparatus	**Description**	**Materials**	**Description**
	HACH DR5000		Ammonia Salicylate Reagent pillows
	25mL Glass Vials		Ammonia Cyanurate Reagent pillows
NO_2^--N concentration (Method 8507)			
Apparatus	**Description**	**Materials**	**Description**
	HACH DR5000		NitriVer® 3 Nitrite Reagent Powder Pillows
	10mL Glass Vials		

NO_3^--N concentration (Method 8192)			
Apparatus	**Description**	**Materials**	**Description**
	HACH DR5000		NitriVer® 3 Nitrite Reagent Powder Pillows
	25mL Graduated Mixing Cylinder		NitraVer® 6 Nitrate Reagent powder pillow
	10mL Glass Vials		

COD (Method 8000)			
Apparatus	**Description**	**Materials**	**Description**
	HACH DR3900		COD Digestion Reagent Vials
	COD Reactor		
	Volumetric Pipet		

BOD (APHA 5210B (21^{st} Edition))			
Apparatus	**Description**	**Materials**	**Description**
	pH Meter		HACH BOD Buffer Pillow
	YSI 5100 DO Meter		

BOD Bottles 	

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

Results of the research are divided into three major parts which are the DO concentration in the acclimation stage, the nitrogen removal efficiency of UMSR and comparison of removal efficiency between UMSR and existing treatment plant. Results are discussed based on the objectives of the research. Changes made during the acclimation stage and its effects to the DO concentration in reactor are discussed in the first part. A clearer view on the relationship between the changes on prototype and DO concentration can be seen in this part. Removal efficiency of the UMSR is determined by 4 parameters which are TN, NH_4^+-N concentration, COD and BOD. NO_2^--N concentration and NO_3^--N concentration are determined to have a clear view on the relationship among the nitrogen compounds during the nitrogen removal process. Removal rates of TN, NH_4^+-N concentration, COD and BOD for UMSR are 78.28%, 83.57%, 80.59% and 25.30%, respectively. The results are then compared with the removal of existing treatment plants. Removal rates of TN, NH_4^+-N concentration, COD and BOD for existing treatment plant are 19.05%, 18.96%, 82.55% and 9.08%, respectively.

4.2 DO Variation in Acclimation Stage

The main purpose of changing the conditions of the reactor is to adjust the DO concentration in reactor in order to meet with the microaerobic conditions which the DO is ranging from 0.3 to 1.0 mg/L. In the phase 1 of acclimation stage, HRT of the treatment process was set at 8 hours with 3 reoxygenation processes and the prototype was exposed to the sunlight. The conditions of prototype in Phase I was shown in the Figure 4.1.The DO of the reactor was ranging within 6.17 to 19.84 mg/L as shown in Figure 4.4 (blue line). Eutrophication phenomenon was occurred during this period as shown in Figure 4.2. This was because the prototype exposed to the sunlight and the wastewater contained high contents of ammoniacal nitrogen. The algae consumed oxygen in the night time and released oxygen as the product of photosynthesis in the day time. Therefore, the DO concentration was maintained within certain range and could not drop anymore.

Figure 4.1: Conditions of Prototype in Phase I

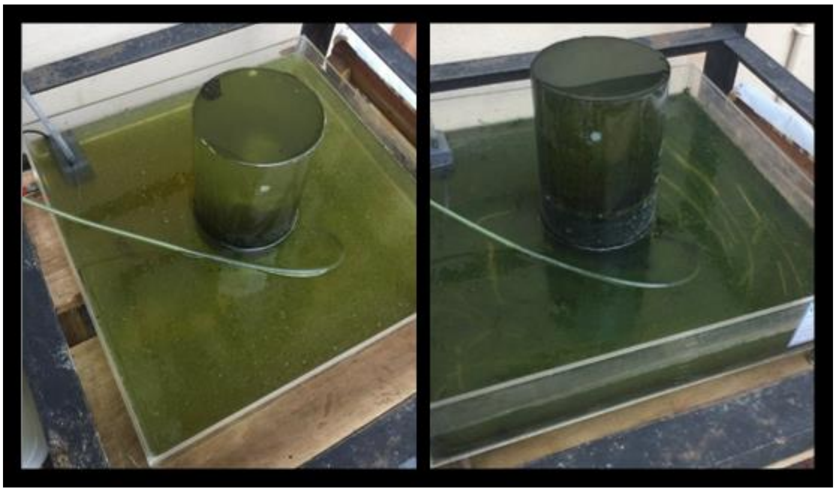


Figure 4.2: Eutrophication on the Surface of Wastewater and Wall of Reactor

Phase II of the acclimation stage can be further divided into two stages which are II-a and II-b. In phase II-a, HRT of the treatment process was set at 8 hours with 3 reoxygenation processes while the prototype was kept in the dark condition as shown in

Error! Reference source not found.. The DO concentration was ranging within 1.26 to 4.08 mg/L as shown in Figure 4.4 (red line). Right after the prototype was kept in the dark condition, DO immediately dropped to 1.26 mg/L which the lowest among the phase II-a. However, it raised and maintained within the range of 2-4 mg/L in the following days. When prototype had just been kept in the dark conditions, photosynthesis of algae was stopped while consumption of oxygen by algae was continued. In results, the DO dropped to the lowest point at the beginning. The algae were then removed from the aeration tank and reactor, therefore the DO was raised back and maintained within certain range. In the conclusion, the growth of algae in the reactor affect the DO concentration of the wastewater. In phase II-b, HRT was set at 12 hours with no reoxygenation process and the prototype was kept in dark condition. DO concentration was ranging within 1.53 to 2.32 mg/L as shown in Figure 4.4 (red line). Since the DO did not drop anymore, the HRT was changed to 12 hours and the reoxygenation process was stopped. Increase in the HRT was resulted in the decrease of influent flow rate. Low influent flow rate caused the microbial activities were taken longer time in the reactor and resulting in the decrease of DO concentrations.

Figure 4.3: Conditions of Prototype in Phase II & III

Lastly, HRT was set as 24 hours with no reoxygenation process and prototype was still kept in dark condition in the phase III of the acclimation stage. DO concentration was ranging within 0.39 to 1.51 mg/L as shown in Figure 4.4 (green line). As mentioned before, increase in the HRT caused the microbial activities took longer time in the reactor and hence resulting in the drop of DO concentrations. In phase III, the DO was finally controlled to meet the microaerobic conditions which within the range of 0.3 to 1.0mg/L.

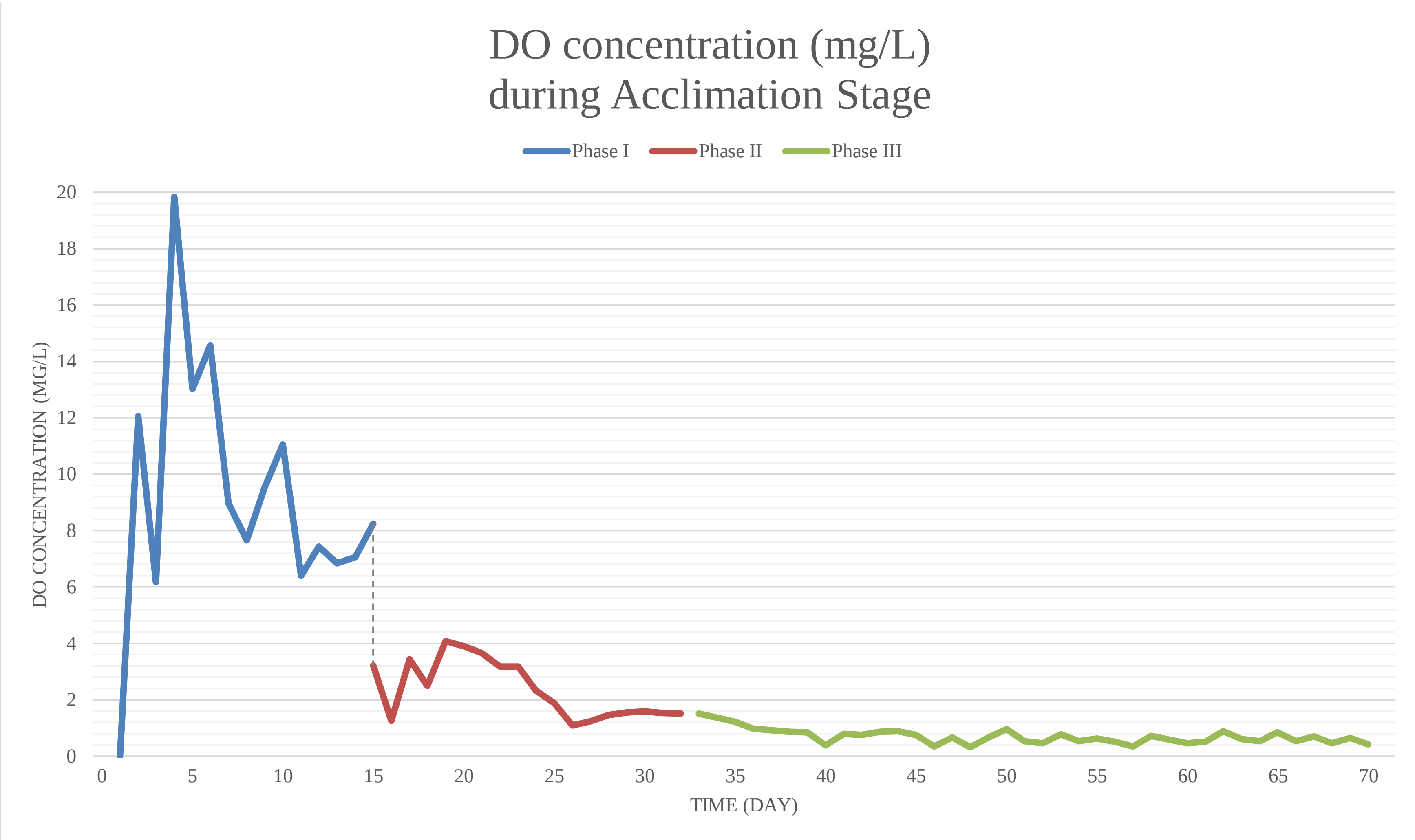


Figure 4.4: DO Variation in Acclimation Stage

4.3 Removal Efficiencies of UMSR

The removal efficiencies of the UMSR were determined based on 4 parameters which were TN, NH_4^+-N, BOD and COD. NO_2^--N concentration and NO_3^--N concentration were determined in order to have a clearer view in mechanisms of nitrogen removal process. Removal efficiencies of UMSR were discussed separately in the following parts.

4.3.1 TN Removal Efficiency of UMSR

Table 4.1: TN Removal Efficiency of UMSR

	Sample		
	1	**2**	**3**
Influent (mg/L)	96	27	64
Effluent (mg/L)	12	10	10
Removal Efficiency (%)	87.5	62.96	84.38
Average (%)		78.28	

According to the Table 4.1, TN concentration in influents for sample 1,2 and 3 were 96, 27, and 64 mg/L, respectively. In the other hand, TN concentration in effluents for 3 samples were 12, 10 and 10 mg/L, respectively. As the results indicate the TN removal rates for 3 samples were 87.50%, 62.96% and 84.38%, respectively. The average TN removal rates for UMSR was 78.28%. According to the results above, sample 1 and sample 3 had TN removal rates over 80% which considered as an excellent efficiency in nitrogen removal. However, the removal rate for sample 2 was just 62.96% which was an unacceptable result for the nitrogen removal. According to the Table 4.1, the TN concentration in effluents were consistent within the range of 10 to 12 mg/L while the TN concentration in influent of sample 2 was much lower than other 2 samples. It could be said that the low removal rate in sample 2 was caused by the low TN concentration of the influent. It might be caused by the freshness of the slaughterhouse wastewater. For sample 1 and sample 3, the influents were the raw wastewater just taken from the Slaughterhouse Kuantan. However, the influent for sample 2 was the raw wastewater that kept in cold room for more than 1 week. Since the raw wastewater was kept for more than 1 week, the contaminants in the wastewater might be treated due to the microbial activities in the wastewater. More study should be done to prove the statement.

Four principal pathways for nitrogen removal and NH_4^+-N conversion were nitrogen assimilation, traditional nitrification and denitrification, short-cut nitrification and denitrification and anammox. Nitrogen assimilation required both organic and inorganic sources as the energy for the removal of nitrogen. Traditional nitrification and denitrification required 2.86g of COD to remove 1g of nitrogen without assimilation. Besides that, shortcut nitrification and denitrification required 1.72g of COD to remove 1g of nitrogen without assimilation. Inorganic carbon was required for anammox as the energy for autotrophic nitrogen removal pathway (Meng *et al.*, 2015). Besides the pathways mentioned above, the reduction of NO_2^-—N to NO and further to N_2O could contribute to the nitrogen removal in slaughterhouse wastewater also. Study on microbial community in activated sludge should be carried out to determine the dominant mechanism in driving the nitrogen removal process in UMSR.

4.3.2 NH_4^+-N Removal Efficiency of UMSR

Table 4.2: NH_4^+-N Removal Efficiency of UMSR

	Sample		
	1	**2**	**3**
Influent (mg/L)	88.4	9.43	58.5
Effluent (mg/L)	4.70	3.90	1.50
Removal Efficiency (%)	94.68	58.64	97.44
Average (%)		83.57	

According to the Table 4.2, NH_4^+-N concentration in influents for sample 1,2 and 3 were 88.4, 9.43, and 58.5 mg/L, respectively. Besides that, NH_4^+-N concentration in effluents for 3 samples were 4.70, 3.90 and 1.50 mg/L, respectively. The results indicated that the NH_4^+-N removal rates for 3 samples were 94.68%, 58.64% and 97.44%, respectively. The average NH_4^+-N removal rates for UMSR was 83.57%. According to the results above, sample 1 and sample 3 had NH_4^+-N removal rates over 90% which show an excellent efficiency in nitrogen removal. However, the removal rate for sample 2 was just 58.64% which was an unacceptable result for the nitrogen removal. Based on the results in Table 4.1, the NH_4^+-N concentration in effluents were consistently less than 5 mg/L while the NH_4^+-N concentration in influent of sample 2 was much lower than the other 2 samples. Low NH_4^+-N concentration in the influent of sample 2 was greatly influence the NH_4^+-N removal rates for sample 2. As mentioned previously, it might be caused by the freshness of the slaughterhouse wastewater. Influents for sample 1 and sample 3 were the raw wastewater just taken from the Slaughterhouse Kuantan. However, the influent for sample 2 was the raw wastewater that kept in cold room for more than 1 week. As mentioned before, the contaminants in the wastewater might be treated due to the microbial activities in the wastewater. Therefore, more study should be carried out to prove the statement.

Based on (Meng *et al.*, 2015), oxidations was driven by the AOB to oxidize part of the NH_4^+-N to NO_2^-—N by using the limited DO in the UMSR. While the residual NH_4^+-N was reacted with the formed NO_2^-—N to N_2 by the helping of anammox bacteria that could be found deep in the sludge floc. Enrichment of AOB would start from the acclimation stage of activated sludge. Enrichment of AOB was the biological foundations for the excellent NH_4^+-N removal in the UMSR. Besides that, the pH within the range of 7.0 to 8.0 and room temperature were favourable conditions for the growth of anammox bacteria (Meng *et al.*, 2015). However, the NO_2^-—N concentration above 30 mg/L might inhibit the activity of annamox bacteria.(Fernández *et al.*, 2012). In the case which treating the wastewater with low COD/TN ratio, annamox was an important denitrification mechanism since no carbon source was needed. However, the wastewater used in present study was the wastewater with high COD/TN ratio. Therefore, heterotrophic denitrification might be the main mechanism in approaching the denitrification process in UMSR. Study on microbial community must be conducted to investigate the actual mechanism in approaching the NH_4^+-N removal in UMSR.

4.3.3 BOD Removal Efficiency of UMSR

Table 4.3: BOD Removal Efficiency of UMSR

	Sample		
	1	**2**	**3**
Influent (mg/L)	10.55	11.70	26.75
Effluent (mg/L)	8.25	10.55	14.90
Removal Efficiency (%)	21.80	9.80	44.30
Average (%)		25.30	

According to the Table 4.3, BOD value in influents for sample 1,2 and 3 were 10.55, 11.70, and 26.75 mg/L, respectively. Furthermore, BOD value in effluents for 3 samples were 8.25, 10.55 and 14.90 mg/L, respectively. The results indicated that the BOD removal rates for 3 samples were just 21.80%, 9.80% and 44.30%, respectively. The average BOD removal rates for UMSR was 25.30%. According to the results above, all 3 samples were showing poor performance in BOD removal efficiency. In Malaysia, the BOD results in effluents were restricted by the Standard A and Standard B that set by the Environmental Quality Act 1974. In Standard A, the BOD in effluent should not more than 20 mg/L while the limitation for Standard B was 50 mg/L. According to the results in Table 4.3, all 3 samples for effluents where passed the standards. The low removal rates of BOD might be caused by the low value of BOD in the influents. The BOD results in the influents of sample 1 and sample 2 even could pass both standards before being treated.

In UMSR, biological treatment process was undergoing to treat the slaughterhouse wastewater. High organic substances and nutrients were contained in the slaughterhouse wastewater. Therefore, it should indicate the high BOD value in the influent samples. Furthermore, the wastewater was taken from the aeration pond which indicates the DO in the wastewater should be higher. BOD value is the relative oxygen required by the microbes in wastewater. Hence, the low BOD value might be caused by the lack of the aerobic bacteria in the wastewater. Aerobic bacteria such as AOB and NOB might not exist in the raw wastewater that just taken from the slaughterhouse. The aerobic microbial activities were not active in the raw wastewater indicate the low BOD value in the influent. However, the microbial communities in the wastewater should be studied to have clearer view on the structure of it in order to find out the exact reason for the abnormal low BOD value in the influents.

4.3.4 COD Removal Efficiency of UMSR

Table 4.4: COD Removal Efficiency of UMSR

	Sample		
	1	**2**	**3**
Influent (mg/L)	3300	580	2410
Effluent (mg/L)	220	210	370
Removal Efficiency (%)	93.33	63.79	84.64
Average (%)		80.59	

According to the Table 4.4, COD value in influents for sample 1,2 and 3 were 3300, 580, and 2410 mg/L, respectively. In the other hand, COD value in effluents for 3 samples were 220, 210 and 370 mg/L, respectively. The results indicated that the COD removal rates for 3 samples were 93.33%, 63.79% and 84.64%, respectively. The average COD removal rates for UMSR was 80.59%. Same as the results in TN removal and NH_4^+-

N removal, sample 1 and sample 3 had the excellent efficiency in the COD removal which the removal rates for both samples were over 85%. Same things happened to the removal rate for sample 2 which its removal rate was just reach an unacceptable value of 63.79%. According to the results in Table 4.4, the COD concentration in effluents were consistently within the range of 210 to 370mg/L while the COD value in the influent of sample 2 was much lower than the other 2 samples. Low COD removal rate in sample 2 might be caused by the low COD value in the influent. As mentioned previously, it might be caused by the freshness of the slaughterhouse wastewater. Influents for sample 1 and sample 3 were the raw wastewater just taken from the Slaughterhouse Kuantan. However, the influent for sample 2 was the raw wastewater that kept in cold room for more than 1 week. As explained before, the contaminants in the wastewater might be treated due to the microbial activities in the wastewater. Therefore, more study should be carried out to prove the statement.

The residual COD in the effluent might be the non-biodegradable organics in slaughterhouse wastewater (Meng *et al.*, 2015). Based on (Meng *et al.*, 2015), the COD removal rate would increase significantly with the decrease of NO_2^--N concentration. It indicated that the decreased NO_2^--N was reduced to N_2 by heterotrophic denitrification via shortcut nitrification-denitrification process and it was believed that the shortcut nitrification-denitrification process was the main mechanism in the COD removal in UMSR. However, just 3 results for NO_2^--N concentrations were not able to find out the trend of the NO_2^--N concentration. Therefore, consistent lab testing should be carried out to ensure the trend of all data could be investigated. To get more evidence in determine the pathway of COD removal, the structure and activity of the microbial community should be investigated.

4.4 Comparison between UMSR and Existing Treatment Plant in Slaughterhouse Kuantan

Table 4.5: Removal Efficiencies of Treatment Plant in Slaughterhouse Kuantan

	TN	NH_4^+-N	BOD	COD
Influent (mg/L)	63	58	29.75	3840
Effluent (mg/L)	51	47	27.05	670
Removal Efficiency (%)	19.05	18.96	9.08	82.55

The removal efficiencies of the existing STP in Slaughterhouse Kuantan were shown in Table 4.5. TN concentration in the influent sample was 63 mg/L and it dropped to 51 mg/L in the effluent sample. The removal rate for TN was 19.05%. Besides that, NH_4^+-N concentration of the influent sample was 58 mg/L and it dropped to 47 mg/L in the effluent sample. NH_4^+-N removal rate for the existing STP was just 18.96%. Furthermore, BOD value in the influent sample was 29.75 mg/L while it came out with 27.05 mg/L in the effluent sample. The BOD removal rate for the existing STP was just 9.08%. Lastly, the COD value in the influent sample was 3840 mg/L while it was just 670 in the effluent sample. The COD removal rate of the existing STP was 82.55%.

TN and NH_4^+-N removal efficiencies were the most important parameters in determine ammoniacal nitrogen removal efficiency of a treatment system. Obviously, the ammoniacal nitrogen removal efficiency for the existing STP was at a very low rate since the TN and NH_4^+-N removal efficiencies were just 19.05% and 18.96% respectively. According to (Indah Water, 2018), wastewater treatment plants or STPs in Malaysia were not designed for ammoniacal nitrogen removal. The results in Table 4.5 were proven the statement above. Besides that, the COD removal efficiency of existing STP was reached 82.55% which indicate the high removal efficiency. However, the BOD removal efficiency of existing STP was just 9.08% which indicate an extremely poor removal efficiency. More research should be carried out to study the problem of low BOD removal efficiency in future.

Table 4.6: Comparisons between UMSR and Existing STP

Removal Rate (%)	**Sample**		
	UMSR	**STP**	**Better Performance**
TN Removal	78.28	19.05	UMSR
NH_4^+-N Removal	83.57	18.96	UMSR
BOD Removal	25.30	9.08	UMSR
COD Removal	80.59	82.55	STP

As mentioned in (Indah Water, 2018), there is no standard for ammoniacal nitrogen removal in Malaysia, therefore the contaminant performances of UMSR were compared with the existing STP in Slaughterhouse Kuantan in 4 parameters which were TN, NH_4^+-N, BOD and COD. The comparisons between removal efficiencies of two reactors were tabulate in Table 4.6. As mentioned before, TN and NH_4^+-N removal efficiencies are the main parameters for the determination of ammoniacal nitrogen removal. TN removal rate for UMSR was 78.28% which higher than 19.05% for existing STP. Besides that, UMSR had 83.57% of NH_4^+-N removal rate while existing STP had only 18.96% of removal rate. Therefore, a conclusion could be made on the UMSR had better ammoniacal nitrogen removal efficiency than the existing STP. Furthermore, UMSR had 80.59% of COD removal efficiency which a bit lower than 82.55% for the exiting STP. Both reactors had removal efficiency exceeded 80% which considered having good performance in COD removal. Lastly, UMSR had 25.30% of removal efficiency which higher than 9.08% for exiting STP in BOD removal performance. Low BOD removal efficiencies in both reactors were caused by the low BOD value in the influents. Since the influent samples for UMSR and existing STP were taken from the same aeration pond, therefore the problem might be caused by the wastewater in the aeration pond. Microbial community and water analysis should be carried out for the wastewater in aeration pond to determine reason for low BOD value in the influent samples.

CHAPTER 5

CONCLUSION

5.1 Conclusion

In recent decades, the rapid growth of livestock industry had generated large volume of highly polluted wastewater. The wastewater generated from slaughterhouse will contain a large amount of organic and inorganic pollutants which are harmful to the environment (Anijiofor and Nik Daud, 2018). NH_4^+-N in the wastewater discharged without proper treatment can lead to the eutrophication of the water bodies (Zhang *et al.*, 2018). Microaerobic treatment process has been introduced for treating wastewater with high NH_4^+-N concentration in recent decades. In the microaerobic conditions, aerobic and anaerobic bacteria are allowed to coexist in the same activated sludge reactor (Zitomer, 1998; Zhang *et al.*, 2018). Previous studies had been proved that UMSR had excellent results in treating piggery water with high NH_4^+-N and low COD/TN ratio. In present study, a prototype of UMSR was constructed for treating the slaughterhouse's wastewater with high NH_4^+-N concentration and high COD/TN ratio and compare its nitrogen removal efficiency with the existing slaughterhouse's wastewater treatment plant.

A prototype of the UMSR was constructed with 5 major elements which are the slaughterhouse wastewater tank, influent tank, reoxygenation tank, aeration tank and reactor. The reactor was controlled in the microaerobic condition which the DO within the range of 0.3 mg/L to 1.0 mg/L and pH value within the range of 7.0-8.0. The temperature varied with the room temperature. The prototype was placed behind the Environmental Lab of FKASA, UMP. The research was started with the acclimation stage which taking around 60 days. The acclimation stage was divided into 3 major phases. Influent samples and effluent samples on Day 61, Day 65 and Day 70 were taken from the prototype for analysis. Influent sample and effluent sample were taken from the aeration pond of wastewater treatment plant in Slaughterhouse Kuantan for the comparison. Removal efficiencies for the wastewater treatment plants were determined by 4 parameters which were TN, NH_4^+-N, COD and BOD.

In the phase 1 of acclimation stage, HRT of the treatment process was set as 8 hours with 3 reoxygenation processes and the prototype was exposed to the sunlight. The DO of the reactor was ranging within 6.17 to 19.84 mg/L. Phase II of the acclimation stage can be further divided into two stages which are II-a and II-b. In phase II-a, HRT of the treatment process was set as 8 hours with 3 reoxygenation processes while the prototype was kept in the dark condition. The DO concentration was ranging within 1.26 to 4.08 mg/L. In phase II-b, HRT was set as 12 hours with no reoxygenation process and the prototype was kept in dark condition. DO concentration was ranging within 1.53 to 2.32 mg/L. Lastly, HRT was set as 24 hours with no reoxygenation process and prototype was still kept in dark condition in the phase III of the acclimation stage. DO concentration was ranging within 0.39 to 1.51 mg/L.

To achieve objective 1, removal efficiencies of UMSR were analysed based on TN, NH_4^+-N, COD and BOD. TN removal efficiency for sample 1, sample 2 and sample 3 were 87.50%, 62.96% and 84.38%, respectively. The average TN removal efficiency for UMSR was 78.28%. It indicated UMSR had an excellent efficiency in nitrogen removal. Besides that, NH_4^+-N removal efficiency for 3 samples were 94.68%, 58.64% and 97.44%, respectively. The average NH_4^+-N removal rates for UMSR was 83.57%. According to the results above, it shows an excellent efficiency in nitrogen removal for UMSR. Furthermore, BOD removal efficiency for 3 samples were just 21.80%, 9.80% and 44.30%, respectively. The average BOD removal rates for UMSR was 25.30%. According to the results above, all 3 samples were showing poor performance in BOD removal efficiency. Lastly, COD removal efficiency for 3 samples were 93.33%, 63.79% and 84.64%, respectively. The average COD removal rates for UMSR was 80.59%. It had indicated that the UMSR had the excellent efficiency in the COD removal.

To achieve objective 2, the removal efficiencies of UMSR were compared with the existing STP in same parameters. The TN removal rate for UMSR was 78.28% which higher than 19.05% for existing STP. Besides that, UMSR had 83.57% of NH_4^+-N removal rate while existing STP had only 18.96% of removal rate. It can be indicated that the UMSR has better performance than the existing STP in ammoniacal nitrogen removal. The low value in TN and NH_4^+-N removal rates were indicated that the existing STP was not designed for the ammoniacal nitrogen removal as mentioned by the (Indah Water, 2018). For COD removal, UMSR had 80.59% of removal rate which a bit lower than 82.55% for exiting STP. For BOD removal, UMSR had 25.30% of removal rate which higher than 9.08% for exiting STP.

In conclusion, UMSR had good performance in nitrogen removal with the average removal rates in TN, NH_4^+-N, BOD and COD of 78.28%, 83.57%, A% and 80.59%, respectively. It had better removal performance in TN, NH_4^+-N and BOD than the existing STP. Although the COD removal rates were lower than existing STP, but it still in the acceptable range. More studies can be conducted on investigating the microbial community in the sludge floc to determine the main mechanism in the nitrogen removal treatment process.

5.2 Recommendations

Although the research had achieved the objectives that set in the beginning, but there were still some improvements can be done to improve the research achievement. There were some problems observed during the whole research period. First and foremost, there were just 3 samples taken for the analysis. Obviously, 3 samples were not enough for the data analysing. Consistent laboratory test shall be carried out every day along the research period to expand the database in order to have a more accuracy data analysing. Besides that, there was a problem may be regarding to the freshness of the raw wastewater samples. The contaminants concentration in one of the samples was comparative lower than others since the raw wastewater sample was kept in the cold room for more than 1 week. Therefore, frequent sample collection shall be done to ensure the freshness of the raw wastewater. Furthermore, the microbial community in the wastewater is one of the interesting topics to be studied in the future. Although there are a lot of researches studying about the microbial community in different treatment systems. However, it just can become the guidance for the different research in the field of wastewater treatment. This is because just a small parameter change in the reactor may come out with a totally different microbial community. Therefore, the microbial community in UMSR shall be studied to determine the microbial structure and its mechanism in nitrogen removal.

5.2.1 Consistent Laboratory Test

In the present study, there were just 3 samples taken for the analysis. 3 samples definitely not enough for the data analysis. According to (Zheng and Cui, 2012; Meng *et al.*, 2015; Meng, Li, Li, Sun, *et al.*, 2016; Zhang *et al.*, 2016, 2018), the sample should be taken every day for the analysis. Daily sample collection can help to expand the database and hence a graph can be plotted in order to get a more accurate removal efficiency. Furthermore, different results can be plotted in the same graph to show the relationship between the parameters. However, increase in number of laboratory test will come out with the increase of research cost. Therefore, a proper planning shall be done in the beginning to determine the suitable number of laboratory test based on the budget given. The more laboratory tests are conducted, the more data observed and hence stronger conclusion can be made.

5.2.2 Frequent Sample Collections for the Raw Wastewater

The prototype for present research was placed behind the Environmental Laboratory of FKASA, UMP. However, the raw wastewater source was the STP in Slaughterhouse Kuantan which located around 30 minutes apart from UMP. Large amount of wastewater was required for the research. Therefore, around 100L to 125L of raw wastewater would be taken from the aeration tank in every collection trip. 25L of raw wastewater was poured into the slaughterhouse wastewater sample tank and the rest were kept in the cold room. The wastewater was then refilled after 5 days duration. It was led to the contaminants in the raw wastewater that kept for long time were digested by the microbial activity. A more frequent sample collection should be done to ensure the freshness of the raw wastewater.

5.2.3 Microbial Community Study

Study in microbial community of the activated sludge can have a better view in observing the mechanisms of the nitrogen removal process. It is an interesting topic to be studied in the future research. There are a lot of results regarding to the microbial community study in different wastewater treatment systems. However, it just can become the guidance in different wastewater treatment study. A small parameter change will lead to a different microbial community in the activated sludge. In different phases of the reactor operation duration, the microbial community will vary with condition changes.

Investigation in microbial community can help in adjusting the controlled variables of the reactor. An easy solution may be applied to achieve the required condition. Therefore, the microbial community in UMSR shall be studied to determine the microbial structure and its mechanism in nitrogen removal.

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APPENDIX A
DATA COLLECTIONS FOR ANALYSIS

	Sample 1		Sample 2		Sample 3	
	Influnt	**Effluent**	**Influnt**	**Effluent**	**Influnt**	**Effluent**
DO_1 (mg/L)	7.24	6.99	7.32	7.44	6.40	6.920
DO_5 (mg/L)	5.13	5.34	4.98	5.33	1.05	3.92
BOD (mg/L)	10.55	8.25	11.70	10.55	26.75	14.90
COD (mg/L)	3300	220	580	210	2410	370
$NH_4^{+-}N$ (mg/L)	88.4	4.70	9.43	3.90	58.50	1.50
NO_2^--N (mg/L)	0.055	0.043	0.054	0.054	0.020	0.030
NO_3^--N (mg/L)	1.03	1.01	1.04	0.87	0.50	0.20

Table of Nitrogen Removal Data Collected from UMSR

	Sample 1	
	Influnt	**Effluent**
DO_1 (mg/L)	6.76	6.26
DO_5 (mg/L)	0.81	0.85
BOD (mg/L)	29.75	27.05
COD (mg/L)	3840	670
$NH_4^{+-}N$ (mg/L)	58	47
NO_2^--N (mg/L)	0.030	0.030
NO_3^--N (mg/L)	0.10	0.10

Table of Nitrogen Removal Data Collected from existing STP in Salughetrhouse Kuantan

APPENDIX B
DATA OF DO VARIATION IN ACCLIMATION STAGE

Day	DO in Reactor (mg/L)	Phase	Day	DO in Reactor (mg/L)	Phase
0	0		**36**	0.98	III
1	12.05	I	**37**	0.92	III
2	6.17	I	**38**	0.86	III
3	19.84	I	**39**	0.84	III
4	13.02	I	**40**	0.39	III
5	14.58	I	**41**	0.8	III
6	8.97	I	**42**	0.75	III
7	7.65	I	**43**	0.87	III
8	9.53	I	**44**	0.88	III
9	11.05	I	**45**	0.76	III
10	6.39	I	**46**	0.34	III
11	7.44	I	**47**	0.67	III
12	6.84	I	**48**	0.32	III
13	7.06	I	**49**	0.67	III
14	8.24	I	**50**	0.96	III
15	3.21	II-a	**51**	0.54	III
16	1.26	II-a	**52**	0.45	III
17	3.43	II-a	**53**	0.78	III
18	2.49	II-a	**54**	0.53	III
19	4.08	II-a	**55**	0.62	III
20	3.9	II-a	**56**	0.51	III
21	3.66	II-a	**57**	0.35	III
22	3.17	II-a	**58**	0.71	III
23	3.17	II-a	**59**	0.59	III
24	2.32	II-b	**60**	0.46	III
25	1.89	II-b	**61**	0.52	III
26	1.09	II-b	**62**	0.88	III
27	1.23	II-b	**63**	0.61	III
28	1.45	II-b	**64**	0.54	III
29	1.55	II-b	**65**	0.85	III
30	1.58	II-b	**66**	0.53	III
31	1.53	II-b	**67**	0.7	III
32	1.52	II-b	**68**	0.45	III
33	1.51	III	**69**	0.64	III
34	1.36	III	**70**	0.43	III
35	1.21	III			

APPENDIX C
REPORT OF TN CONCENTRATION FROM CENTRAL LAB, UMP

Attached at behind.

APPENDIX D
APPROVAL LETTER FROM DEPARTMENT OF VETERINARY SERVICES

Attached at bebind.