MICROBIAL POWERED DOWN-FLOW HANGING SPONGE FOR NUTRIENT REMOVAL IN SEWAGE TREATMENT

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A project report submitted in partial fulfilment of the
requirements for the award of Bachelor of Engineering
(Honours) Chemical Engineering

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DECLARATION

I hereby declare that this project report is based on my original work except for citations and quotations which have been duly acknowledged. I also declare that it has not been previously and concurrently submitted for any other degree or award at UTAR or other institutions.

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APPROVAL FOR SUBMISSION

I certify that this project report entitled **"MICROBIAL POWERED DOWN-FLOW HANGING SPONGE FOR NUTRIENT REMOVAL IN SEWAGE TREATMENT"** was prepared by **TAN WEI YANG** has met the required standard for submission in partial fulfilment of the requirements for the award of Bachelor of Engineering (Honours) Chemical Engineering at Universiti Tunku Abdul Rahman.

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ABSTRACT

Significant water pollution has been the great concern to the public due to the discharge of untreated or poor treated wastewater to the environment. Nutrients, particularly phosphorus and nitrogen, are the pollutants that causes the eutrophication and damage of ecosystem due to their enrichment effect. Conventional wastewater treatment plants in Malaysia are ineffective in nutrient removal due to the sewage discharge regulation was only enforced after year 2009 and only applicable to new plant that build thereafter. This research was conducted to design a Down-Flow Hanging Sponge (DHS) system at low cost as a post-treatment of the conventional wastewater treatment plant to remove nutrient. The sewage used in this study was synthetized by using the laboratory chemicals. During the start-up period (Phase 1), the working volume of the DHS reactor was set at 1.5 L per day and was increased to 2 L per day during the commencement of parameter study (Phase 2). Throughout the operation of Phase 1, the DHS reactor showed a simultaneous nitrogen and phosphorus removal. However, negative phosphorus removal was observed due to inadequate operating condition coupled with the enhanced biological pohosphorus removal (EBPR) process carried out by PAOs. Positive phosphorus removal efficiency was then observed during Phase 2 of the study (3.42 – 14.27 %), which was attributed to the proper operating condition that discovered from Phase 1. On the other hand, the removal of nitrogen was much significant than the phophorus removal with nitrification efficiency of 6.91 – 39.43 % in Phase 1 and 2.39 – 21.72 % in Phase 2. The following denitrification efficiency remained high at 20.62 – 98.56 % and 4.39 – 97.42 % at Phase 1 and 2 respectively due to the presence of anaerobic environment at the depth of 0.75 cm from the sponge surface. In addition, the DHS reactor performed well on the removal of COD with removal percentage of more than 90 % at both phases. The sponge matrix with small pores in it acted as a filter to retain the organic matters that flow through it and the high sludge retention time characteristic of the DHS reactor allowed sufficient time for the degradation of organic matters. Therefore, no additional sludge treatment is needed downstream of DHS reactor. Throughout the study, the operating condition or DHS environment had favoured the growth of nitrifiers and denitrifiers, which result in significant nitrogen removal. The removal of phosphorus was suppressed due to the presence of anaerobic zone and accumulation of extracellular polymeric substances (EPSs) that restrict the penetration of air into the system. In short, introduction of DHS reactor in conventional sewage treatment for nutrient removal is recommended because the system is able to remove both nutrient and COD. Moreover, it is cost effective and required only small area to build.

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ATU	allylthiourea
DHS	down-flow hanging sponge
DPAOs	denitrifying phosphorus accumulating organisms
EPSs	extracellular polymeric substances
HRT	hydraulic retention time
IST	individual septic tank
OLR	organic loading rate
PAOs	phosphorus accumulating organisms
PHB	polyhydroxy-butyrate
SCFAs	short chain fatty acids
SPS	sponge pore size
SRT	sludge resident time
T-BOD	total oxygen demand
T-COD	total chemical oxygen demand
T-N	total nitrogen
UASB	upflow anaerobic sludge blanket
VSS	volatile suspended solids

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CHAPTER 1

INTRODUCTION

1.1 Global Water Pollution Issue

Water is essential for human to stay alive. Thus, the ancient settlements were always near to water sources. Unfortunately, the amount of clean water was reducing along with the technology's advancement during mid-19th century, which was the beginning of industrial revolution. The production of consumer goods in various industries during the industrial revolution generated large number of by-products, which became water contaminants when discharge into water bodies. Along with the increase in world population, the water pollution issue is worsened due to the wastes dispose from households. The polluted water discharge from industrial activities and households are known as wastewater and sewage respectively. According to the Surfer Today (2019), 80 % of the ocean pollution are coming from the land activities. Moreover, more than 80 % of untreated sewage is discharged and polluted the water in developing country. It is reported that 3.6 million of life is lost every year due to water pollution, which is equivalent to 10 000 people every day. The reasons of increasing water pollution crisis are due to the lack of public awareness, inadequacy of education on water pollution issue, and economic concerns.

Apart from the common water pollution caused by chemical discharge from industrial activities, nutrient is another pollutant that must be focused on. It is important to control the concentration of nutrient in wastewater before discharge into environment. Nutrient is chemicals such as nitrogen, phosphorus, carbon, calcium, sulphur, etc. that promote the growth of living things. When exceeding amount of nutrient is present in water, they allow the excessive growth of aquatic plants, particularly algae and lead to eutrophication. Eutrophication occurs when green algae starts to grow and form a thick layer on water surface (algae blooms). This phenomenon is considered as water pollution as the algae limits the light penetration into water and the dead algae will promote the growth of bacteria, which causes the competition of oxygen under water becomes critical. As a result, the dissolved oxygen in water decreases and aquatic life cannot survive. Therefore, new technology must be developed to solve the problem of excess nutrient found in the effluent of conventional wastewater treatment plant (National Ocean Service, 2019).

1.1.1 Wastewater and Sewage

Wastewater and sewage are the main causes of water pollution. Wastewater represents any used water that comprises waste products. The sources of wastewater include industrial activities, agricultural, restaurants, hospitals etc. In contrast, sewage is polluted water with suspended solid wastes such as faeces, urine and laundry waste. The polluted water usually consists of contaminants like pathogens, oxygen demanding wastes, heat, nutrient, sediments, radioactive substances, heavy metals, pesticides, and persistent organic matter (UNESCO, 2017).

Water pollution is classified into point source and non-point source. For point source, polluted water is collected in networks of pipe or channels before discharge. Examples of point source are sewage treatment plant and industrial discharges. On the other hand, non-point source is usually more intermittent and characterize by many points of entry to the receiving water such as runoff from agricultural lands and small construction site.

1.1.2 Sewage Treatment Technology

Sewage treatment is a process of removing contaminants and converting the sewage into cleaner water, which is safe to discharge into the environment or use directly. The technology used in sewage treatment can be classified into three major groups, namely physical, chemical and biological treatment. sewage that contains large amount of dissolved or suspended solid can be treated by physical process. The suspended materials are removed as sewage flows through filter media and screening devices, or they can be removed by gravity settling. Next, chemical process is capable to remove small particles that are too small to settle by gravity and cannot remove by simple filtration. For instance, coagulation and flocculation make use of some chemical reactions to get rid of small size particles. In addition, chemical process like chlorination is useful for disinfection and it decelerates the rate of decomposition of treated water. Physical and chemical treatments are usually employed together to improve the performance of wastewater treatment system.

In biological treatment, microorganisms (primarily bacteria) are used to convert the biodegradable wastes into simple substances. Moreover, biological treatment is further subdivided into aerobic and anaerobic conditions, depending on the availability of dissolved oxygen (Toprak Wastewater Engineering, 2006).

1.1.3 Practices in Malaysia

Over the years, the sewage treatment plants in Malaysia continue to practice the treatment technologies that established in the old day. There is no new invention or breakthrough in the sewage treatment technology to solve the increasingly serious water pollution problem. The conventional sewage treatment process comprises of three major phases: primary, secondary and tertiary. The first two phases are practiced by Indah Water Konsortium, a national sewerage company in Malaysia. The main role of primary stage is to remove the suspended solids, while secondary stage is responsible for the removal of organic matter in the wastewater. Furthermore, the nutrient and toxic substances in the sewage are removed through tertiary treatment (Lai and Issabeyeva, 2016).

Unfortunately, tertiary treatment is not practiced in Malaysia sewage treatment plant. In the old regulation for sewage discharge that established before January 1999, there is no nutrient discharge limit. Therefore, this results in neither the largest sewerage services authority nor the other public treatment plants in Malaysia were designed for nutrient removal (Indah Water, 2019). There is no facility for nutrient removal in the treatment plant that binds on old regulation. However, due to the increasing water pollution issue arises from the sewage discharge, the regulation is revised. The Environment Quality (Sewage) Regulations 2009 had revised to make phosphorus removal as part of the sewage discharge standard, but this regulation is only enforced on new sewage treatment plant that built after the effective date of the regulation (Department of Environment, 2010). Therefore, tertiary treatment becomes vital for sewage treatment as the available sewage treatment systems are not able to cope with the freshwater demand due to fast growth of population.

According to the Environmental Quality (Sewage) Regulations 2009, the phosphorus concentration in standard effluent that discharge by the sewage treatment system into a stagnant water body must be below 5 mg/L and 10 mg/L for Standard A and B respectively. Standard A water is allowed to release into any inland waters listed in the Third Schedule, while Standard B water can discharge to the remaining inland waters or Malaysian waters.

1.1.4 Down-Flow Hanging Sponge

Down-flow hanging sponge (DHS) is a system that has similar principle to the trickling filter. It was developed as aerobic post treatment of the upflow anaerobic sludge blanket (UASB) by Professor Hideki Harada in Japan. The wastewater will enter the system at the top and trickles down through the supporting media by gravitational forces. The main difference between DHS and trickling filter is DHS uses sponge (polyurethane foam) as supporting media instead of the conventional rock. The highly porous sponge becomes excellent site for the attachment and growth of biomass. The microorganism presence in the sponge will contact with the down coming wastewater and effectively oxidize the biodegradable organic wastes. The sponge packing inside DHS can be of various shapes with different arrangement and different configurations have their own pros and cons.

The advantages of DHS over conventional biological sewage treatment are low cost, energy effective, environmentally friendly, and simple construction. Recently, the interest in DHS for wastewater treatment has emerging due to its high efficiency over traditional suspended and attached growth treatment (Nurmiyanto and Ohashi, 2019). It was believed that the DHS system is capable to remove nutrient from wastewater because the sponge media support in the system can create both aerobic and anaerobic environments that are important for decomposition of nutrient.

1.2 Problem Statement

Water quality has become a great global challenge for societies in this 21st century. It is threatening multiple aspects include social, economic and environmental. Newly emerging technologies have created more pollutants and reduced the availability of freshwater. In facts, human is accessing less than 1 % of the Earth’s clean water and consumption of untreated or partially treated water kills more life than the war. Generally, the most common problem with water quality is high nutrient load that result in eutrophication. The examples of nutrient are nitrogen and phosphorus which originated from food processing industries, livestock manure, surface runoff from agricultural activities and runoff from sewage treatment plant.

In Malaysia, the main sources of water pollution are from sewage, tin mining, palm oil and natural rubber industries. Due to the increase in freshwater demand pressure, Malaysia is focusing in sewerage services to deal with the pollution arises from household wastewater. However, the existing sewerage treatment plants are only equipped with primary and secondary treatment, not the removal of nutrient in the wastewater. Although new regulation has been established in 2009 by taking phosphorus removal into account for sewage treatment plant, but this regulation is only enforced on new plant that built thereafter.

As discussed in Section 1.1.4, DHS is an excellent system over traditional attached and suspended growth treatment to remove nutrient from wastewater. Study has shown that, the DHS is capable to reduce phosphorus and nitrogen concentration in treated effluent. However, DHS is a newly developed technology where only few plants are currently practicing in the world. In order to establish DHS system in Malaysia, extensive research has to be carried out due to the minimal information on this fresh technology. Moreover, the existing plants' information are only served as reference as the operating conditions in other countries may not applicable to Malaysia due to difference in climate and handling capacity. In addition, the arrangement and configurations of sponge on DHS performance is another topic to be focusing on.

1.3 Aim and Objectives

Incorporation of proper designed DHS with nutrient removal ability in current wastewater or sewage treatment plant in Malaysia is vital to overcome the water pollution issue. However, DHS system is a newly-developed technology and most of the existing plant are practicing overseas. The operating condition of the DHS system will vary depending on the sewage treatment plant location. For instance, the weather, handling capacity and content of wastewater may vary from one location to another. Therefore, extensive research is needed to identify the optimum operating condition of DHS system that suit for local operation. There are many factors that will affect the performance of DHS system, which include hydraulic retention time (HRT), sludge resident time (SRT), organic loading rate (OLR), sponge pore size (SPS), ventilation and accumulation of biomass in the sponge. The objectives of this project are:

(i) To design a laboratory-scale DHS system that can incorporate in local sewage treatment plant.
(ii) To establish DHS system for nutrient removal in sewage treatment.
(iii) To study the factors that affecting the performance of nutrient removal in DHS system.

1.4 Scope and Limitation of the Study

The first step in this study is to select the generation of DHS system to be used. The third generation of DHS system is selected as the investigation target and some modification has been made on it. Originally, the sponge medium is randomly packed inside the third generation DHS. It is suggested to place the sponge inside a supporting medium before pack in the DHS reactor. The purpose of the supporting medium is to enhance the air transfer inside the system as well as to increase the mechanical strength of the sponge media to prevent deformation. Secondly, it is to investigate the ability of DHS system in the removal of nutrient. Studies have shown that DHS system is capable to remove various pollutants in wastewater such as biochemical oxygen demanding waste, chemical oxygen demanding waste, total nitrogen, nutrient, suspended solids, etc. Nevertheless, the focus of this study is on the nutrient removal of DHS system. The removal of nutrient is important to prevent the deterioration of water quality due to eutrophication. Moreover, there are various factors that tend to affect the efficiency of DHS such as HRT, SRT, OLR, SPS, ventilation and configuration of DHS reactor. The factor being study in this project is the configuration DHS reactor.

The main limitation of this study is the lack of suitable information on operating conditions. Since most of the research is carried out in temperate countries with different seasons and environment condition. Therefore, the justifications and results provided by other researchers may not be suitable for local operation. In addition, the difference in the sewage composition may also affect the performance of DHS system differently.

1.5 Contribution of the Study

This study emphasized on the use of a biological method to remove the nutrient, particularly phosphorus and nitrogen, from the sewage treatment. DHS reactor emerged as a low cost down-stream processing unit after the UASB reactor, which it exhibited high performance in the removal of organics and nitrogenous compounds. It is believed that by cultivating the microorganisms from the activated sludge of conventional wastewater treatment process on the sponge media of DHS reactor, the phosphorus and nitrogen in the sewage can be removed by the phosphorus accumulating organisms (PAOs) and nitrifying and denitrifying bacteria, respectively. The highly porous sponge media provides a vast surface for the attachment of microbes and it allows air to transfer into the system without the need of external aeration. On the other hand, the sponge media promotes a longer retention time of sewage and sludge, hence, the DHS system can achieve high removal of nutrient and minimize the generation of excess sludge. The main focus of this experiment is to study the ability of DHS reactor on nutrient removal for sewage treatment. Once confirmed its nutrient removal capability, the DHS reactor can be simply installed at the downstream of conventional sewage treatment plant to remove the nutrient. The construction of DHS reactor is way cheaper and easier as compared to the conventional biological treatment process.

1.6 Outline of the Report

There were five chapters in this report which included introduction, literature review, methodology and work plan, results and discussion, as well as conclusion and recommendation.

First, Chapter 1 provides a brief background on the global water pollution issue such as the sources of pollutants and their effects to the water body. The wastewater treatment technologies available nowadays and the water treatment practices in Malaysia were also studied. The problem statement pointed out the limitations facing by conventional sewage treatment plant in Malaysia and the remedy for this situation was introduced, which is the DHS reactor. Therefore, the aim of this study was to overcome the challenges encountered through the development of DHS reactor as a biological method to remove nutrient from sewage.

In Chapter 2, eutrophication that caused by excess nutrient in the water body was discussed in details. The classification and standard of water quality were also studied to acknowledge the usage and discharge limit on different type of water. Besides, the practices and regulations on sewage treatment in Malaysia were also studied to identify the problem that lead to water pollution by nutrient. The pros and cons of current technologies to remove the nutrient from water were discussed together with the mechanisms involved during the treatment process. On the other hand, the existing researches on DHS reactor was reviewed to provide information for the design phase of this study. In addition, the factors that will affect the performance of DHS reactor were studied.

Next, the work plans and experimental procedures were stated in Chapter 3. The selection of DHS design was carried out and a draft of schematic diagram on DHS reactor set-up was constructed. The experimental procedures on synthetic wastewater preparation and cultivation of microorganisms were written in details. Besides, the instruments or techniques used to monitor the performance of DHS reactor were introduced. The details of working principles and samples preparation steps were also included.

In Chapter 4, a more detailed explanation on the DHS system design had been included. This is because one of the objectives of this study was to design a laboratory-scale DHS reactor that can be incorporated in local sewage treatment plant for the removal of nutrient.

The following Chapter 5 is focusing on the establishment of DHS reactor for nutrient removal in sewage treatment. The performances of DHS reactor on chemical oxygen demand (COD), phosphorus and nitrogen removal were discussed.

Furthermore, the effect of phosphorus concentration on DHS reactor removal efficiency was also studied during the Phase 2 of this experiment and the discussion was included in Chapter 6. The phosphorus concentration was reduced to lower level as compared to that in Phase 1 (establishment phase) and the performance of the DHS reactor was closely monitored.

Lastly, Chapter 7 summarized the conclusions of this study based on the findings and results obtained in Chapter 4, 5 and 6. Besides, recommendations for future work were also included to provide useful information for research work.

CHAPTER 2

LITERATURE REVIEW

2.1 Water Pollution and Water Crisis

Access to sufficient quantity and quality of water are the fundamental human rights. Water is important to achieve sustainable development in various dimensions include health, food security and poverty reduction. Moreover, the water-related ecosystems that are essential to human beings such as providing natural purification, transportation, flood protection, irrigation, etc. are also strongly dependent on the availability of water. However, urbanization and population growth had overwhelmed nature's ability to supply the fresh water. Water is very vulnerable to pollution due to its "universal solvent" properties. It allows more substances to dissolve in it than any other liquid in this world. Therefore, the pollutants produced from sources such as surface runoff and factory discharge are readily to dissolve and mix with water, which result in water pollution. The pollutants that released into the water bodies will interfere with the natural functioning of ecosystems and the beneficial use of water. According to Helmer and Hespanhol (1997), the standard statistical classification of water quality for the maintenance of aquatic life was listed in Table 2.1. Moreover, the classes of water and their respective uses were summarized in Table 2.2.

Table 2.1: ECE Standard Statistical Classification of Water Quality (Helmer and Hespanhol, 1997).

Variables	**Class I**	**Class II**	**Class III**	**Class IV**	**Class V**
Oxygen Regime					
Epilimnion (stratified waters) DO (%)	90-110	70-90 or 110-120	50-70 or 120-130	30-50 or 130-150	< 30 or > 150
Hypolimnion (stratified waters) DO (%)	70-90	50-70	30-50	10-30	< 10

Table 2.1 (Continued)

Unstratified waters DO (%)	70-90	50-70 or 110-120	30-50 or 120-130	10-30 or 130-150	< 10 or > 150
DO (mg/L)	> 7	6-7	4-6	3-4	< 3
COD-Mn (mg O_2/L)	< 3	3-10	10-20	20-30	> 30
COD-Cr (mg O_2/L)	-	-	-	-	-
Eutrophication					
Total P (μg/L)	< 10 (< 15)	10-25 (15-40)	25-50 (40-75)	50-125 (73-190)	> 125 (> 190)
Total N (μg/L)	< 300	300-750	750-1500	1500-2500	> 2500
Chlorophyll a (μg/L)	< 2.5 (< 4)	2.5-10 (4-15)	10-30 (15-45)	30-110 (45-165)	> 110 (> 165)
Acidification:					
pH[2]	6.5-9.0	6.3-6.5	6.0-6.3	5.3-6.0	< 5.3
Alkalinity (mg $CaCO_3$/L)	> 200	100-200	20-100	1020	< 10
Metals:					
Aluminium (μg/L; pH 6.5)	< 1.6	1.6-3.2	3.2-5	5-75	> 75
Arsenic (μg/L)[3]	< 10	10-100	100-190	190-360	> 360
Cadmium (μg/L)[4]	< 0.07	0.07-0.53	0.53-1.1	1.1-3.9	> 3.9
Chromium (μg/L)[3]	< 1	1-6	6-11	11-16	> 16
Copper (μg/L)[4]	< 2	2-7	7-12	12-18	> 18

Note: Value in bracket represents the standard for flowing waters

Table 2.2: Water Classes and Their Uses.

Class		**Uses**
I		Conservation of natural environment. No treatment is needed for water supply. Suitable for very sensitive aquatic species.
II	**A**	Conventional treatment is required for water supply. Suitable for sensitive aquatic species.
	B	Recreational use body contact.
III		Extensive treatment is required for water supply. Suitable for common, economic value and tolerant species; livestock drinking
IV		Irrigation.
V		None of the above.

Water can be polluted by various substances, including putrescible organic wastes, toxic chemicals, radioactive substances, plant nutrient, pathogenic microorganisms, sediments, petroleum, and heat. The main sources of water pollutions are coming from the waste produced by human activities and the limitations in the handling of domestic sewage. The polluted water bodies will contain unacceptable amount of the substances that used to measure the water quality (those listed in Table 2.1) and this water will have adverse effects on human health when consumed. Moreover, the amount of dissolved oxygen in the polluted water will reduce drastically due to the decomposition of organic wastes that uptake high amount of oxygen in water. According to Lenntech (2017), the concentration of dissolved oxygen in water must be maintained above 5 mg/L to prevent from putting aquatic life in stress. However, this requirement will never achieve in a polluted water. Next, the major substances in domestic sewage is nutrient, which consist of mainly nitrates and phosphates. The untreated or poor-treated sewage that contain high concentration of nutrient will result in a phenomenon known as eutrophication.

When excess nutrient is present in water, the nitrates and phosphates will act as growing factors that promote the growth of algae. The algae can rapidly grow into a dense layer that cover up the water surfaces and this is known as algae blooms. The growth of algae will compete the dissolved oxygen in water with other aquatic life such as fishes and underwater flora. Moreover, the coverage of algae on the water surface can prevent the sunlight from penetrating into the water, thereby impede the oxygen production by underwater plants through photosynthesis. Furthermore, the availability of dissolved oxygen in the water become more critical when the algae die. Oxygen is used extensively by the microorganisms when they are digesting the dead algae during decomposition process. In addition, methane and hydrogen sulphide gases that are harmful to aerobic life are being produced during the anaerobic decomposition of organic wastes. During eutrophication process, the water body changes from a clean and healthy state with low concentration of dissolved nutrient to a nutrient-rich and algae-filled conditions with limited oxygen concentration that fall below healthy level. Naturally, eutrophication is a slow and inevitable process. However, the water pollution arises from human activities can accelerate the eutrophication process and result in premature aging and death of water body (Nathanson, 2019).

As mentioned above, the culprit of excess nutrient discharge into water bodies are poor-treated or untreated sewage. According to Department of Economics and Social Affairs (2019) in United Nations, untreated sewage from households had degrades the overall water quality. It was estimated that twenty-two out of seventy-nine high- and middle-income nations have less than 50 % of their household sewage being treated safely. The statistics also reported that only 18 % of the sewage are treated on-site, while the remaining are treated by households with their own sewer connection. In the past, the old sewage treatment plants were mainly designed to treat organic wastes for the removal of COD and BOD, but not for the removal of nutrient. Therefore, poor-treated sewage with excess nutrient is being discharged from these plants, as their design could not cope with nutrient removal. The condition is worsened in some developing countries such as India, which they do not even have enough sewage treatment plants to cater for the whole country. As a result, the sewage from households are left untreated and discharge directly into the water.

The same condition is happening in Malaysia. The existing sewage treatment plants in Malaysia that permitted after January 1999 have no discharge standard on phosphorus and nitrates. This is because there was no regulation being set on the removal of nutrient before 2009. Until year 2009, only the Environmental Quality (Sewage) Regulations are being amended to take the removal of nutrient into account for the new sewage treatment plant that build thereafter (Department of Environment, 2010).

2.2 Sewage Treatment in Malaysia

In Malaysia, the sewage is characterized base on its physical, chemical and biological contents. The main constituents in physical properties include colour, temperature, solids and odour. The chemical properties are further classified into organic: surfactants, oil and grease, fats, proteins, carbohydrates; inorganic: pH, chlorides, sulphur, phosphorus, nitrogen; and gases: oxygen, methane, hydrogen sulphide. There are three major methods for sewage treatment, which include physical, chemical and biology processes. Physical processes involve the employment of physical forces to separate pollutants from the sewage through processes such as screening, sedimentation, flocculation, filtration, flotation and mixing. In chemical processes, chemicals are added to remove the pollutants through chemical reactions such as adsorption, precipitation and disinfection. In the case of biological processes like oxidation pond, the pollutants are converted into gases and smaller form of wastes through the use of microorganisms. The wastes are then removed by settling (Indah Water, 2019).

When the sewage first reaches the sewage treatment plant, it will go through preliminary treatment such as screening and grinding to remove large substances that tend to cause operational problems. Moreover, flotation is used to remove the oil and grease float on sewage. Next, the screening and gravitational settling in primary treatment will get rid of some of the organic matter and suspended solids. The effluent from primary treatment that contains high amount of organic matters will then treat in secondary treatment. Biological unit operations are being used to remove the remaining suspended solids and the biodegradable organic substances. Disinfection may be part of the secondary treatment if needed. Lastly, tertiary treatment is responsible to remove the nutrient and toxic substances in the sewage. The organic matters and suspended solids are further removed in tertiary treatment to produce a high standard water that can be discharged. However, the sewage treatment plants in Malaysia are focusing to provide a basic standard of all stages of treatment, except tertiary treatment. Therefore, the discharge of water is high in nutrient concentration (Indah Water, 2019).

In Malaysia, the practices of sewage treatment involving the use of primary systems that include Imhoff tanks and communal septic tanks, as well as the secondary systems such as oxidation ponds. Moreover, Individual Septic Tank (IST) is adopted in large urban areas to treat the sewage, which estimated over one million IST is being used. Sewage is only partially treated in these ISTs and the effluent is still rich in nutrient and organic materials, which are potential causes of water pollutions, particularly in urban areas. Besides, about 38 % of the public sewage treatment plants in Malaysia are classified under mechanical plants that utilize mechanical equipment to accelerate the decomposition of sewage. Indah Water Konsortium is responsible for the rationalizing and organization of public sewerage facilities to eliminate the use of "multipoint concept", instead of standardize the types of plants used to ensure efficient and equal quality of sewage treatment. However, there are many issues associated with the operation of sewage treatment plant. For instance, high operating cost, required large area for construction as well as sludge treatment (for treatment involving biological systems). Among those, disposal of the sludge is an important issue as the treatment of excess sludge are expensive and there are limited disposal fields due to public perception. Hence, the latest water treatment technology has started to shift from conventional activated sludge process that will generate high amount sludge into anaerobic treatment with relative low sludge production. The future sewage treatment plant in Malaysia will be mechanical-based such as rotating biological contactors, sequenced batch reactors, extended aeration, and trickling filters (Indah Water, 2019).

2.3 Malaysia's Sewage Treatment Regulation

The government of Malaysia has established legal arrangements to overcome the water pollution issue that is getting serious as day goes by. Starting from October 2009, any new source of sewage, industrial discharges and leachate discharges or releases must be notified to the Director General. Without prior written notification, no person shall carry out activities that would generate new source of waste. Moreover, discharge of new effluent is also prohibited.

Furthermore, the discharge of sewage and industrial effluent as well as leachate must comply with their respective standards.

Under the previous Environmental Quality Regulations, the effluent from the sewage treatment, industrial discharge and leachate in landfill are excluded of nutrient concentration. By looking at the regulations that set for existing sewage treatment plant before January 1999, discharge standard was only made on the ammoniacal nitrogen with extremely high limit (ranged from 60 – 100 mg/L). Next, for the existing sewage treatment plant that enforced after January 1999, the regulations were again included only ammoniacal nitrogen but with lower discharge limit, which was 50 mg/L. Nonetheless, owing to the fast growth of population and urbanization, the issue of nutrient pollution had arisen. Therefore, a new regulation has been set under Environmental Quality (Sewage) Regulations 2009 whereby the nutrient (primarily nitrogen and phosphorus) in the effluent of sewage must fall below the established standards. However, there is no requirement on nutrient control for industrial effluent and leachate discharge (Department of Environment, 2010). Table 2.3 to 2.5 below show the standards of water discharge from sewage treatment plant before and after the amendment of Environmental Quality Regulations (Department of Environment, 2010). This information can also be obtained from Appendix A.

Table 2.3: Existing Sewage Treatment System that Approved Before January 1999 (Department of Environment, 2010).

Parameter	**Type of Sewage Treatment System**									
	Communal Septic Tank		**Imhoff Tank**		**Aerated Lagoon**		**Oxidation Pond**		**Mechanical System**	
	A	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**
BOD_5 at 20 ℃ (mg/L)	200	200	175	175	100	100	120	120	60	60
COD (mg/L)	-	-	-	-	300	300	360	360	180	240
Suspended Solids (mg/L)	180	180	150	150	120	120	150	150	100	120

Table 2.3 (Continued)

Oil and Grease (mg/L)	-	-	-	-	-	-	-	-	20	20
Ammoniacal Nitrogen (mg/L)	-	-	100	100	80	80	70	70	60	60

Table 2.4: Existing Sewage Treatment System that Approved After January 1999 (Department of Environment, 2010).

Parameter	**Unit**	**Standard**	
		A	**B**
BOD_5 at 20 ℃	mg/L	20	50
COD	mg/L	120	200
Suspended Solids	mg/L	50	100
Oil and Grease	mg/L	20	20
Ammoniacal Nitrogen	mg/L	50	50

Table 2.5: New Sewage Treatment System that Approved After October 2009 (Department of Environment, 2010).

Parameter	**Unit**	**Standard**	
		A	**B**
Temperature	℃	40	40
pH Value	-	6.0 - 9.0	5.5 - 9.0
BOD_5 at 20 ℃	mg/L	20	50
COD	mg/L	120	200
Suspended Solids	mg/L	50	100
Oil and Grease	mg/L	5.0	10.0
Ammoniacal Nitrogen (enclosed water body)	mg/L	5.0	5.0
Ammoniacal Nitrogen (river)	mg/L	10.0	20.0
Nitrate – Nitrogen (river)	mg/L	20.0	50.0
Nitrate – Nitrogen (enclosed water body)	mg/L	10.0	10.0
Phosphorus (enclosed water body)	mg/L	5.0	10.0

In Table 2.3 to 2.5, Standard A represents the treated water that can be discharged into any inland waters within catchment areas that listed in the Third Schedule of Environmental Quality Regulations 2009. In contrast, Standard B water is allowed to discharge to any other Malaysian waters or inland waters.

2.4 Phosphorus and Nitrogen Removal

Phosphorus in wastewater present as orthophosphate that can form dihydrogen phosphate ($H_2PO_4{}^-$), hydrogen phosphate ($HPO_4{}^{2-}$), and phosphate ($PO_4{}^{3-}$) through the loss of hydrogen ions. Phosphorus can be removed by two basics which are chemical and biological processes. The conventional phosphorus removal involves the incorporation of phosphorus into suspended solids and subsequently remove by sedimentation or filtration. Chemical precipitation aims at removing the inorganic phosphate with the use of coagulant such as lime ($Ca(OH)_2$) and alum ($Al_2(SO_4)_3$). When the pH value of sewage increases above 10, the excess calcium ions in lime will precipitate phosphate to form hydroxylapatite. The removal of phosphorus using lime is very much depending on the alkalinity of sewage. Therefore, the subsequent treated water is high in pH and must go through neutralization process such as recarbonation with the use of carbon dioxide (CO_2) to bring the pH down to neutral before disposal. In the case of alum coagulant, the aluminium ions will bind to phosphate and form the precipitate called aluminium phosphate. Alum coagulant is sometime added in the aeration tank during activated sludge process in secondary treatment, thus eliminating the need of additional clarifier. However, high dosage of alum coagulant in aeration tank can bring adverse effects on the microbial population in activated sludge process. In addition, chemical precipitation of phosphorus generates large amount of sludge (Lenntech, 1998).

On the other hand, cell biomass that containing PAOs is being used in the biological treatment for phosphorus removal. The advantage of biological process over chemical process is relatively less sludge generation. The removal of phosphorus is carried out in reactor with special configuration that promotes the growth of PAOs over other microorganisms in the cell biomass to consume phosphorus. The reactor comprises of anaerobic zone in the anaerobic tank and aerobic zone in an activated sludge tank. During the anaerobic stage, the PAOs take in fermentation products such as short chain fatty acids (SCFAs) in the sewage and store them within the cell as polyhydroxy-butyrate (PHB). The energy required for this uptake is derived from the hydrolysis of polyphosphates in cells body and the subsequent hydrolysed polyphosphates are discharge into sewage as orthophosphates. Next, the stored PHB is metabolised to produce carbon for cell growth and energy for oxidation in the aerobic stage. When the PHB storage is depleted, the PAOs will uptake the dissolved phosphorus from the sewage and store them as polyphosphates. Part of the PAOs must be wasted from the biomass to remove the stored phosphorus and ultimately dispose as sludge. Biological process requires high capital costs for construction and the process is complex yet with low phosphorus removal (Cooper, Day and Thomas, 1994).

Similarly, nitrogen in sewage may be eliminated by either chemical or biological processes. Ammonia stripping and ion exchange are examples of chemical processes to remove nitrogen in sewage. The chemical processes for nitrogen removal have not been widely adopted than biological processes due to their high costs. Moreover, the processes do not produce high-quality effluent and additional steps are required to treat the water to acceptable standard. For example, the sewage treated by ion exchange process will contain high amount of ammonium ions that has to be removed before discharge. In contrast, biological processes allow nitrogen to recycle back into the atmosphere through a natural and low costs pathway. In the removal of nitrogen, the aerobic microorganism (or simply bacteria) will first convert ammonia ($\mathrm{NH_4}^+$) to nitrate ($\mathrm{NO_3}^-$) through nitrification under aerobic conditions. After that, the anaerobic bacteria will responsible for the denitrification process where nitrate is converted to nitrogen gas ($\mathrm{N_2}$) under anoxic conditions. Usually, the detention time of nitrification is around 15 days or more and additional nutrient is required, which is methanol ($\mathrm{CH_3OH}$). Under anoxic condition, the dissolved oxygen is not available and the only source of oxygen in the water is nitrate ions. Therefore, the heterotrophic bacteria will uptake nitrate as oxygen source through the modification of their mechanism (Cooper, Day and Thomas, 1994).

To summarize, there is still some room for improvement in the available technologies for nitrogen and phosphorus removal from sewage. The major drawback of chemical processes for nutrient removal is high costs, while for the biological treatment, the removal efficiency is low and the process requires long time. These limitations had provided an opportunity for the DHS system to be incorporated in future sewage treatment process. The removal of phosphorus and nitrogen from sewage involve both aerobic and anaerobic performances of bacteria. DHS system that contains the biomass cell inoculate on the outer surface and interior surface of the sponge media can provide both aerobic and anaerobic conditions. According to Araki, et al. (1999), the study had shown that both nitrification and denitrification processes were observed on the sponge media, which correspond to the presence of aerobic and anaerobic environment. The study had proved that the surface of the sponge media was under aerobic condition up until the depth of 0.75 cm. The interior of the sponge media at the depth beyond 0.75 cm was in anoxic conditions. The study carried out by Machdar, et al. (2000) and Machdar, et al. (1997) also reported the similar conclusion as Araki, et al. (1999). Therefore, the presence of aerobic and anaerobic conditions in DHS system made it a potential system for the removal nutrient through the mechanisms discussed above. Furthermore, the sludge production is relatively low in the DHS system. Lastly, the DHS system is a simple process with low construction cost as compared to the conventional biological and chemical processes for nutrient removal.

2.5 DHS Process for Sewage Treatment

DHS system is an aerobic biological treatment analogous to trickling filter. Inside a DHS reactor, there are packing made up of polyurethane sponge with the presence of activated sludge. The microorganisms or bacteria that are responsible to decompose the organic wastes are populated on the surface and within the pores of sponge. High porosity sponge provides enormous surface area for the attachment of bacteria. Moreover, the high porosity characteristic enhances the contact between sewage that flowing down from the top of reactor and bacteria, which is important for the treatment process. The sponge can be packed into different configurations, which will pose different removal efficiency on the sewage. The newly developed DHS is a potential alternative treatment system to conventional treatment technologies with the advantages of lower cost and high removal efficiency.

Currently, there are six types of DHS configuration have been developed, which correspond to first-generation DHS to sixth-generation DHS. The emergence of DHS was by connecting a series of polyurethane sponge cube with side length of 1.5 cm to the UASB reactor, which serve as a pre-treatment of sewage. This is known as first-generation of DHS (G1). DHS-G1 exhibited high performance in removal of organics and nitrogenous compounds, which could produce effluent with only 2 mg/L BOD. However, DHS-G1 was not compatible in full scale operation due to the low mechanical strength of nylon string that used to hold the cubes in place. Furthermore, channelling had occurred whereby the flow of wastewater on the hanging sponge was not distributed uniformly (Harada, 2008).

With regards of the drawback of DHS-G1, DHS-G2 was developed thereafter to overcome the weakness of previous generation. Triangular prism sponge in DHS-G2 had replaced the original cube shape in DHS-G1 and the G2 configuration was stick on both side of a plastic sheet forming a curtain-like arrangement. The curtain was 2 m in height and the triangular prism sponges was 3 cm on triangular sides and 75 cm in length. The DHS-G2 had successfully operated on pilot scale to treat sewage in Karnal India with handling capacity of 1 000 m^3/day. The second generation was able to achieve higher total ammonia nitrogen removal than previous, yet, the problem of wastewater distribution over the sponge is remained. In addition, the sponge media in curtain like configuration is weak in retaining biomass and causes biomass washout, which in turn reduce the process efficiency (Harada, 2008).

In the third generation DHS, the design was relatively simple. The packing medium in DHS-G3 was constructed by randomly packing the sponge units that enclosed in a net-like polypropylene plastic supporting media (2.7 cm in diameter) inside the reactor. The wastewater permeates the sponges continuously on its way down the reactor. According to Tawfik, et al. (2006), on top of the organic removal performance similar to previous generation, the DHS-G3 was capable of separating fecal coliform out from the wastewater. In contrast, the drawback of DHS-G3 was degradation of nitrification process after few month operations due to poor air transfer between sponge media that packed randomly. The subsequent treated water is having low dissolved oxygen concentration (Harada, 2008).

Next, the design of DHS-G4 was an improvement over DHS-G3 where similar plastic support media and sponge was used but longer in length. The main concept of DHS-G4 was to enhance penetration of air into wastewater and prevent clogging of reactor by minimizing the sludge accumulation. Therefore, the long sponge strips with dimensions of 2.5 cm × 2.5 cm × 50 cm were arranged in a row and stacked on top of each other to form a box module. DHS-G4 was excellent in removing total chemical oxygen demand (T-COD), total biochemical oxygen demand (T-BOD), suspended solids, and total nitrogen (T-N). The major imperfection of DHS-G4 was the biomass accumulate inside the sponge strips tend to deform the sponge module over the time due to their weight (Harada, 2008).

In year 2004, the DHS-G5 concept was developed on the foundation of DHS-G2 with multiple curtains hanged next to each other in a rectangular frame supported by a hanger assembly. This configuration was then stacked together for the operation of pilot plant with dimensions of 85 cm × 49.5 cm × 49.5 cm. The DHS-G5 exhibited comparable performance to the conventional activated sludge process. The main issues with DHS-G5 were detachment of sponge curtain and deformation of curtain holder arise from poor strength of material to support the load accumulation (Harada, 2008).

Lastly, the sixth generation of DHS was constructed in a different way from the previous generations. The medium for sponge was produced by copolymerizing polyurethane with epoxy resin. Unlike the soft-sponge type in G1 to G5, the resulting sponge medium was rigid in DHS-G6. The design of DHS-G6 can prevent the sponge deformation problem without the need of any support such as plastic case. Moreover, higher sponge surface area is exposed without the supporting medium, which increase the contact between the wastewater and biomass in sponge. The performance of DHS-G6 was comparable to all other generations. However, this design reduced the void volume in sponge and lowered the amount of biomass retained by the sponge (Nurmiyanto and Ohashi, 2019). The historical development of DHS system was illustrated in Figure 2.1.

Nowadays, there is an increasing interest in attached growth biological treatment of sewage, which the biomass is growing on the support media. Attached growth systems are favoured over conventional activated sludge process due to the advantages of lower energy consumption, minimum biomass production, and comparably shorter HRT (Nurmiyanto and Ohashi, 2019). DHS system is one of the attached growth systems for treatment of sewage. DHS is a system similar to trickling filter, but differ in its packing material. The sponge in DHS is highly porous with void space up to 90 %, which provided enormous surface area for the growth of microorganisms. Moreover, the high porosity condition ensures that the biomass concentration is retained on the internal and external of the sponge, which leads to longer SRT. Higher SRT allows sufficient time for the self-degradation of organic wastes, whereby reducing the excess sludge generation of the process. Next, sponge is an excellent media to hold wastewater and this extends the HRT. Henceforth, DHS does not require large area to provide sufficient contact time between biomass and microorganisms as compared to conventional wastewater treatment. Thus, the capital cost for DHS system is low. Furthermore, sponge packing used in DHS is made of non-biodegradable material that is abundantly available, inexpensive and cheap. Besides, study had found that both aerobic and anaerobic conditions are presence in DHS system, which favour the denitrification process. In addition, there is no aeration devices are needed in DHS although it is being aerobic. As the sponge is hung freely in the reactor without submerge in wastewater, the oxygen is free to dissolve into the wastewater when it trickles down. This phenomenon is able to maintain high level of dissolved oxygen concentration in wastewater, which exceeds the need of microorganisms. Therefore, the only energy require is for wastewater distribution pump and this made the operating cost of DHS low.

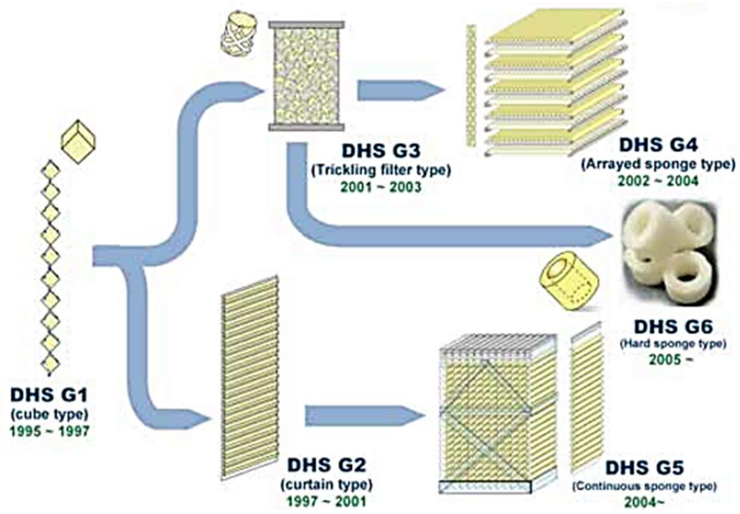

Figure 2.1: Evolution of DHS System (Harada, 2008).

2.6 Parameters Affect the Performance of DHS Reactor

The DHS reactor is a system that having similar design or operating concept as the conventional trickling filter. In trickling filter system, the microorganisms are just attached on the surface of supporting media, which they will form a slime layer and act as a biological filter to treat the wastewater. In contrast, the DHS reactor was developed to overcome the shortcoming of trickling filter. The microorganisms in the DHS reactor are retained on the surface and inside the pore of sponge media. Therefore, the highly porous sponge media provides larger surface area per unit volume for the microorganisms to attach as compared to conventional trickling filter system. On the other hand, the DHS reactor also poses high sludge retention time up to 100 days (Tawfik, et al., 2006) and consequently, produce a low amount of sludge. There are some factors affecting the performance of DHS reactor such HRT, HLR, OLR, ventilation, sponge size and sponge pore. Below is the brief discussion on each factor.

2.6.1 Hydraulic Retention Time

HRT indicates the average length of time that a compound remains in a treatment tank or unit. In other words, it is the time taken by the wastewater to pass through the DHS reactor. It is typically expressed in unit hour. HRT is an important parameter as the wastewater requires necessary period of time in the DHS reactor in order to be sufficiently treated. The greater organic removal efficiency can be achieved when the wastewater remains in the reactor for longer time; longer contact time between the microbes and wastewater. The time that the wastewater spend inside the DHS reactor is depending on the flowrate of wastewater and the porosity and volume of the sponge media used. The increase in flowrate of wastewater discharge into the DHS reactor will result in a shorter HRT. In contrast, the increase in the volume of sponge media will lead to longer HRT.

Based on the theory, the longest HRT will provide greatest removal efficiency. In other words, the nutrient in sewage can be completely removed if the HRT becomes infinity. However, it is impractical to have too long HRT in actual world especially for the sewage treatment plants that need to process extremely large amount of sewage on daily basis. Optimum HRT that is sufficient to treat the sewage to discharge standard is therefore crucial for efficient performance of DHS reactor. According to Machdar, et al. (2018); Yoochatchaval, et al. (2014); Takahashi, et al. (2011); the removal efficiency of DHS reactor increased when the HRT becomes longer. The findings of these researchers are in close agreement with the theory. The performance of DHS reactor was evaluated based on the physio-chemical parameters: COD, BOD, ammonium-nitrogen ($\mathrm{NH_4-N}$), nitrite-nitrogen ($\mathrm{NO_2-N}$), nitrate-nitrogen ($\mathrm{NO_3-N}$), TN, TSS, and volatile suspended solids (VSS). On the other hand, it was observed from the results of Machdar, et al. (2018) that the dissolved oxygen concentration in the effluent at different HRTs were similar. This finding suggested that the HRT does not much affect the diffusion of oxygen from the air into the sponge media, which eventually dissolve into the sewage. Moreover, the research of Yoochatchaval, et al. (2014) showed that the amount of sludge produced from the DHS reactor was negligible. This can be explained by the self-degradation of attached biomass or sludge in the sponge media when adequate HRT is provided.

In short, the increase in the HRT has enhanced the performance of DHS reactor in treating the wastewater. Longer HRT allows longer contact time between microorganisms and wastewater, which provides sufficient time for the decomposition of organic matters. However, optimum HRT must be obtained as too short HRT may result in poor removal efficiency, while too long HRT may lead to long operation time and reduces handling capacity at a given time. There is no such thing as the best HRT because the HRT will vary according to the situation. The examples of factor that affecting the HRT in a particular plant include handling capacity (or flowrate), type of sewage (concentration of each physio-chemical parameters), total volume of the sponge media, temperature, ventilation, etc. Therefore, the optimum HRT is only available through continuous experimental work.

2.6.2 Hydraulic Loading Rate

According to Daniel Theobald (2016), the hydraulic loading rate (HLR) in a wastewater treatment process is the volume of wastewater applied to the surface area of the process unit per unit of time. In this project, the process unit is referring to the sponge media. The increase in the surface area of sponge media or decrease in the volume of wastewater (or simply low HLR), can often improve the quality of the treated water. In other words, lower HLR results in longer HRT and could enhance the treatment efficiency.

The relationship between the removal efficiency of DHS reactor and the HLR was investigated by Mahmoud, Tawfik and El-Gohary (2010). The study had shown that the COD removal was significantly influenced by the increase in HLR during organic shock load test (a sudden increase in the flowrate). However, the overall performance of the DHS reactor was almost unaffected by the change in HLR in long term operation. During organic shock load test, the high value of COD at the DHS effluent was attributed to the slough off of attached biomass and sludge at this intense flowrate. From the study, the increase in the flowrate (HLR) of the influent tends to shorten the retention time of sewage in the DHS reactor. High value of HLR indicates shorter contact time between the microorganisms and organic matters in the wastewater. Furthermore, the findings of this study can be supported by the researches that were discussed in Section 2.6.1. The previous researches in Section 2.6.1 suggested that the performance of DHS reactor will be improved when the HRT becomes longer. As mentioned earlier, longer HRT demonstrates a lower HLR, which will lead to higher removal efficiency of the DHS reactor.

In addition, the study of Mahmoud, Tawfik and El-Gohary (2010) also showed that the nitrogen concentrations in the DHS effluent increased when HLR became higher. At the same time, the phosphorus removal efficiency was slightly improved. According to theory, the high HLR will reduce the contact time between sewage and the microorganisms, therefore, the phosphorus removal efficiency should drop instead of increased. The decreased in phosphorus concentration in the effluent may attributed to the phosphorus uptake by denitrifying bacteria during denitrification process. Besides, the phosphorus can also be removed through the adsorption of phosphorus particle on the sponge media in DHS reactor.

To conclude, higher value of HLR will drive the sewage across the sponge media at a faster rate, which means the residence time or contact time between microorganisms and the sewage is shortened. As a result, there is insufficient time for the microorganisms to completely decompose the organic matters presence in the sewage and lead to poor removal efficiency. Moreover, too high HLR may flush away the microorganisms inside the sponge media that are crucial for sewage treatment. Although the sudden increase in HLR (hydraulic shock load) will reduce the removal efficiency of DHS reactor, but it has no significant effect on long term performance of DHS reactor. Again, there is no constant value for the most feasible HLR. Different situations need to employ different HLR values in order to achieve efficient treatment.

2.6.3 Organic Loading Rate

OLR is defined as the amount of soluble and particulate organic matter being applied to the surface area of the treatment unit. It is usually expressed in the mass of BOD over unit area of the sponge media per unit time. OLR can be controlled through pre-treatment of sewage to reduce the amount of TSS and BOD concentration. It is vital to determine the appropriate OLR and study the ability of DHS reactor to react upon organic shock load to ensure the optimum performance. Organic shock load is the sudden increase in the BOD concentration of the incoming sewage.

The effect of OLR on DHS removal efficiency was studied by Tandukar, et al. (2005); Miyaoka, et al. (2017); and Nomoto, et al. (2018). All three researches came to the same conclusion that the fluctuation in OLR has insignificant effect on the overall performance of DHS reactor in long run. The removal of COD, BOD, NH_4-N and TSS in the DHS reactor were apparently stable and almost completed at different OLR values. However, all the three researches reported that there was a deterioration in NH_4-N and TSS removal during the organic shock load. When the COD (or OLR) in the influent increases, the amount of dissolved oxygen in the DHS reactor will reduce drastically. This statement is proven by the Nomoto, et al. (2018), where the dissolved oxygen on the top layer of DHS reactor was equal to zero when the OLR is high. It was proposed by the researchers that the oxygen supplied to the DHS reactor was lesser than the oxygen uptake by the microorganisms during the decomposition of organic matters at high OLR; eventually, the dissolved oxygen level was reduced. Under constant oxygen supplied rate, DHS reactor with high OLR will have a lower dissolved oxygen value than the one with low OLR as the microorganisms are consuming more oxygen to decompose the large amount of organic matters in the sewage.

During high OLR, the dissolved oxygen concentration in the DHS reactor are lower and there exists an intense competition between heterotrophic microorganisms and the denitrifying bacteria over the limited dissolved oxygen in the system. The result had clearly shown that, the denitrifying bacteria was defeated by the heterotrophic microorganisms, which result in deterioration of NH_4-N removal when OLR increased. In addition, the biosolid escape phenomenon that occurs at high loading rate may also result in low NH_4-N removal efficiency. The retained sludge that sloughed off from the sponge media as a result of hydraulic shear force will contribute to the high TSS concentration at the DHS effluent.

In a nutshell, nitrification efficiency decreased as the OLR in the influent increased due to the dissolved oxygen competition between heterotrophic bacteria and denitrifying bacteria. However, the overall efficiency of DHS reactor is not much affected by the changing of OLR. Nitrification and denitrification are taking place simultaneously in the DHS reactor. Nitrification takes place on the surface of the sponge media up to 0.75 cm depth, while denitrification exists in the anoxic zone of the sponge media (Araki, et al., 1999; Machdar, et al., 2000; Machdar, et al., 1997). The ammonia is aerobically converted to nitrate through nitrification process, thereby explaining the competition of dissolved oxygen with the heterotrophic bacteria that require oxygen during metabolism in the system. In general, the DHS reactor is able to cope with the fluctuated OLR values. However, the influent of DHS reactor still needs to undergo pre-treatment to prevent extremely high OLR entering the reactor. The OLR can be adjusted by filtering the sewage to remove large organic matters prior entering the system.

2.6.4 Sponge Size and Sponge Pore Size

Sponge size and SPS have significant effect on the quality of water being treated. The surface area of the sponge media can be maximized by reducing the sponge size and increasing the porosity. High surface area of sponge media is paramount as it provides large place for the microbial immobilisation, which is responsible for the treatment of sewage. Moreover, high surface area allows easy penetration of sewage into the sponge media and promotes the contact between microorganisms and the sewage, which could possibly improve the removal of organic matters. Other than that, small sponge size with large SPS may provide better oxygen transfer to the sewage flowing down through the reactor.

The study of sponge size on the DHS reactor removal efficiency was carried out by Uemura, et al. (2012). According to the results, all the DHS reactors with different sponge size possessed satisfactory removal of COD, ammonium nitrogen, and fecal coliform at a fixed HRT. However, there is still some difference in the removal of above parameters between different sizes of sponge media. There was a tendency for the DHS reactor with smaller sponge size to achieve slightly higher COD removal as compared to reactor with larger sponge size. Moreover, the study of Machdar, et al. (2018) and Bundy, et al. (2017) also showed similar result to the research done by Uemura, et al. (2012) when the SPS become larger. Both studies found that the removal of total COD and soluble COD in the influent decreased significantly when the SPS increased.

According to Uemura, et al. (2012) the dissolved oxygen was the highest for the DHS reactor with smallest sponge size. When the size of the sponge media becomes smaller, the specific surface area is increased and oxygen diffuses more easily into the wastewater. The removal efficiency is to certain extent depends on the amount of dissolved oxygen in sewage as some of the decomposition processes are under aerobic condition such as denitrification. In addition, the smaller sponge media provides larger surface area for the interaction between microorganisms and sewage, thereby improve the removal efficiency. Therefore, the findings of higher COD removal in reactor that employed smaller sponge size is obeyed the theory. Although reactor with larger sponge size does show a poorer COD removal, but the effluent of the reactor still gives an acceptable result. This phenomenon is due to the merits of DHS system, in which the organic substances can be adsorbed temporary onto the sponge media, hence, results in satisfactory removal efficiency.

In contrast, the study of Machdar, et al. (2018) and Bundy, et al. (2017) indicated that the SPS had no effect on the concentration of dissolved oxygen in the effluents. High dissolved oxygen concentration was achieved even with small SPS and provided a favourable environment for the removal of COD and nitrogen. However, there was a significant decrease in COD removal when the SPS increased. Similarly, the concentration of NH_4 in the effluent of DHS reactor increased with increasing SPS. This result is observed because the ability of sponge to accumulate or retain the sludge will be reduced with large SPS. The study by Chu and Wang (2011) reported that the high nitrification rates in sponge media were arise from the high amounts of biomass accumulated. Therefore, the accumulation of sludge is vital as it affects the removal efficiency of COD. On the other hand, the results also presented that the removal percentage of TSS decreased when the SPS became larger. This indicates that the sponge media with smaller SPS improve the retention of solids in sewage. The sludge is first accumulated on the surface of sponge, followed by trapping inside the pores when sewage penetrating the sponge media. Furthermore, the high amount of sludge holds by sponge media with smaller SPS effectively increased the effective volume of the sponge and help in the dispersion of water over the sponge matrix, thereby reduced the chances of sewage short-routeing. Therefore, dead zone can be avoided and all the microorganisms inside the sponge media will be used effectively to treat the sewage.

2.6.5 Ventilation

The only source of oxygen that is needed by the microorganisms in DHS reactor is obtained through the ventilation system. The differences in temperature and humidity between the air outside and inside the DHS reactor create a concentration gradient, which provides natural draft ventilation. The concentration gradient is required to generate the needed force that draw oxygen into the system.

The effects of ventilation on oxygen concentration in the 6th generation DHS reactor were investigated by Onodera, et al. (2013). It was found by the researchers that adequate natural ventilation was able to provide sufficient oxygen to the DHS reactor, whereby the oxygen concentration in the DHS reactor was comparable to that outside the reactor. The high concentration of oxygen had increased the amount of dissolved oxygen in the wastewater and enhanced the removal efficiency of T-BOD and T-N. In contrast, the oxygen content in the reactor was lower when the natural ventilation was reduced when limited ventilation was provided by closing of windows on the DHS reactor. The concentration of oxygen was much lower as compared to the ambient air throughout the reactor, especially in the first segment. The first segment of reactor has to cope with the high organic loading and thus, leads to high oxygen consumption by the bacteria that decomposing the organic matters.

Generally, the DHS reactor can be distributed into three distinct regions. The regions near to the inlet of wastewater is responsible for the removal of organic matter and nitrogenous components. Next, the middle portion is mainly used to treat the residual matter from first region, as well as some ammonia. Lastly, most of the ammonia and nitrogen oxides are eliminated in the last portion. The removal of organic matters by heterotrophic bacteria in first portion of reactor had reduced the organic loading in the following portions. Therefore, ammonia and nitrogen removal were primarily occurred in the later portions with low organic loading and high dissolved oxygen concentration, which favours the nitrification process.

To conclude, proper ventilation is required to maintain sufficient oxygen concentration inside the DHS reactor. High dissolved oxygen concentration in sewage is important to ensure good removal efficiency especially for the aerobic process: removal of ammonia and nitrogen. Moreover, adequate natural ventilation is enough to supply the needed oxygen by the microorganisms even the difference in temperature between the ambient air and reactor air is less than 1 ℃. Therefore, mechanical pumping of air into a DHS reactor is not required.

2.6.6 Summary

The performance of a DHS reactor is depending on various factors such as HRT, HLR, OLR, SPS and ventilation. Optimum HLR must be set by controlling the flowrate of influent as it is directly related to the values of HRT and OLR. Low HLR indicates a longer HRT with lower OLR, which will increase the residence time of sewage in the DHS reactor. Long residence time coupled with the high specific surface area of the sponge media (small SPS) allows the maximum contact or interaction between the microorganisms inoculated on the sponge media and the sewage. As a result, the removal efficiency of the DHS reactor increased. On the other hand, proper ventilation must be done to provide sufficient oxygen for the growth of microorganisms as well as for the use in aerobic processes such as nitrification. Table 2.6 below shows the summary of how various parameters affect the performance of a DHS reactor.

Table 2.6: Parameters Affect the Performance of DHS Reactor.

Parameter	To achieve efficient removal	Sources
Hydraulic retention time	Long hydraulic retention time allows sufficient removal of organic matters.	(Machdar, et al., 2018) (Yoochatchaval, et al., 2014) (Takahashi, et al., 2011)
Hydraulic loading rate	Low value of hydraulic loading rate allows longer contact time between the sewage and microorganisms.	(Mahmoud, et al., 2010) (Machdar, et al., 2018) (Yoochatchaval, et al., 2014) (Takahashi, et al., 2011)
Organic loading rate	Small organic loading rate allows sufficient removal of organic matters.	(Tandukar, et al., 2005) (Miyaoka, et al., 2017) (Nomoto, et al., 2018)
Sponge size and sponge pore size	Small sponge size and sponge pore size are desired to achieve large specific surface area and longer sludge retention time.	(Uemura, et al., 2012) (Machdar, et al., 2018) (Bundy, et al., 2017)
Ventilation	Adequate natural ventilation is important.	(Onodera, et al., 2013)

CHAPTER 3

METHODOLOGY AND WORK PLAN

3.1 Introduction

In this study of nutrient removal in sewage treatment using DHS, the sponge media was a vital part. Sponge media provided place for the inoculation of bacteria, which was responsible for the treatment of sewage. Different arrangement or module of sponge media will have their own advantages and disadvantages; hence, the selection of sponge module was largely depending on the operating conditions such as handling capacity, organic loading rate, height of DHS reactor, etc.

The third generation of DHS sponge module was selected to study the applicability of DHS reactor in the removal of nutrient from sewage. In third generation system, the sponge media is randomly packed inside the DHS reactor without any support. Some modifications had been made on the selected third generation of DHS sponge module to improve the mechanical strength of the sponge as well as to achieve higher surface area per unit volume of sponge. First, each sponge media was placed in an individual support material before packing all the sponge inside the DHS reactor. The introduction of support material was mimicking the configuration of sixth generation DHS, whereby epoxy resin is added to the polyurethane network to generate rigid sponge media that can resist deformation without any support material. With the support material, the sponge media were spaced away from each other, thereby increasing the available specific surface area for the attachment of bacteria as well as for the contact of sewage. At the same time, the ventilation was also improved where the air from the environment can easily diffuse deep into the sponge media as the sponges were spaced away, which supply the needed oxygen for bacteria. On the other hand, the modification was done on the arrangement of sponge media inside the DHS reactor. The sponge media were arranged with order and stacked up layer by layer instead of randomly packed as shown in the original third generation of design. This arrangement was important to prevent short-routing of the sewage. The random packing of sponge media will have the chances to drive the sewage down the DHS reactor through the path with least obstruction, thereby creating death zone. Death zone with no sewage penetration is undesired because the bacteria located in this area are not being utilized for the treatment of sewage, hence, results in poor removal efficiency.

In this study, the plastic hair roller had been selected as the ‘casing’ to support the sponge media. The plastic hair roller was selected because it is easily available in the market. Figure 3.1 below depicts a plastic hair roller.

Figure 3.1: Plastic Hair Roller.

There were many researches that utilized net ring, a type of support material with shape similar to plastic hair roller, as the support material for third generation DHS sponge media in the treatment of sewage or wastewater. The examples of research and the dimension of sponge media used were listed in Table 3.1.

Table 3.1: Researches that Employed Plastic Hair Roller as Support Material.

Research title	**Dimension of sponge media**	**Sources**
Direct treatment of high-strength soft drink wastewater using a down-flow hanging sponge reactor: performance and microbial community dynamics	Rectangle sponge unit: 3.5 cm × 3.5 cm × 3.0 cm	(Liao, et al., 2017)

Table 3.1 (Continued)

Development of a down-flow hanging sponge reactor for the treatment of low strength sewage	Cylindrical sponge unit: Diameter = 3.3 cm Length = 3.3 cm	(Yoochatchaval, et al., 2014)
A pilot-scale study on a down-flow hanging sponge reactor for septic tank sludge treatment	Cylindrical sponge unit: Diameter = 3 cm Length = 3 cm	(Machdar, et al., 2018)
Hydraulic behaviour in the downflow hanging sponge bioreactor	Cylindrical sponge unit: Diameter = 3.5 cm Length = 3 cm	(Machdar, 2016)

By using the plastic hair roller as the support material, all the advantages mentioned above can be achieved. Moreover, the horizontal hollow lines on the plastic hair roller allow the access of sewage and air to the bacteria that located deep in the pores of sponge media. In short, the plastic hair roller as a supporting material for the sponge media may enhance the performance of DHS reactor in treating sewage.

3.2 Experiment Set-up

The model of DHS reactor was constructed using a mineral water bottle with volume of 1.5 L. The mineral water bottle was placed upside down with both the top and bottom covers removed to facilitate the inlet and outlet of reactor. The DHS reactor was divided into two stages with a sewage distributor installed on the top of the first stage. The sewage distributor was made from a bowl shape plastic sheet with small holes on it to imitate the appearance of a shower head that allow even distribution of sewage. Other than that, there was a sewage reservoir in between the two stages. This sewage reservoir was serving two purposes. It acts as a stage to collect the sewage flowing down from first stage and at the same time, it plays the role of a sewage distributor to evenly disperse the sewage that flowing down to second stages. Both sewage distributor and sewage reservoir were important to maximize the control on sewage flowing to optimise the performance of DHS reactor.

Moving forward, the sponge media that seeded with microbes were trimmed and placed inside the plastic hair roller. The plastic hair roller was used as a support for the sponge media that were trimmed into rectangular shape. The plastic hair roller was filled by multiple sponge media that were thin rather than a single big sponge to prevent the extra void space between the cylindrical plastic hair roller and the rectangular sponge media. In addition, multiple thin sponge media was having the advantage of achieving higher specific surface area per unit volume than a big single sponge media. Lastly, the plastic hair rollers filled with sponge were packed vertically on the sewage distributor located at different heights in the mineral water bottle. Some overhead space was remained empty to prevent overflow of sewage. Figure 3.2 and 3.3 depict the schematic diagram of DHS reactor set-up and the plastic hair roller filled with sponge media, respectively.

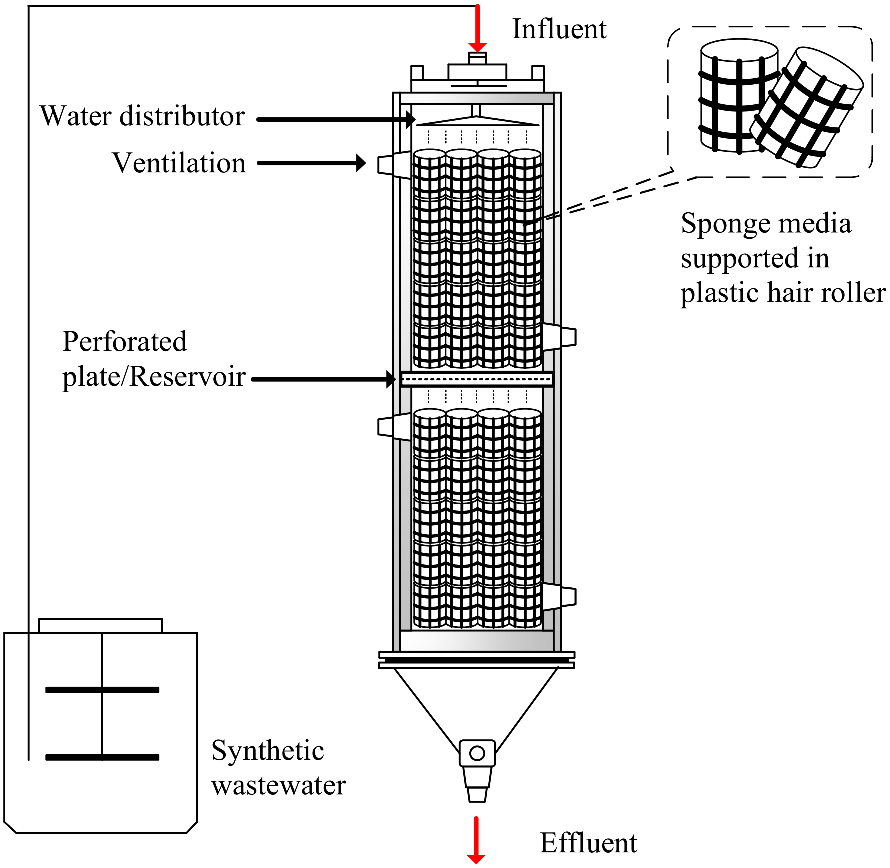

Figure 3.2: Schematic Diagram of DHS Reactor Set-up.

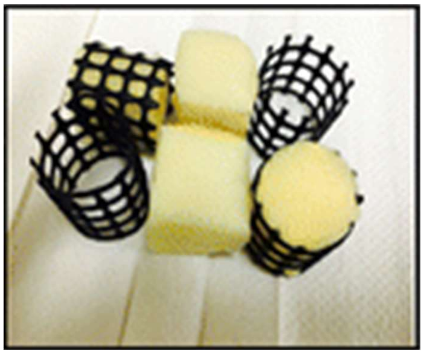

Figure 3.3: Plastic Hair Roller Filled with Sponge Media (Liao, et al., 2017).

3.3 Experimental Procedures

This study was expected to last for 50 days and would be separated into two major parts or phases. The cultivation of sponge media was carried out in the first 7 days (Phase 0) and then followed by 21 days of Phase 1. Under Phase 1, the suitable operating conditions for the nutrient removal in DHS reactor were studied. Moving forward to Phase 2, another 21 days were allocated for parameter studies. The phosphorus concentration in the influent was varied to study its effect on the DHS reactor performance. Table 3.2 below summarizes the progress of the study.

Table 3.2: Description of Activities Throughout the Study Period.

Phase	Activities
0	➢ Cultivation of activated sludge on sponge media
1	➢ Start-up the DHS reactor with concentrated feed and "P-water" dilute at a ratio of 1:20 ➢ Identify the suitable operating conditions to achieve nutrient removal in DHS reactor ➢ Monitoring the nutrient removal efficiency and make adjustment to improve the results
2	➢ Study the effect of phosphorus concentration in the wastewater feed to the performance of DHS reactor ➢ The dilution factor of concentrated "P-water" changes to 1:30, whereas the dilution factor of concentrated feed remains at 1:20

Next, the start-up of the DHS reactor will be discussed, which including the preparation of synthetic wastewater, cultivation of seedling sludge on sponge media, and the set-up or configuration of DHS reactor.

3.3.1 Preparation of Synthetic Wastewater

The source of the wastewater used in this experiment was synthesized from the laboratory chemicals. During the synthesis of wastewater, two different sets of mixtures were prepared to produce one concentrated feed and one concentrated "P-water". The concentrated feed was playing the role of wastewater in real life, while the "P-water" acted as a source of nutrient (or simply phosphorus). The source of nitrogen in this synthetic wastewater was coming from the ammonium chloride (NH_4Cl). The combination of synthetic wastewater and "P-water" can therefore mimicking the condition of sewage. However, the concentrated "P-water" was not added to the concentrated feed directly as the high concentration of feed would lead to the formation of precipitate during the mixing. The precipitation may cause blockage in the system, which is undesired. Therefore, they were prepared separately. Before mixing the two concentrated mixtures, both were diluted with distilled water at a dilution factor of 1:10. After mixing, the final mixture would have a dilution factor of 1:20 as compared to the original stock solution (concentrated feed and "P-water"). The chemicals or compounds that added during the preparation of concentrated feed and "P-water" were listed in Table 3.3 below.

The glassware and apparatus needed during the preparation of synthetic wastewater were spatula, weighing paper and Scott bottle with volume of 1 L. Weighing paper was selected instead of weighing tray because its smooth surface prevent the chemicals from sticking on it. This was important as some of the chemicals used in this experiment are in very small amount, which the attachment of chemicals on the weighing tray will drastically affect the concentration of synthetic wastewater. First, two Scott bottles were filled with distilled water to the mark of 1 L. Next, the chemicals were weighed accordingly using the weighing paper and then pour inside the Scott bottle. After all the chemicals were poured into respective Scott bottle, the cap was fastened and the bottle was inverted up and down for evenly mixing. The concentrated feed and "P-water" were then stored inside the fridge to prevent any self-degradation or contaminants from consuming the nutrient and organic matters. The concentrated stock solution was diluted according to the operating condition and used as the synthetic wastewater in the influent tank of DHS reactor.

Table 3.3: Contents of Laboratory Synthetic Wastewater.

Chemicals / Compounds	Chemical Formula	Concentration (g/L)
Concentrated feed		
Sodium acetate	$C_2H_3NaO_2$	2.79
Peptone	$C_{13}H_{24}O_4$	0.93
Yeast extract	$C_{19}H_{14}O_2$	0.15
Ammonium chloride	NH_4Cl	0.32
Magnesium sulfate heptahydrate	$MgSO_4 \cdot 7H_2O$	0.68
Magnesium chloride hexahydrate	$MgCl_2 \cdot 6H_2O$	1.21
Calcium chloride hydrate	$CaCl_2 \cdot H_2O$	0.32
Allylthiourea (ATU)	$C_4H_8N_2S$	0.02
Concentrated “P-water”		
Dipotassium phosphate	K_2HPO_4	0.34
Monopotassium phosphate	KH_2PO_4	0.39

3.3.2 Preparation of Seeding Sludge

The microorganisms or bacteria that responsible for the treatment of sewage were obtained from the activated sludge that originates from a sewage treatment plant based in Selangor, Malaysia. The activated sludge was chosen as the source of microorganisms due to its rich microbial diversity. The main groups of microorganisms typically found in activated sludge include bacteria, protozoa, metazoa, filamentous bacteria, and algae (Theobald, 2014). The presence of rich microbial diversity provides efficient treatment of sewage. Moreover, it was important to study whether the microbial being used in the conventional activated sludge process can function efficiently in a DHS reactor. If the same microbial from existing sewage treatment plant can work well in a DHS reactor, hence, the DHS reactor can be incorporated to the existing plant without any modification or need for a new source of microbial.

The sponge media that inoculated with microorganisms have to be prepared prior to the experiment. First, the sponge media that were already loaded inside the plastic hair roller were soaked or immersed in the activated sludge as shown in Figure 3.4. This process was expected to last for one week to provide enough time for the microbes to migrate to the sponge media. The level of the activated sludge must be sufficient to cover all the sponge media. In other words, all the sponge media must be submerged below the activated sludge. This was to ensure the whole area of sponge media is covered with microorganisms. Air was provided all the time during cultivation by using an external aerator. After one week, the seeded sponge media can then be arranged in the DHS reactor and use for experiment.

Figure 3.4: Cultivation of Activated Sludge on Sponge Media.

3.4 The Monitoring of DHS Reactor Performance

Composite sampling method was adopted during the sample collection in this study where the individual sample was extracted every 2 hours. The samples were collected at 9 a.m., 11 a.m., 1 p.m., 3 p.m., and 5 p.m. on each sampling day. Furthermore, each sampling day was counted as one complete cycle. Before the commencement of a new cycle, the remaining synthetic wastewater that left inside the influent tank was discarded and replaced with fresh one to ensure the accuracy of result as the synthetic wastewater might undergo self-degradation after exposed to room temperature for a long period. During the sampling process, around 35 mL of sample was drawn from effluent of DHS reactor by using a syringe to ensure the amount is sufficient for both COD and ion chromatography analysis as well as for backup purpose. The sample collected in the syringe was then passed through a syringe filter with pore size of 0.45 μm before collecting in a 50 mL centrifuge tube to filter out the suspended solid such as undissolved chemicals and detached activated sludge. Next, the COD concentration in the treated sewage was measured by HACH method while the phosphorus and nitrogen concentration were analysed through ion chromatography.

3.4.1 Analysis of COD

HACH method was employed to measure the concentration of chemical oxygen demand (COD) in the raw and treated sewage. The analysis of the sewage was carried out on a DR 3900 spectrophotometer using reactor digestion method under method 8000. Method 8000 is capable of analysing the chemical oxygen demand in a sample that range from 3 to 150 mg/L.

According to Boyles (1997), the COD test can be categorized into two steps, which are the digestion of the sample in a close-reflux digester and the light-absorbing test in a spectrophotometer. The first step of COD test is the closed-reflux digestion of sample. Concentrated sulphuric acid ($\mathrm{H_2SO_4}$) inside the COD vial will act as a primary digestion catalyst and then follow by the secondary catalyst, which is silver sulfate ($\mathrm{AgSO_4}$) that oxidized the straight-chain hydrocarbons in the sample. The digestion of sample was carried out inside a digital reactor block where the sample will be heated at 150 ℃ and the heat also plays the role of a catalyst. During the digestion process, the organic carbon (C) present in the sample will be oxidized through the reaction with hexavalent dichromate ion ($\mathrm{Cr_2O_7}^{2-}$) that originated from potassium dichromate ($\mathrm{K_2Cr_2O_7}$). The $\mathrm{Cr_2O_7}^{2-}$ ion readily generates oxygen atom to react with the organic carbon atom and form carbon dioxide ($\mathrm{CO_2}$). Meanwhile, the $\mathrm{Cr_2O_7}^{2-}$ ion is reduced to a trivalent ion, $\mathrm{Cr^{3+}}$, due to the transaction of oxygen to form $\mathrm{CO_2}$. The principle behind the COD test is to measure the amount of oxygen reacted with the sample and from there, the concentration of organic carbon is calculated (Boyles, 1997).

Second step of COD test is the light-absorbing ability of digested sample that carry out by using a spectrophotometer. The analysis works as the two chromium ions, $Cr_2O_7{}^{2-}$ and Cr^{3+}, will absorb the light in visible range but at dissimilar wavelengths. The $Cr_2O_7{}^{2-}$ ion will absorb the light at wavelength of 420 nm, while the Cr^{3+}ion is visible at around 600 – 620 nm. The $Cr_2O_7{}^{2-}$ ion does not absorb at the wavelength of Cr^{3+} range, whereas Cr^{3+} does absorb a small amount of light under $Cr_2O_7{}^{2-}$ wavelength. However, this overlapping interference can be eliminated during the calibration step. For the analysis of low range COD with concentration below 150 mg/L (or ppm), the spectrophotometer will determine the organics in the sample by measuring the increase in Cr^{3+} ion. The higher the number of Cr^{3+} ion measured, the higher the concentration of organic present in the sample (Boyles, 1997).

During the sample preparation using reactor digestion method, 2 mL of sample was charged into the vial at an angle of 45 degree using pipette. The vial must be chosen according to the selected range of COD, which is 3 to 150 mg/L. Furthermore, deionized water will act as the blank and charge into the vial using the same methods as the preparation of sample. Furthermore, the closed vials were rinsed with water and wipe with clean paper towel. This step is important to remove any contaminants on the vials that will scatter the light during analysis. After that, the vials were inverted up and down for several times to mix the sample. The well-mixed vials were then be put in a preheated digital reactor block (DRB 2000) and heat for 2 hours at 150 °C. After the 2 hours, the vials were allowed to cool for approximately 20 minutes until the temperature drops to 120 °C or below. Lastly, the vials were removed from the digital reactor block and inverted up and down again to mix. The vials were allowed to cool to room temperature on a tube rack before the measurement of COD. Next, the colorimeter procedure for the measurement of COD in the sample was carried out in the DR 3900 spectrophotometer. The program number 430 COD LR with the measurement range of 30 to 150 mg/L was used to measure the COD of sample. First, the blank sample was put into the cell holder to read its COD value, which was recorded as 0.0 mg/L, then follow by the measurement of sample. The COD concentration for the sample was read and displayed on the monitor. Figure 3.5 and 3.6 depict the digital reactor block and DR 3900 spectrophotometer respectively.

Figure 3.5: Digital Reactor Block.

Figure 3.6: HACH DR 3900 Spectrophotometer.

3.4.2 Analysis of Phosphorus and Nitrogen

Ion chromatography was used to determine the concentration of phosphorus and nitrogen in the sample. Chromatography is a technique to separate the molecules of interest from a mixture of solution based on the difference in absorption and elution of the species. In ion chromatography, the molecules are separated through the ionic interactions between different charges. In this study, the concentration of phosphorus and nitrogen were determined by measuring the concentration of phosphate ($\mathrm{PO_4}^{3-}$) and nitrate ($\mathrm{NO_3}^{-}$) species through ion chromatography (861 Advanced Compact, IC, Metrohm, Switzerland) with column methrom of A sup 5 150/4.0 mm. Ultra-pure water with conductivity value less than 0.1 μS/cm was used as the reagents, while eluent solution that made up of 3.2 mM Na_2CO_3, 1 mM $NaHCO_3$, and 0.01 M H_2SO_4 was employed as regenerant solution. After the ion exchange in chromatographer, the concentrations of $\mathrm{PO_4}^{3-}$ and $\mathrm{NO_3}^{-}$ were calculated by comparing the chromatogram areas of samples to the blank or standard solutions.

During the sample preparation for phosphate test, the samples that stored inside centrifuge tube were further filtered by a 0.2 μm syringe filter to prevent any blockage happen during the analysis as the tubing in ion chromatography are too delicate. Next, the filtered sample was charged into the vial up to two-third of the vial's volume for proper detection by the instrument. Blank that made up of deionized water was placed in the position before and after the samples of each cycle to prevent contamination. After the analysis, the result was generated and displayed on the computer. Figure 3.7 below shows the ion chromatography being used in this experiment.

Figure 3.7: Ion Chromatography for Phosphate Test.

CHAPTER 4

DESIGN DETAILS OF DHS SYSTEM

4.1 Introduction

The details design of the DHS system for nutrient removal in sewage treatment was discussed in this chapter. The equipment used, their functions, and some of the dimensions were included as well.

4.2 Design of DHS Reactor

A laboratory scale DHS system was set up for the study of nutrient removal in sewage treatment. The schematic diagram and laboratory set-up of DHS reactor were shown in Figure 4.1.

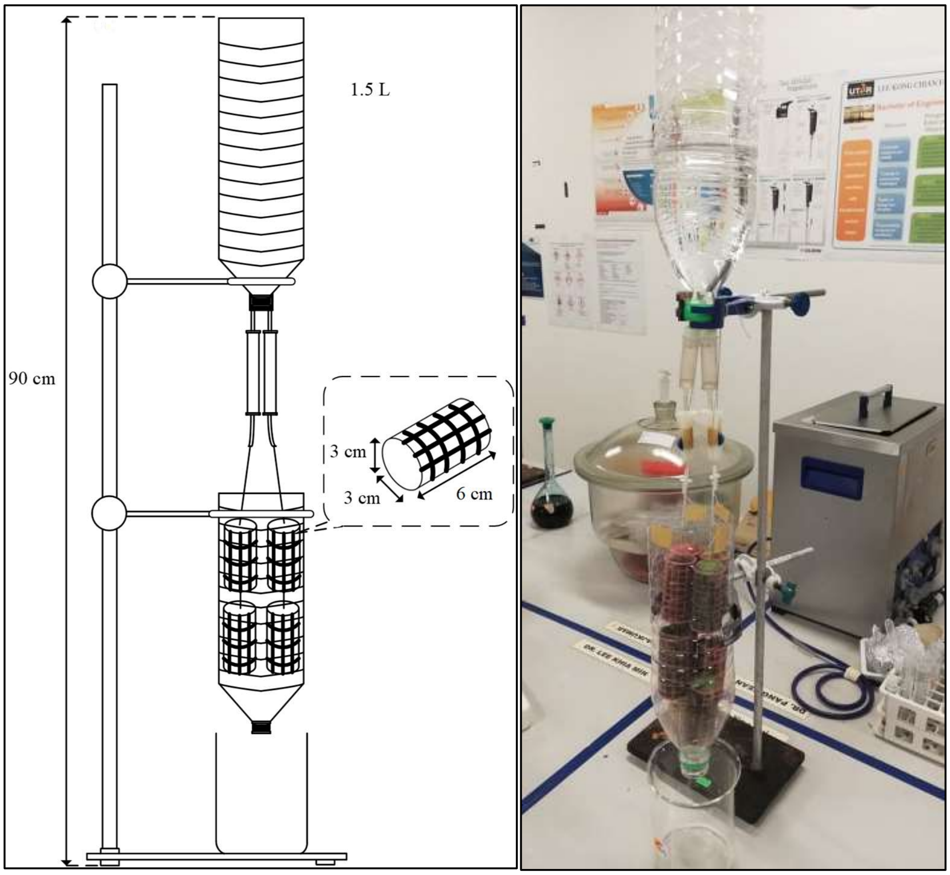

Figure 4.1: Design of DHS Reactor: (a) Schematic Diagram of DHS reactor, (b) Laboratory Set-up of DHS Reactor.

The total height of this system was around 90 cm and it consisted of three parts, which included the influent tank, DHS reactor and effluent tank. The influent tank that responsible for the storage of fresh synthetic sewage was made from a mineral water bottle with volume of 1.5 L that placed upside down. The bottom of the bottle was cut to ease the sewage filling process as well as to ensure the flowing of sewage down the bottle through the combined effects of atmospheric pressure and gravitational force. Furthermore, two small holes were punched on the bottle cap for the insertion of infusion sets. Infusion sets played the role of flow controller in this DHS system as they controlled the flowrate of sewage flowing down the reactor.

Next, the core of the system that is DHS reactor composed of sponge media that hanging vertically inside a column. There were four strings of rectangular sponge media that encapsulated by cylindrical plastic hair roller being used for the inoculation of microbes. The string was used to connect and hold the sponge media in place as the water absorption properties of string can also act as a sewage distributor to direct the flow of sewage from the infusion set outlet onto the sponge media. In addition, the cohesion and adhesion properties of water further improve the flow directing process. On each string, there were two sponge media connected diagonally in series relative to each other. The dimension of the sponge media was 6 cm × 3 cm × 3 cm. As compared to the dimension of sponge media that was used in existing researches that employed plastic hair roller support (refer to Table 3.1), the length of sponge media in this experiment was longer than practice; 3 cm longer than the common practice. The reason of this extra length was to create an anaerobic environment for the removal of nutrient since the biological removal of phosphate by the PAOs and denitrification are occurring during the anaerobic phase. To support this statement, the study of Araki, et al. (1999); Machdar, et al. (2000); and Machdar, et al., (1997) had reported that the oxygen concentration inside the sponge media was reduced when it getting deep to the centre of the sponge. Therefore, the sponge media with longer length was used for the DHS reactor to ensure and promote the existence of anaerobic condition for nutrient removal. Next, the sponge media were hanged inside a mineral water bottle. The bottle cap was opened and the bottom was cut. The purpose of this bottle was to direct the effluent treated by sponge media to the effluent tank.

Moreover, it also contributed to the anaerobic condition as the diffusion of air into the sponge media was diminished by the presence of the bottle.

The last part of this system is the effluent tank. The treated effluent flowing down from the sponge media was collected by a beaker, which acted as a temporary effluent collector. During the collection of samples at certain time, the beaker with previously stored effluent was replaced with a new one and allow the system to flow for some time. The purpose of changing new beaker for sample collection is to prevent cross contamination and to ensure the accuracy of result. Once the volume of effluent reaches the desired volume (around 35 mL), a syringe was used to transfer the sample into a 50 mL centrifuge tube. A syringe filter with pore size of 0.45 μm was used to filter out the large particles during the transfer. Lastly, the remaining effluent or wastes was discarded into waste bottle prior to disposal.

The experiment was conducted at ambient temperature and pressure. Due to the small size of DHS system, the working volume of the reactor was set at 1.5 L per day. The low working volume allows the sewage to flow at a slower rate, which increases the retention time of sewage inside the sponge media. Retention time is one of the important keys to achieve better removal efficiency as it is related to the contact time between sewage and the microbes. The DHS reactor was initiated by seeding the sponge media with activated sludge solution obtained from a sewage treatment plant based in Selangor, Malaysia. Activated sludge was chosen as the source of microbes because it contains rich microbial diversity. It was done with the hope that the microbial community would contain heterotrophs and autotrophs that uptake nutrient and organic matters as food. The seeding process was lasted for one week to provide sufficient time for the microbes to migrate or grow onto the sponge media and adapt to the new environment. After that, the synthetic sewage prepared from the chemicals was added to the influent tank and continuously trickled down onto the sponge media by gravitational force. The direction of sewage flowing out from the infusion set to the sponge media was controlled by string.

On the operating period wise, the DHS reactor was running continuously for 24 hours from Monday to Friday. Due to the office hours, the laboratory will be closed on Saturday and Sunday. The access to the DHS model and the routine of refilling the influent wastewater to the system would therefore prohibit by the shutting down of the laboratory. Hence, the DHS system was dismantled on every weekend and the sponge media that containing microorganisms were stored inside the refrigerator at around 2 ℃. This was to prevent the microorganisms to die of starvation due to no influent flow during weekend. Temporary storage of microorganisms under low temperature environment (or refrigeration) would slow down their activity dramatically and reduce their demands on food. As a result, the microorganisms could survive over the weekend even there was no food provided.

Besides, some precaution steps were taken before starting a new cycle after the weekend. The sponge media were taken out from the refrigerator and allowed to slowly rise back to room temperature. Directly exposure of the microorganisms at very low temperature to the influent wastewater at room temperature was avoided to prevent them from lyse. This is because a sudden increase in the temperature would create pores on the cell wall of the microorganisms and also capable of leading to cell lysis. The difference between the temperature inside and outside the cell would exert pressure on the cell wall and might be able to tear it. Therefore, the practice was to slowly bring the temperature of sponge media close to room temperature before the new cycle start. Moreover, there was no sample collection on the cycle where the sponge media were just out from the fridge. The system was allowed to run for some time for stabilization and sample collection would carry out on the next day.

There were two phases of experiment where the first phase was the establishment of nutrient removal in DHS reactor and the second phase was the study of parameter affecting the performance of DHS reactor. During the establishment phase, the performance of the reactor was constantly monitored and changes were made according to the result obtained after analysis. The main purpose of this phase was to identify the suitable operating conditions to achieve removal of nutrient in DHS reactor. Besides, the concentration of phosphate in the synthetic sewage was adjusted during the second phase to analyse the effect of phosphorus concentration on the performance of DHS reactor. The effect was studied by changing the dilution factor of concentrated “P-water” from 1:10 in Phase 1 to 1:30 in Phase 2. In other words, the concentration of phosphorus inside the synthetic wastewater in Phase 2 of the experiment was lower as compared to Phase 1.

CHAPTER 5

ESTABLISHMENT OF NUTRIENT REMOVALPROCESS IN DHS SYSTEM

5.1 Introduction

After the set-up of the laboratory scale DHS reactor, the Phase 1 experiment that lasted for 21 days was started. The capacity of the reactor was fixed at 1.5 L synthetic wastewater per day while the other parameters such as influent concentration, monitoring period and ventilation were varied depending on the performance of DHS reactor on phosphorus and nitrogen removal.

5.2 Operating Condition During Establishment Phase

In Phase 1, the performance of the DHS reactor was monitored continuously for a period of 21 days. This phase was to examine the ability of DHS reactor to remove nutrient as well as to identify the suitable operating condition that promote the removal efficiency. Modification and adjustment on the operating condition of DHS reactor were constantly carried out after the analysis of result to ensure a promising performance of nutrient removal.

On Day 0, the DHS reactor was started up with the concentrated synthetic sewage being diluted at a dilution factor of 1:20; 1 part of concentrated sewage mixed with 19 parts of distilled water. The calculation of dilution factor is governed by the Equation (5.1) below.

$$C_1V_1 = C_2V_2 \tag{5.1}$$

where

C_1 = initial concentration of stock solution, mg/L

V_1 = initial volume of stock solution, mL

C_2 = final concentration of final solution, mg/L

V_2 = final volume of final solution, mL

Taking 1 L of final solution as an example, the calculation of volume to be used to achieve dilution factor of 1:20 is performed by using Equation (5.1). The dilution factor of 1:20 means that the initial concentration of the stock solution is 20 times more concentrated than the final solution ($C_1 = 20C_2$).

$$C_1V_1 = C_2V_2$$

$$\frac{C_1}{C_2} = \frac{V_2}{V_1}$$

$$\frac{20C_2}{C_2} = \frac{1000}{V_1}$$

$$V_1 = 50\ mL$$

According to the calculation above, the final solution with a dilution factor of 1:20 will contain 50 mL of concentrated feed, 50 mL of concentrated “P-water”, and 900 mL of distilled water.

Throughout the operation of Phase 1, the synthetic wastewater was freshly prepared on daily basic by diluting the concentrated feed and concentrated “P-water” that stored inside the fridge. This step was to minimize the fluctuation of nutrient and organic loading concentration inside the wastewater that might arise due to self-degradation or consumption by the bacteria that enter through opened air. Besides, the top of the influent tank was covered with aluminium foil to prevent contaminants in the air from entering the synthetic wastewater. However, a small hole was poked on the aluminium foil by necessity to ensure the synthetic sewage can flow down the DHS reactor with the aid of atmospheric pressure and gravitational force.

There were 5 samples being collected per day with interval of 2 hours between each sample. The five samples were collected on 9 a.m., 11 a.m., 1 p.m., 3 p.m. and 5 p.m. respectively. Each sampling day was taken as one cycle. The collection of samples was carried out frequently during the early stage to closely monitor the efficiency of nutrient removal and the frequency reduced when the system became relatively stable. Figure 5.1 and 5.2 below show the concentration profile of phosphate and nitrogen, in Phase 1, respectively, whereas concentration of COD profile was shown in Figure 5.3. According to the concentration profiles, DHS reactor performed better on the removal of nitrogen and COD, whereas the phosphorus removal was insignificant. The details discussion on the results was included in following section.

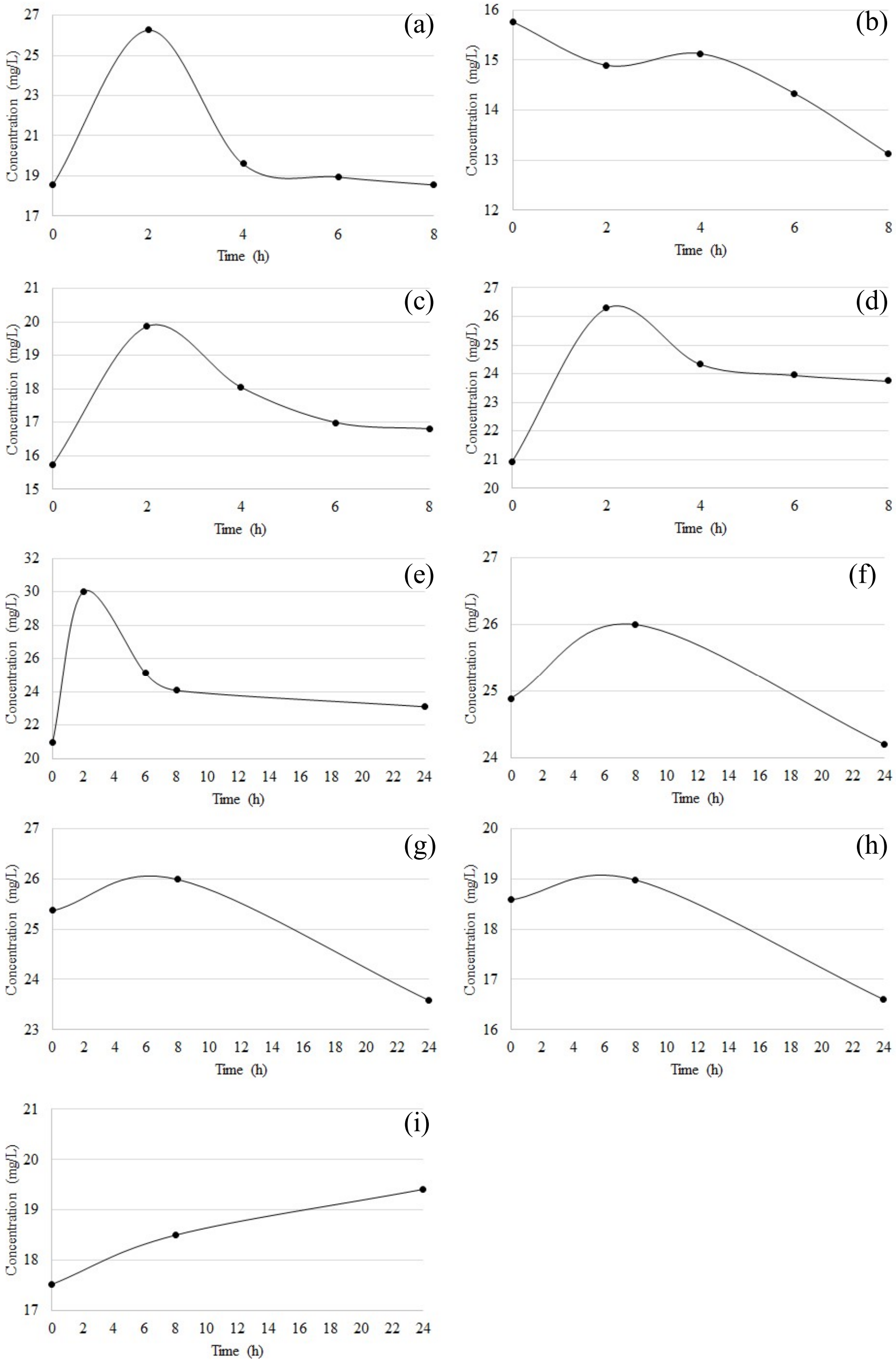

Figure 5.1: Phosphate Concentration Profile on Different Days during Phase 1 at (a) Day 0, (b) Day 1, (c) Day 3, (d) Day 6, (e) Day 8, (f) Day 13, (g) Day 16, (h) Day 18, (i) Day 21.

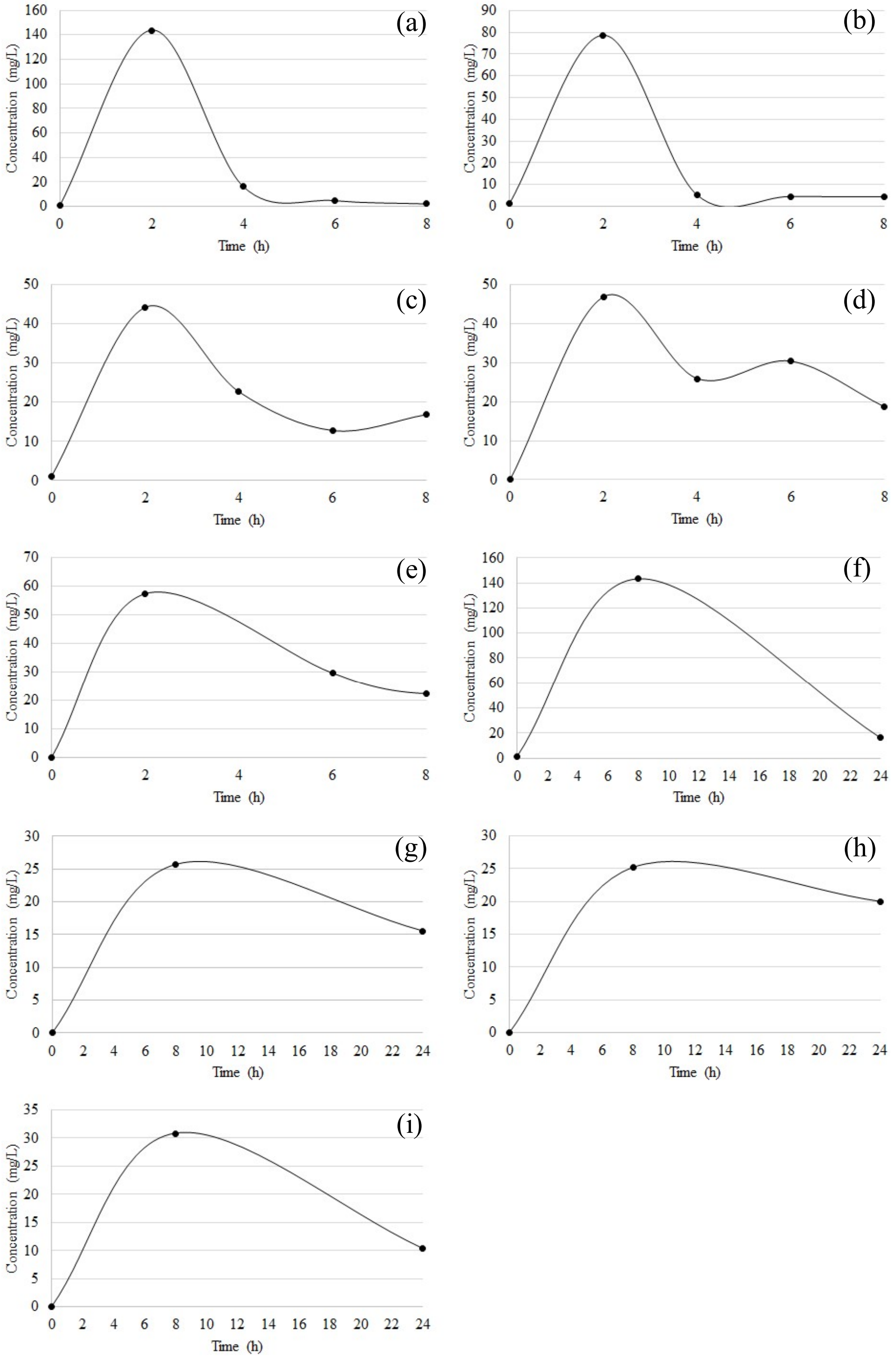

Figure 5.2: Nitrate Concentration Profile on Different Days during Phase 1 at (a) Day 0, (b) Day 1, (c) Day 3, (d) Day 6, (e) Day 8, (f) Day 13, (g) Day 16, (h) Day 18, (i) Day 21.

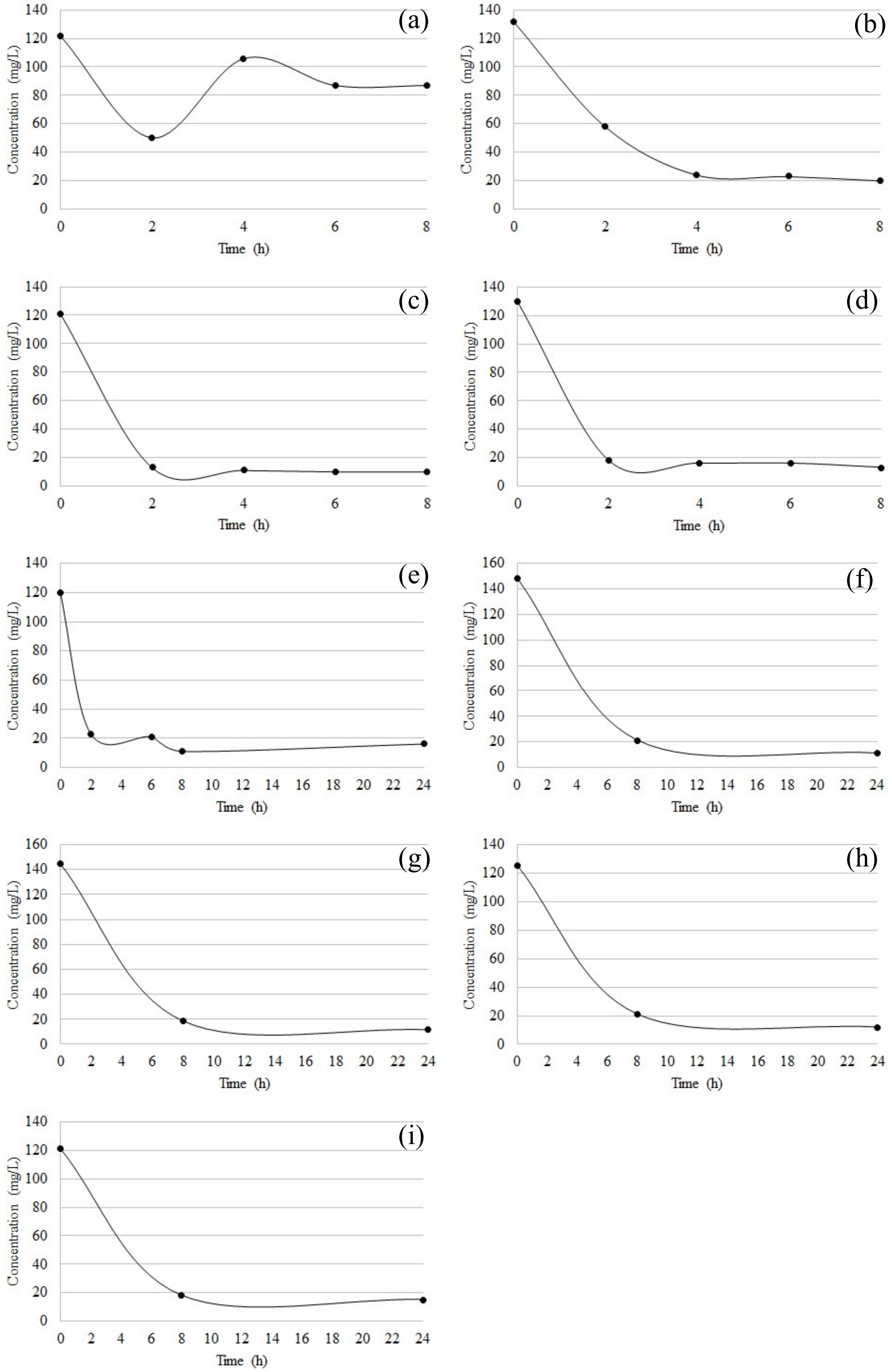

Figure 5.3: Chemical Oxygen Demand (COD) Concentration Profile on Different Days during Phase 1 at (a) Day 0, (b) Day 1, (c) Day 3, (d) Day 6, (e) Day 8, (f) Day 13, (g) Day 16, (h) Day 18, (i) Day 21.

5.3 Phosphate Removal

During the establishment phase of DHS reactor, the phosphorus removal exhibited a negative performance for most of the cycles. Therefore, modifications on the operating condition of the reactor were made with the anticipation to achieve a positive phosphorus removal. In this section, the step by step modification based on the performance of phosphorus removal was discussed. Next, the performance of DHS reactor on phosphorus and COD removal were illustrated in the Figure 5.4. The concentration profiles of phosphate and COD in the influent and effluent of DHS reactor were plotted as shown in Figure 5.5 to study the relationship between phosphate and COD removal. The detailed discussion on the result in Phase 1 was included in the following sections. Also, the percentage removal for phosphate and COD was calculated by using the Equation (5.2) below. A sample calculation for phosphate removal on Day 16 was performed; the data was extracted from the Table B-1 in Appendix B.

$$Removal\ percentage(\%) = \frac{Initial\ Conc. - Final\ Conc.}{Initial\ Conc.} \times 100\% \tag{5.2}$$

$$Phosphate\ removal\ percentage\ (Day\ 16) = \frac{25.371 - 23.592}{25.371} \times 100\%$$

$$= 7.02\%$$

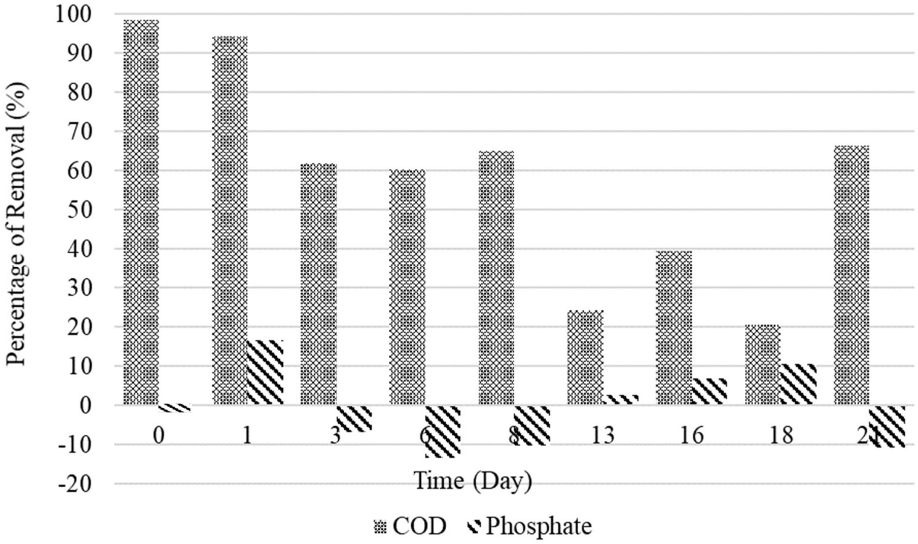

Figure 5.4: Phosphate and COD Removal Efficiency.

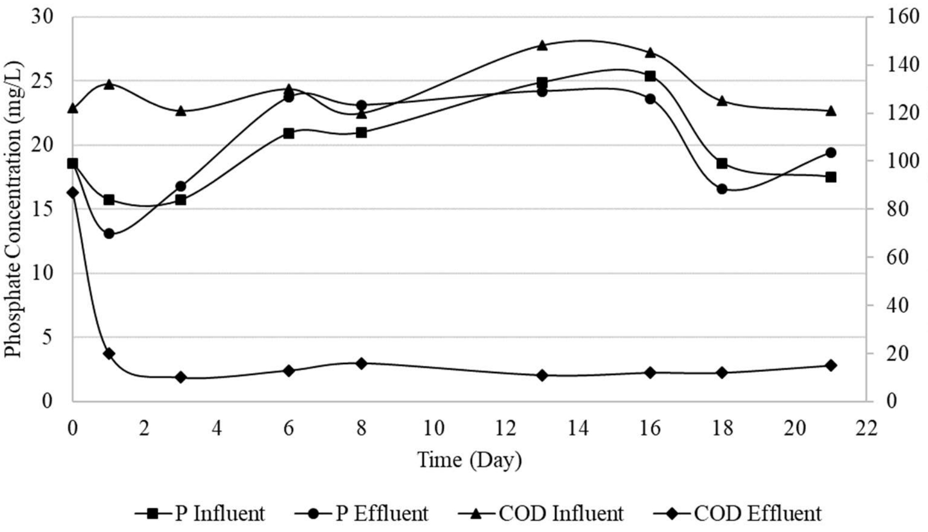

Figure 5.5: Variation of Phosphate and COD Concentration in DHS Reactor Influent and Effluent.

The results of phosphate removal on Day 0 to Day 6 showed a negative value except for Day 1 (refer to Figure 5.4). In other words, the concentration of phosphate in the effluent of DHS reactor was higher than the influent. According to the theory, the concentration of phosphate should be reduced after passing through the DHS reactor as the microbial activities of phosphate accumulating organisms (PAOs) tend to uptake phosphate from the sewage (Mahmoud, et al., 2010; Hatamoto, et al., 2015). As shown in Figure 5.1, the phosphate concentration of second sample in all cycles reached a peak that was higher as compared to the initial source or the first sample. However, the phosphate concentration in the following samples (third to fifth sample) did showed a reducing trend. In short, the phosphate concentration rose at the beginning of each cycle and then decreased gradually. The sudden surge of the phosphate concentration can be explained by the microbial activities of PAOs that known as Enhanced Biological Phosphorus Removal (EBPR). According to Haandel and Lubbe (2007), EBPR is a two-step process carry out by the PAOs that lead to the removal of phosphate from the sewage. The first step is happened under anaerobic condition where sufficient amount of volatile fatty acids (VFAs) are present in the system. These VFAs will trigger the growth of bacterial population. However, this phenomenon is not observed on PAOs. During the anaerobic phase, there exists limited suitable electron acceptor (oxygen, nitrate and nitrite) for PAOs to utilize the available substrates. Alternatively, PAOs will uptake the VFAs from the liquid into their cells and stored as PHB, a type of internal reserved polymers. During the uptake and storage of VFAs, the PHB will be formed from glycogen and produce an intermediate energy carrier, $NADH_2$. The energy required for this process comes from the hydrolysis of polyphosphate in PAOs and ATP molecules will be produced. At the same time, the hydrolysed polyphosphate will be spilt into orthophosphate (PO_4^{3-}) that is then release out from the cell. The release of PO_4^{3-} during the anaerobic phase explained the increase in the phosphate concentration during the experiment. Moving forward, the bacterial growth of PAOs is initiated in the subsequent aerobic phase where the stored PHB will be used as a source of energy supply. The oxidation of PHB will produce carbon dioxide and energy in the form of $NADH_2$. Meanwhile, part of the energy derived from PHB will be consumed for the regeneration of polyphosphate from the liquid outside the cell. The purpose of polyphosphate uptake during aerobic condition is to replace the released polyphosphate during anaerobic condition. This EBPR process was illustrated in Figure 5.6.

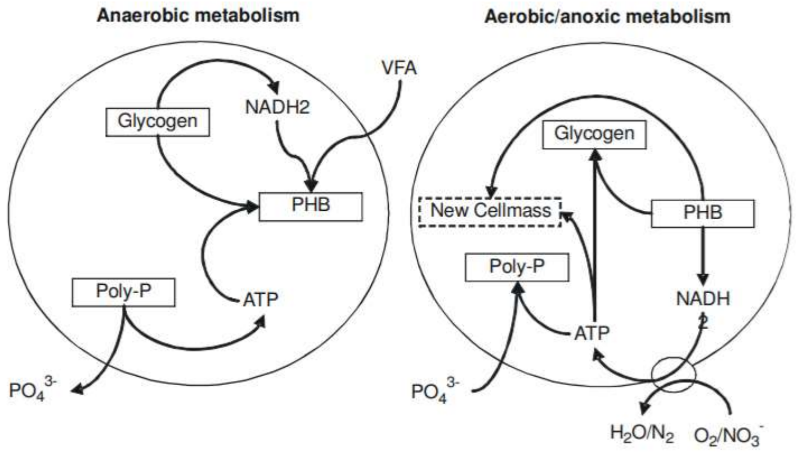

Figure 5.6: Metabolism of PAOs under Aerobic and Anaerobic Conditions (Haandel and Lubbe, 2007).

The study carried out by Nurmiyanto, et al. (2017) also observed the occurrence of EBPR process in a DHS system, which supported the results obtained in this experiment. Therefore, the second phase of EBPR process described the decreasing trend of phosphate concentration observed from each cycle during the experiment. Unfortunately, the phosphate concentration in the effluent of last sample (8 hours) of the cycle did not fall lower than the influent, hence, presenting a negative removal efficiency. Apart from that, the result on Day 1 that did not obey the EBPR process might be due to the early stage of operation. At this stage, the microbes were still adapting to the environment and undergone growing phase at which they might uptake more phosphorus to supply with the substrates needed during cell growth. In addition, the analytical error will also contribute to the result that did not obey the theory. These were the possible reasons that contributed to the high phosphate removal percentage of 16.71 % on Day 1.

Next, it was suspected that the phosphate concentration would be further decreased after the 8th hour monitoring period. This is because the phosphate profile of DHS reactor effluent posed a decreasing trend from 2nd hour to 8th hour. Therefore, there was a change in monitoring period of each cycle from original 8 hours to 24 hours starting from Day 8 to study the extend of phosphate removal. In other words, the samples of each cycle were collected on five different period, which were 0 hour, 2 hours, 6 hours, 8 hours and 24 hours. Regrettably, the result on Day 8 still demonstrating a negative removal even after the monitoring period had been extended. This situation might be due to the changing in influent flowrate during night time. Due to the operating time of laboratory, the ventilation system and air conditioner in the laboratory tend to shut down after office hours (between 8th hours to 24th hours). The absence of air ventilation system will lead to poor air transfer into the laboratory and this will result in change in pressure that slowing down the flow of synthetic sewage. In addition, the reduction of water level in the influent tank also contributed to the change in flowrate after laboratory office hours. Once the water level become lower, the pressure exerted on the exit of influent tank was reduced. Therefore, the flow of synthetic sewage is slowing down when the water level reduces and eventually stop. As stated by Ross (2013), the slowing or stopping of influent into the DHS reactor may have some effects on the biological phosphorus removal by PAOs. First, the uptake of phosphorus by the PAOs during aerobic condition may be incomplete if the dissolved oxygen in the sponge media is too low. The oxygen needed by the PAOs during aerobic condition can be obtained through two processes, which are the direct diffusion of oxygen from the atmosphere to sponge media or by diffusing into the flowing influent and then into the sponge media. In addition, Machdar, et al. (1997) also stated that the migration of wastewater from the anterior of sponge to the posterior position through permeation may act as a supply of dissolved oxygen to the system. Therefore, the stop of influent flow will reduce the oxygen being dissolved into the sponge media through dripping influent. Secondly, the stopping of influent flow also leads to insufficient supply of VFAs to the PAOs that result in poor phosphorus uptake (Ross, 2013). As discussed above, the uptake of phosphate ions from the medium and the subsequent formation of polyphosphate granules in the cells requires energy. This energy is obtained from the metabolism of the PHB absorbed during the anaerobic phase. Since there was a stop of influent flow, the amount of VFAs available in the feed for the PAOs to store as PHB will greatly reduce. Hence, there is insufficient PHB to be metabolised to provide energy for the phosphorus uptake.

In short, the slowing down or stopping of influent flow was the main culprit that led to the negative removal of phosphate on Day 8. Some modifications had been made to minimize the effect of slowing influent flow to the removal of phosphate. The restriction on shutting down of ventilation system and air conditioner after laboratory office hour were not controllable, therefore, the only changes can be made is on the design and operating condition of the DHS system. The aluminium foil cover on the influent tank was removed after the office hour to allow easier air transfer into the influent tank. This is to ensure there is a continuous atmospheric pressure being exerted on the water surface. On the other hand, the influent tank was refilled with additional 500 mL of synthetic sewage at 8th hour so that the sewage can be maintained at a higher water level to induce the flow. The leftover synthetic sewage in the influent tank was also discarded before the beginning of a new cycle to prevent the inaccuracy of result due to self-degradation of sewage. The phosphate removal efficiency was being monitored continuously after the modifications.

Moving forward to Day 13 and 16, the number of samples collected on each cycle was reduced to 3 that were collected on 0 hour, 8 hours and 24 hours. Since all the previous cycles showed an increase in the phosphate concentration in the beginning and then followed by a trend of decreasing, it can be concluded that EBPR process will presence in every cycle. Therefore, the intermediate samples (2 hours and 6 hours) were not taken as the trend of phosphate concentration can be confirmed by the result from previous cycles. According to the result on Day 13 and 16, both cycles presented a positive phosphate removal with percentage of 2.78 % and 7.01 % respectively. At the same time, it could be observed from the DHS model that the problem of stopping or slowing of influent flow was remediated by the adjustments made on Day 8. By ensuring the continuous flow of influent into the DHS reactor, the phosphate removal showed an affirmative result that obey the theory proposed by Ross (2013). However, the removal of phosphate by the DHS reactor was fairly low. The low phosphate removal might be caused by the high concentration of phosphate in the influent. The DHS reactor consisted of only four sponge media supported with plastic hair roller and the amount of PAOs present within the system might be too little to uptake all the phosphate in the influent. Thus, the concentration of phosphate was lowered by adjusting the dilution factor of both concentrated feed and concentrated "P-water". The dilution factor of synthetic sewage was increased from the original 1:20 to 1:25. Moreover, by observing the concentration of phosphate in the influent (sample at 0 hour) of Day 0 until Day 16, the concentration was gradually increased. This might be due to the contamination during the process of dilution. Hence, the remaining concentrated feed and "P-water" that stored inside the fridge was discarded and a new set was prepared. This is to prevent any contaminations or self-degradation that might affect the concentration of phosphate in the influent. The phosphate removal percentage was expected to improve after the changes.

On Day 18, the phosphate concentration in the influent was lowered to 18.582 mg/L after changing the dilution factor and the phosphate removal efficiency was risen as expected. The percentage removal was 10.68 %, which was higher than previous two cycles. From the result, it can be affirmed that the phosphate removal percentage will increase when the phosphate concentration in the influent is reduced. It also inferred that a DHS reactor with larger number of sponge media is required to handle a source with high phosphate concentration in order to achieve higher removal percentage. In contrast, the result on Day 21 showed a negative phosphate removal which was unusual. This abnormal result can be explained by the sloughing off of activated sludge or biomass cell that observed in the effluent of DHS reactor on Day 21 at 24th hour.

Figure 5.7 below illustrates the presence of detached activated sludge in the effluent. When the PAOs died and detached from the sponge media, they will flow down to the effluent tank. After that the dead cells lyse and the stored polyphosphate granules in the cell will be decomposed and become part of the effluent (Ross, 2013). Hence, this phenomenon described why the phosphate concentration in the last sample of Day 21 would increase.

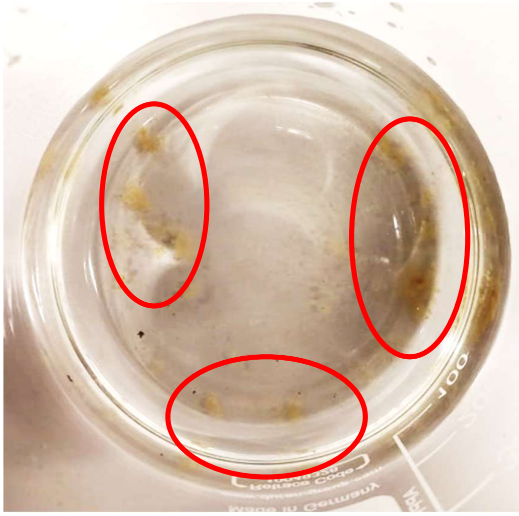

Figure 5.7: Detached Activated Sludge found in the Effluent on Day 21.

Throughout the operation of Phase 1, the DHS reactor showed a negative percentage removal of phosphate on most of the cycles. This was contributed by the improper operating conditions. After understanding and inferring the possible causes of poor removal, adjustments were made to prove the ability of DHS reactor in phosphate removal. Ultimately, the result on Day 13, 16 and 18 showed a positive removal. According to the results, DHS reactor is a potential bio-trickling reactor to remove phosphorus from the sewage. The highest percentage removal achieved in Phase 1 was 10.68 % with the initial concentration of phosphate at 18.582 mg/L. The removal percentage on Day 1 was not taken into account as the DHS reactor was still under the phase of adaption and stabilization. Out of the nine sample collection cycles, three cycles showed a positive removal on phosphate. The remaining cycles with negative removal percentage were all due to the improper operating condition as well as the sloughing off of dead activated sludge. Furthermore, the low percentage of phosphate removal was mainly due to the low sponge volume. As discussed above, when the number (or volume) of the sponge media increases, there will be larger surface for the activated sludge to attach on and enhance the contact between the synthetic sewage and microbes, hence, the removal efficiency will be greater. Next, too high concentration of phosphate in the influent will also reduce the removal efficiency because the number of PAOs presence is insufficient to uptake all the phosphate in the sewage. By referring to the results on Day 16 and Day 18 in Figure 5.4 and 5.5, when the concentration of phosphate in the influent reduced from 25.371 mg/L to 16.598 mg/L, the percentage removal of phosphate was increased from 7.01 % to 10.68 %. There were few findings obtained from the establishment phase of DHS reactor: (i) PAOs will undergo EBPR process and lead to the increase in phosphate concentration that is higher than source; (ii) water level and atmospheric pressure must be always maintain to induce the flow of influent; (iii) the removal of phosphate increases as the concentration of phosphate in the influent decreases. As a conclusion, it was proven that DHS reactor is able to remove phosphorus from the wastewater. The effect of phosphate concentration in the source to the removal efficiency of nutrient was further studied in Phase 2.

5.4 Nitrogen Removal

In this study, the source of nitrogen was coming from the ammonium chloride (NH_4Cl) that added to the concentrated feed during the preparation of synthetic wastewater. Since the ion chromatography only measures the concentration of ions, the concentration of nitrate ions was used to plot the graph in Figure 5.2 above as an indication of nitrogen. During the preparation of synthetic wastewater, the solid NH_4Cl will dissolve in water and dissociate into ammonium ion (NH_4^+) and chloride ion (Cl^-). Next, the ammonium ion will further undergo nitrification and denitrification processes to convert into nitrogen gas, hence, achieving the removal of nitrogen nutrient in water. The Equation (5.3) and (5.4) below illustrate the chemical formula of nitrification and denitrification respectively.

$$\text{Nitrification: } \mathrm{NH_4}^+ \rightarrow NO_2^- \leftrightarrow NO_3^- \tag{5.3}$$

$$\text{Denitrification: } NO_3^- \leftrightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \tag{5.4}$$

During nitrification process under aerobic condition, the ammonium (NH_4^+) is dissociated into nitrite ion (NO_2^-) that is a non-stable intermediate product that prone to oxidation. The presence of oxygen in the water will oxidize the NO_2^- to nitrate ion (NO_3^-), which is more stable and soluble in water. Next, the subsequent denitrification that occurs under anaerobic condition will convert the NO_3^- back to NO_2^- again, then followed by the conversion to nitric oxide (NO) and nitrous oxide (N_2O). Ultimately the N_2O is converted into nitrogen gas (N_2) that is harmless to the environment. Although the nitrification inhibitor (ATU) was added to the synthetic wastewater, the nitrification process still occurred. Due to the extremely small amount of ATU (0.02 mg/L) being used, it might be insufficient to completely inhibit the nitrification process during the experiment. Moreover, the ATU might loss its inhibition characteristic during the long storage period inside the fridge; the activity degraded over time.

The removal of nitrogen by the DHS reactor was not study by direct measurement of NH_4^+ presence at the influent and treated effluent. Instead, it was separated into two parts that were nitrification and denitrification efficiency. First, the nitrification efficiency was calculated by comparing the concentration of nitrogen atom in the NH_4Cl of influent and the NO_3^- of effluent. In each cycle, the sample with highest value of nitrate concentration was chosen and it was assumed to represent the peak of nitrification for that particular cycle. The difference between the concentration of nitrogen atom in the NH_4Cl and NO_3^- signified the nitrification efficiency. Second, the denitrification efficiency was determined by the degree of nitrate removal. The difference between the highest and the lowest concentration of nitrate in each cycle represented the extend of denitrification. The performance of DHS reactor on nitrification and denitrification was plotted in Figure 5.8 together with the COD removal efficiency to study the relationship between organic matters and nitrogen removal. On the other hand, the variation of nitrate and COD concentration in the influent and effluent of DHS reactor was also shown in Figure 5.9. Next, sample calculation was performed by using the result on Day 0 as example to show how the nitrification and denitrification efficiency were calculated. All the values were extracted from Table B-3 in Appendix B. The concentration of nitrogen atom in a chemical compound is governed by the Equation (5.5) below.

$$Concentration\ of\ N = \frac{MW_N}{MW_{CP}} \times C_{CP} \tag{5.5}$$

where

MW_N = molecular weight of the nitrogen atom, g/mol

MW_{CP} = molecular weight of the chemical compound, g/mol

C_{CP} = concentration of the chemical compound, mg/L

$$\begin{aligned} N\ in\ NH_4Cl &= \frac{MW_N}{MW_{NH_4Cl}} \times C_{NH_4Cl} \\ &= \frac{14.0067\frac{g}{mol}}{54.491\frac{g}{mol}} \times 320\frac{mg}{L} \\ &= 82.255\frac{mg}{L} \end{aligned}$$

$$\begin{aligned} N\ in\ \mathrm{NO_3}^- &= \frac{MW_N}{MW_{\mathrm{NO_3}^-}} \times C_{\mathrm{NO_3}^-} \\ &= \frac{14.0067\frac{g}{mol}}{62.0037\frac{g}{mol}} \times 143.564\frac{mg}{L} \\ &= 32.431\frac{mg}{L} \end{aligned}$$

$$\begin{aligned} Nitrification\ Efficiency(\%) &= \frac{N_{\mathrm{NO_3}^-}}{N_{NH_4Cl}} \times 100\% \\ &= \frac{32.431}{82.255} \times 100\% \\ &= 39.43\% \end{aligned}$$

$$\begin{aligned} Denitrification\ Efficiency(\%) &= \frac{{NO_3}^-{}_{highest} - {NO_3}^-{}_{lowest}}{{NO_3}^-{}_{highe}} \times 100\% \\ &= \frac{143.564 - 2.065}{143.564} \times 100\% \\ &= 98.56\% \end{aligned}$$

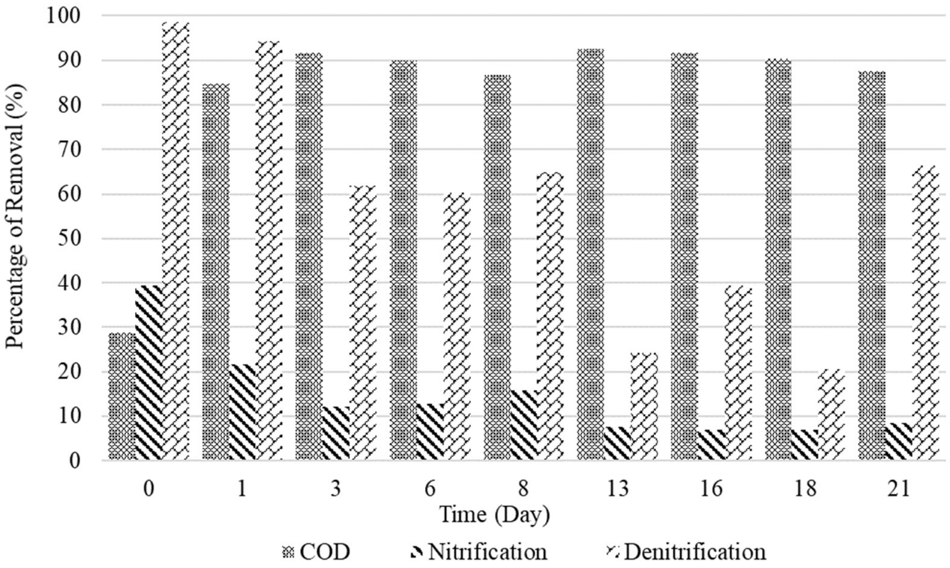

Figure 5.8: Nitrification, Denitrification and COD Removal Efficiency.

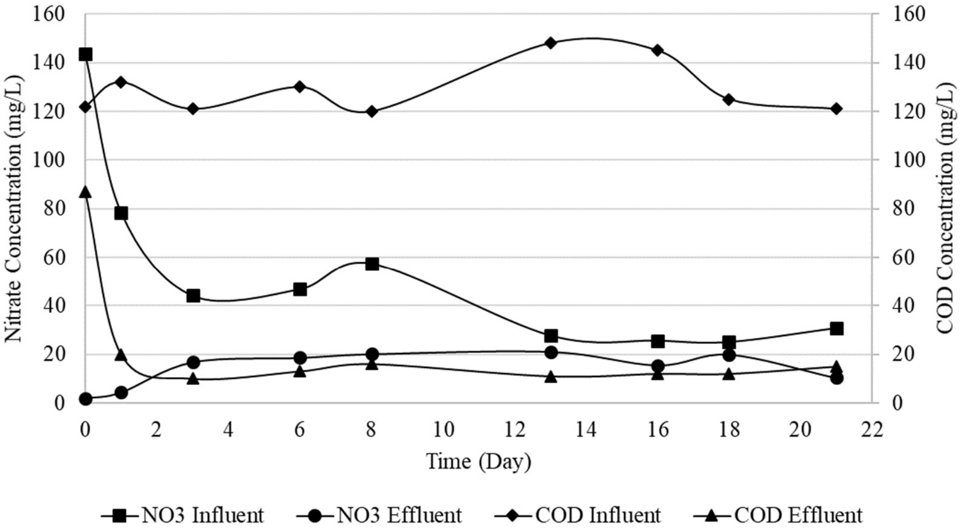

Figure 5.9: Variation of Nitrate and COD Concentration in DHS Reactor Influent and Effluent.

As depicted in Figure 5.9, there was always presence of nitrate in the DHS system, which suggested that nitrification process was happening to oxidize the ammonium ion in the NH_4Cl to nitrate. Moreover, the reduction of the nitrate concentration in the treated effluent described the occurrence of denitrification where the nitrate was being converted to nitrogen gas (refer to Figure 5.2). The percentage of nitrification was ranged between 6.91 % to

39.43 %, while the denitrification was ranged from 20.62 % to 98.56 %. In addition, there was also residual nitrite that found in the effluent, which has not yet been converted into nitrogen gas. However, the result for nitrite was not presented due to its very small and negligible concentration. The results from the experiment advocated that DHS system is a potential bioreactor for nutrient removal. An interesting finding was that the removal of phosphorus and nitrogen were happening simultaneously within a single system. The nitrification process that produces the nitrate creates an anoxic environment for the removal of phosphate by the denitrifying PAOs. The detailed discussion on the simultaneous nitrogen and phosphorus removal was included in Section 4.4 later.

There are many existing researches that reporting the ability of DHS reactor in nitrogen removal. For example, the DHS reactor studied by Araki, et al. (1999) achieved a nitrogen removal efficiency of 78 %; experiment by Agrawal, et al. (1997) reported an average nitrogen removal of 84 %; and Mahmoud, et al. (2009) that attained 83 % reduction in influent ammonia concentration. Nitrifiers are the important microbes that responsible for the oxidation of ammonium ion through nitrification process. The merit of DHS reactor with sponge matrix that is suitable for the retention of biomass and sludge and the consequential long sludge retention time had created a suitable environment for the slow growing nitrifiers (Tandukar, et al., 2005). Other than the nitrifiers, the results in Figure 5.8 also implied that the coexistense of denitrifying bacteria. The reduction of nitrate concentration in the effluent of DHS reactor suggested that the products of nitrification (nitrate) were instantly utilized by the denitrifying bacteria in the DHS reactor. It was noticed that the DHS reactor exhibited reatively high denitrification performance as compared to nitrification. Percentage of denitrification achieved more than twice as great as the ammonia oxidation acitivity by the nitrifiers. The activity of denitrification could be attributed to the presence of a distintive dissolved oxygen gradient along the sponge-inward depth (Machdar, et al., 2000). As mentioned earlier, the inner section (0.75 cm from the surface) of the sponge media in DHS reactor is lack of oxygen and this created an anaerobic zone, which is favourable to the denitrification process (Araki, et al., 1999; Machdar, et al., 2000). Therefore, the dissolved oxygen concentration gradient along the sponge media depth provided a nitrification region at the aerobic surface of the sponge and a denitrification region at the inner anoxic zone of sponge material. The presence of nitrification and denitrification activities in the non-aerated DHS system showed an advantage over conventional treatment process that required external aeration to remove the nitrogen. According to Nguyen and Luong (2012), the aeration is needed for other biological treatment to maintain the dissolved oxygen level of at least 3 mg/L, which could imposed additional operating costs. The merit of high oxygen transfer capacity in the DHS system make it a cost effective treatment process that can save up to 97 % of power consumption (Tanikawa, et al., 2016).

On the other hand, the trend showed in Figure 5.8 reported that the nitrification efficiency decreased when the COD removal efficiency increased. This phenomenon could be ascribed to nitrifiers being outcompeted by the heterotrophs over oxygen. The study of Machdar, et al. (2000) and El-Kamah, Mahmoud and Tawfik (2011) stated that the nitrification acitivity was diminished, especially at the upper portion of DHS reactor, when the COD loading became higher. At high organic matters loading conditions, the heterotrophs bacteria will consume the dissolved oxygen available rapidly to degrade the organics at the upper part of the reactor. Under circumstance of limited dissolved oxygen, the nitrifying bacteria is usually defeated by the heterotrophic microoganisms and result in poor nitrification efficiency (El-Kamah, Mahmoud and Tawfik, 2011). Hence, nitrification would usually took place at the bottom portion of the DHS system where the concentration of organic matters becomes insignificant. In short, the nitrification activity of nitrifiers has an inversely proportional relationship with the organic loading because they cannot compete oxygen and space with the heterotrophs (Fleifle, et al., 2013). Nevertheless, the denitrification activity was relatively unaffected by the influent COD loading. This was contributed by the length of sponge media that created an anaerobic habitat for the denitrifiers. Moreover, the thick and dense layer of biofilm that covered on the sponge surface could impede the penentration of oxygen into the inner part of sponge. Hence, a dissolved oxygen depleted zone was formed in favour of the denitrifiers (Machdar, et al., 1997). In addition, the competition between heterotrophs and nitrifiers on the oxygen would become more intense due to the formation of biofilm.

5.5 COD Removal

Besides the study of nutrient removal in DHS reactor, COD removal should also be investigated. It is desired to develop a DHS system that can use to treat raw sewage in addition of using it as a downstream system for existing sewage treatment units. Therefore, the performance on COD removal was also a great concern. COD is a measure of the organic strength of wastewater that directly reflect the quality of water. It serves as a guideline to examine the quality of discharged wastewater and the effect it will have on the receiving water body. Moreover, the concentration of COD is also important in the wastewater treatment process as it determines the amount of aeration to be supplied for treating the incoming wastewater (Merck, 2016). The classification of water based on COD concentration was illustrated in Table 2.1 under Chapter 2. A wastewater that has high COD concentration will consist of large amount of oxidizable organic matter that will consume the dissolved oxygen in the receiving water body. Consequently, the amount of dissolved oxygen in the water body will be reduced and result in anaerobic condition that is deleterious to aquatic life.

In this study, the COD concentration of DHS effluent was studied in addition to the nutrient removal. It could be observed from Figure 5.4 that the COD removal percentage of DHS reactor was lied low at 28.69 % on Day 0 and then follow by a surge to 84.85 % on Day 1. The trend of COD removal efficiency remained stable after Day 0, in which the value fluctuated between 80 % and 95 %. The poor removal efficiency on Day 0 was due to the effect of influent loading or OLR. Before the set-up of DHS reactor, the sponge media were cultivated inside the activated sludge solution for a week. The microbes attached to the sponge media already adapted to the environment during cultivation and the concentration of organic matters in the cultivation environment was relatively stable after a long time of cultivation. When the set-up was done and the influent started to flow down the sponge media, the sudden change in the influent loading rate as compared to the cultivation environment created the organic shock load phenomenon. In other words, the OLR was increased at the time the influent charged to the sponge media. Similarly, the flow of influent also resulted in the increase in hydraulic loading rate. Originally the sponge media were under a condition with no flow and then the flow was introduced after the start-up of DHS reactor. During the period of increase in influent loading rate, there was an intense separation of attached activated sludge from the sponge media due to hydraulic shear force that contributing to high COD concentration in the effluent. Therefore, the low removal efficiency on Day 0 can be concluded as the biosolid escape from the DHS reactor under high loading rate during start-up phase.

Other than the biosolid escape, the absent of accumulated sludge and suspended particles inside the sponge pore might also gave rise to the poor removal efficiency. During the start-up, the sponge media was relatively clean and contained no sludge or inert but microbes. Without the presence of sludge in the pores, the entrapment of suspended solids became hard and most of the solids would just flow through without captured by the sponge. The entrapment of organic matters inside the sponge media is of importance as the microbes will uptake them as food for growth and provide energy for phosphate removal. This also explained the poor phosphate removal efficiency on Day 1 as the microbes did not obtained enough food for metabolism that supply energy. As the system continued to run, the sludge started to retained inside the sponge media and eventually formed a filter cake, which illustrated by the outstanding COD removal efficiency for the following cycles. The combination of pores inside the sponge media and the sludge filter cake formed an excellent bio-filter to remove COD from the wastewater.

In a study conducted by Mahmoud, Tawfik and El-Gohary (2010) reported the DHS system achieved a COD removal efficiency of 89 % at the organic loading of 1.2 kg COD/m^3·d, but when the organic of 3.4 kg COD/ m^3·d was applied, the removal efficiency dropped to 56 % with a residual of 121 mg/L COD in the effluent. Similarly, the research carried out by Yoochatchaval, et al. (2014) also observed a decrease in COD removal efficiency when the HRT was reduced. The decrease in HRT means that the HLR and OLR were also increased. The COD removal efficieny reduced from 67 % to 57 % when the hydrualic retention time adjusted from 4 houts to 1 hour. Also, Machdar, et al. (2018) obtained a reduction of COD removal percentage from 33 % to 22 % when the hydrualic retentiont time reduced from 4 hours to 3 hours. The past researches work all pointed out that the DHS reactor will have a poor COD removal efficiency during organic shock load.

Nevertheless, after some time, the removal efficiency of COD in above three researchers' studies climbed up to a level that close to or above the original value (before the application of organic shock load). This findings suggested that DHS reactor was relatively unaffected by the change in HRT or OLR. The sudden change in the two parameters will reduce the removal efficiency of COD for a short time, but soon it will adapt and back to normal. Generally, the DHS reactor is capable to cope with variation in OLR. The results on Day 0 onwards show a close agreement with the findings of these researchers as shown in Figure 5.4. After the start-up, the removal percentage of COD shooted up to 84.85 % on Day 1 and remained high at around 90 % in the following cycles. The reason behind this high removal was due to the sponge media that contained large amount of void space. A colonized matrix that is suitable for the attachment of microbes and entrapment of sludge was formed by the reticulated structure of sponge media. The presence of microbes together with the long water residence time characteristic of sponge provide sufficient time for the digestion of organic matters. Moreover, the entrapment of sludge within the matrix of sponge also reduced the generation of excess sludge at the effluent, thereby achieving high COD removal percentage.

On the other hand, the removal efficiency of COD on Day 22 was slightly lower than Day 13, 16 and 18. The value dropped to 87.60 %, which is relatively lower than the previous three cycles with removal more than 90 %. The same trend of removal efficiency was also observed for the phosphate. The reduction of COD removal efficiency was mostly contributed by the sloughing off of activated sludge that occurred on Day 21. Since the DHS system was running continuously for 21 days, the storage capacity of sponge media might be exhausted and ultimately the unintentional washout of dead microorganisms or sludge by the down-flowing sewage took place. Therefore, it can be concluded that when the microbe cells died and lysed, the intracellular stored organic carbon will release in addition of the phosphate and contributed to the COD in the effluent. The removal efficiency of COD on Day 21 was in consistent with the trend of phosphate removal.

In a nutshell, the DHS reactor exhibited an excellent performance on the removal of COD. The removal efficiency was relatively higher (28.69 – 92.57 %) when compared to other researchers' studies. Tandukar, et al, (2005) achieved

76 % on COD removal in a DHS reactor that treated the effluent from a UASB reactor. Furthermore, study by Yoochatchaval, et al. (2014) and Machdar, et al. (2018) also showed a removal efficiency that was lower than 70 %. The performance of DHS reactor was stable in average even under the fluctuating influent condition such as changing flowrate and OLR. Moreover, the long water retention time and porous sponge media that are suitable for innoculation of microorganisms had provided the outstanding COD removal efficiency in this study.

CHAPTER 6

PARAMETER STUDIES OF NUTRIENT REMOVAL PROCESS IN DHS SYSTEM

6.1 Introduction

There are several parameters that will affect the nutrient removal efficiency of DHS reactor such as HRT, SRT, OLR, SPS, etc. Here, the effect of phosphate concentration on the nutrient removal efficiency in DHS reactor was studied.

6.2 The Effect of Phosphate Concentration on Nutrient Removal

The effect of phosphate concentration in the influent on the performance of DHS reactor in nutrient removal was studied during the Phase 2 of the experiment. The performance of DHS reactor on changing phosphate concentration in the influent was conducted in continuous process. The study started directly after the end of Phase 1 with the concentrated "P-water" being diluted at dilution factor of 1:30. However, the concentration of concentrated feed remained unchanged to ensure the supply of organic matters to the system. Therefore, the synthetic wastewater with volume of 1.5 L would have 50 mL of concentrated "P-water", 75 mL of concentrated feed and 1375 mL of distilled water.

During the operation of Phase 2, similar biological activities were observed as Phase 1. First, the phosphate removal was achieved with the observation of EBPR process by the PAOs. Second, nitrification and denitrification activities were coexisted with the removal of phosphate, which again confirmed the ability of DHS reactor to remove nutrient from the wastewater. Lastly, the removal of organic matters or COD was also detected. Out of the three removals, the DHS reactor showed some improvement on the removal of phosphate and COD, whereas the removal of nitrogen became inferior as compared to Phase 1. The variation of DHS reactor performance under different operating condition suggested there was a relationship between removal of COD, phosphate and nitrogen. Any changes in one of the parameters would affect the removal efficiency of the others. The relationship between the three parameters was discussed in paragraph below. The concentration profile of phosphate and nitrate were demonstrated in Figure 6.1 and 6.2 respectively.

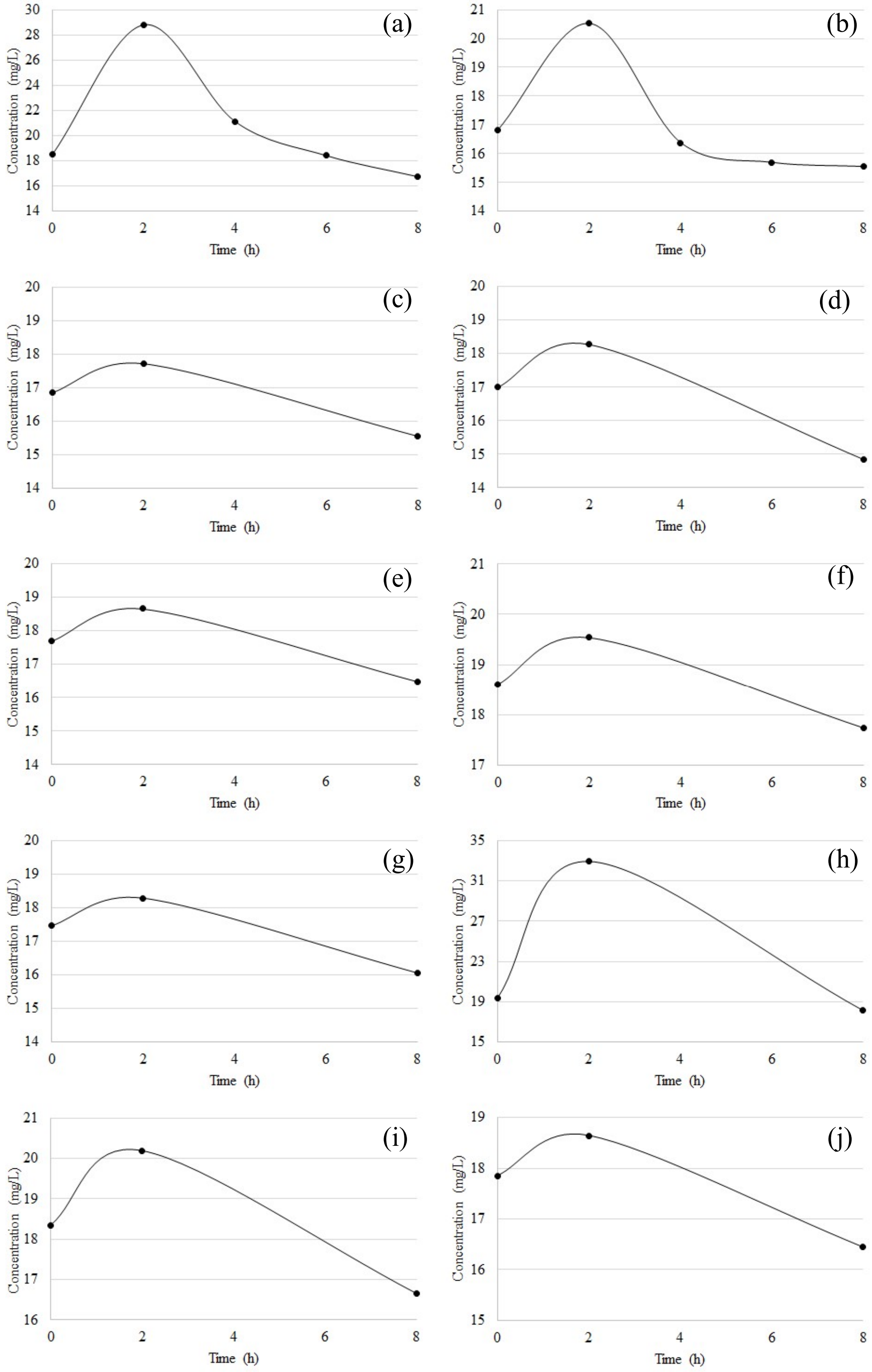

Figure 6.1: Phosphate Concentration Profile on Different Days during Phase 2 at (a) Day 0, (b) Day 1, (c) Day 3, (d) Day 6, (e) Day 8, (f) Day 13, (g) Day 16, (h) Day 18, (i) Day 21, (j) Day 23.

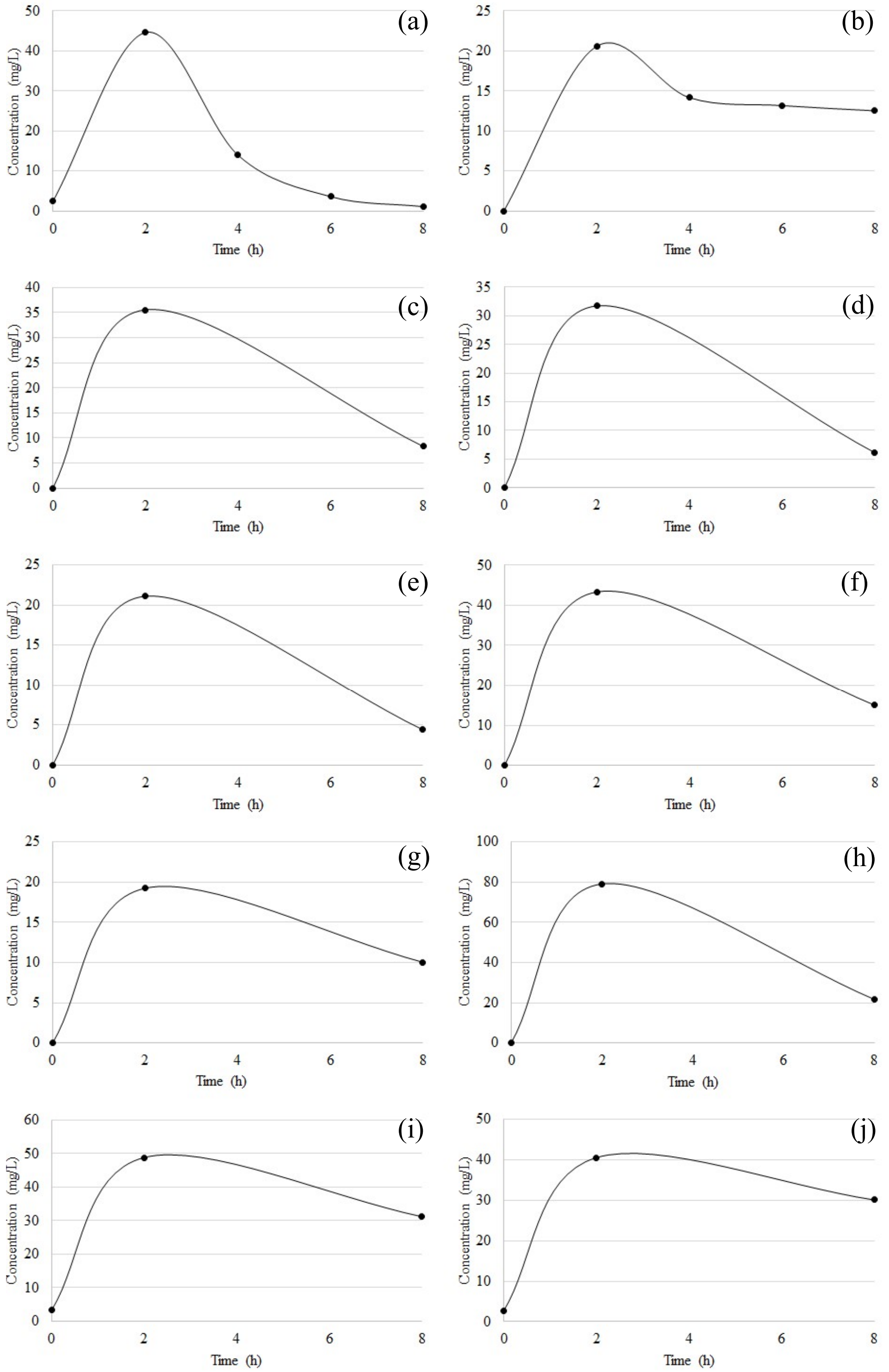

Figure 6.2: Nitrate Concentration Profile on Different Days during Phase 2 at (a) Day 0, (b) Day 1, (c) Day 3, (d) Day 6, (e) Day 8, (f) Day 13, (g) Day 16, (h) Day 18, (i) Day 21, (j) Day 23.

The results in Figure 6.1 and 6.2 verified the simultaneous nitrogen and phosphorus removal within the single DHS system. Both trends of the phosphate and nitrate concentration indicated the presence of EBPR process and nitrification and denitrification activities. Next, the removal efficiency of the three performance indicators were plotted in Figure 6.3, while their concentration profile in the influent and effluent of DHS reactor was shown in Figure 6.4. The DHS reactor posed a higher COD removal efficiency in Phase 2 with the value fluctuated between 86.23 % and 94.24 %. The result was improved as compared to Phase 1 (ranged from 28.69 % to 92.57 %) and this could be attributed to the accumulation of sludge during the operation of Phase 1. In DHS reactor, the organic matters to be digested by the microbes should be captured by the sponge media before they can be digested through biodegradation processes (El-Tabl, Wahaab and Younes, 2013). Throughout the operation of 21 days in Phase 1, the entrapment of sludge and particulate matters had created a sludge filter cake that promote the adsorption and retention of sludge in Phase 2. This can be explained by the rapid establishment of excellent COD removal on Day 0 of Phase 2. Unlike Phase 1 that had a poor COD removal on Day 0 due to the start-up problem, Phase 2 achieved an outstanding removal on the first day itself.

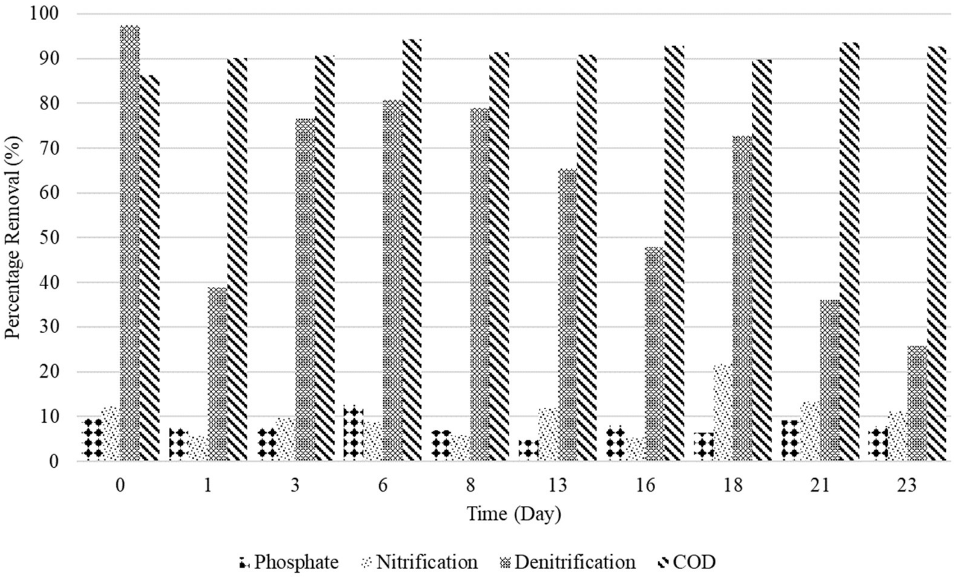

Figure 6.3: Phosphate, Nitrification, Denitrification and COD Removal Efficiency.

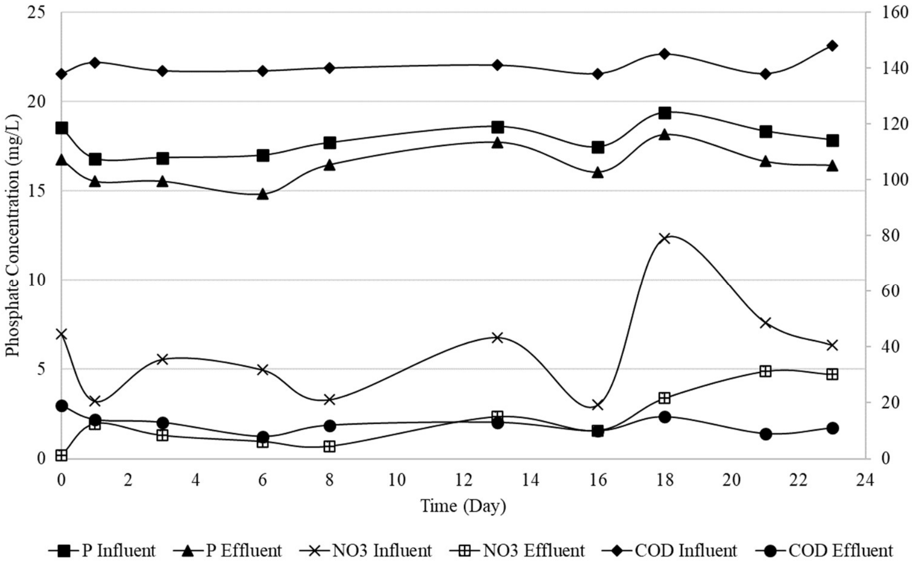

Figure 6.4: Variation of Phosphate, Nitrate and COD Concentration in DHS Reactor Influent and Effluent.

Next, the DHS reactor presented a positive phosphate removal throughout the operation of Phase 2 due to the suitable operating condition that established during Phase 1 of the experiment. The removal efficiency of phosphate was also improved when the phosphate concentration in the influent reduced. In Phase 1, the phosphate concentration was ranged from 18.569 mg/L to 25.371 mg/L, which corresponded to the percentage removal of 2.78 % to 10.68 %. In contrast, the phosphate removal efficiency was improved to the range of 4.70 % to 12.68 % when the phosphate concentration in the influent was between 16.812 mg/L and 19.395 mg/L during Phase 2. When the phosphate concentration in the wastewater decreased, the PAOs and other microorganisms would compete over the small amount of phosphate (as compared to Phase 1) for cell growth. Therefore, with a same amount or number of microbes, a reduction in the phosphate supply would increase the amount of phosphate uptake by the microbes, hence, lead to the improvement in phosphate removal efficiency. On the other hand, the phosphate removal efficiency of DHS reactor experienced a drop between Day 16 and Day 18. This situation was mainly caused by the production of EPSs by the microorganisms in the system. The EPS is a natural polymer that secreted by microorganisms in the natural environments and is usually encountered with bacteria, fungi, algae and yeast (Racho, et al., 2012). They usually present as biofilm, floc or sludge, which serve as the construction material for microbial aggregates. The EPSs have the advantage of holding the biomass cells together and stabilize their attachment on the sponge surface to prevent detachment. It was found out by Tawfik, et al. (2008) that the EPSs also served as an adsorption media to remove ammonium from the wastewater due to their negative charge that can attract the positively charged ammonium ion. Therefore, various cations pollutants in the wastewater that are having mono-, di- and trivalent bond can be bound to the EPSs (Temmink, Klapwijk and Korte, 2001).

Despite the advantages brought by the EPSs, there are several studies that suggested the limit of trickling type bioreactors in biological treatment of wastewater owing to the disadvantages caused by the production of EPS through metabolism of microorganisms. According to the biological clogging experiment carried out by Thullner, et al. (2004), physical plugging and biological clogging phenomenons were observed due to the excessive amount of biomass and accumulation of EPSs, suspended solids and inert. Out of the total organic carbon retained inside a porous media sample, there was approximately 5 % present as bacterial biomass, whereas the remaining 95 % was contributed by the EPSs. In addition, the total volume of the biomass was occupied less than 0.01 % of the total pore space. Therefore, Thullner, et al. (2004) concluded that the observed biological clogging effects in the system were mainly attributed to EPSs. On the other hand, the studied of Racho, Jindal and Wichitsathian (2012) on DHS reactor observed that the formation of EPSs will contribute to the poor effluent quality. Racho, Jindal and Wichitsathian (2012) stated that the DHS system with highest bounded EPS concentration was associated with lowest microbial cell yield and growth rate. Obviously, the biofilm formed by EPSs prevented the influent from penetrating into the pore and posed a negative effect on the biomass consumption rate. Ultimately, the biomass cells that grow underneath the EPSs will die of starving and reduce the available number of bacteria, which is responsible for treating the wastewater. Furthermore, EPSs are soluble in water and the soluble components may give rise to the residual soluble COD (Racho, Jindal and Wichitsathian, 2012). Next, under critical situation where the accumulation of EPSs on the surface become excess, slough off could occur as reported by Liao (2016). As stated by Liao (2016), the EPSs accumulated on the sponge surface could lead to microbial endogenous decay as the wastewater fed cannot penetrate into the sponge. The excessive microbial population on the sponge surface was the trigger to the accumulation of EPSs and eventually led to the occurrence of sloughing; EPSs layer weaken the attachment of biomass on the sponge surface due to increasing hydraulic resistance (Liao, 2016). In short, the generation of EPSs on the surface of the sponge media was the main cause that led to the reducing phosphate removal efficiency between Day 16 and Day 18. Figure 6.5 illustrates the accumulation of EPSs layer on the sponge media in Phase 2 of the experiment. As shown in the figure, the thickness and area covered by the EPSs layer increased as the experiment proceed. The EPSs that accumulated on the top of the sponge media prevent the influent from penetrating into the pores of sponge media. Moroever, overflow and short circuiting of wastewater from the sponge started to occur when the EPSs layer grown thicker and eventually covered up a large area of the sponge. Other than the surface of the sponge media, the EPSs might also accumulate inside the pores of the sponge and in between two sponge media. As a result, some of the wastewater might not be treated and their retention time in the DHS reactor was also reduced. Due to the occurrence of overflow and short circuiting caused by EPSs, the poor removal efficiencies were observed.

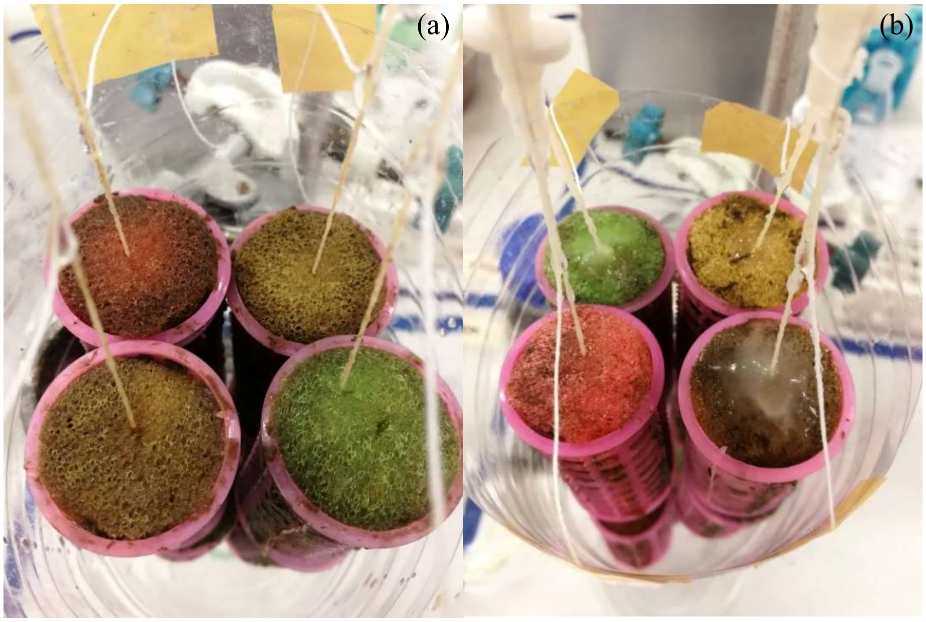

Figure 6.5: Accumulation of Extracellular Polymeric Substances (EPSs) on the Surface of Sponge Media During Phase 2: (a) Day 0, (b) Day 16.

On the other hand, the EPSs layer accumulated on the sponge media was removed on Day 17 to prove the hypothesis of poor performance on phosphate removal due to the EPSs. A spatula was used to scratch off the thick EPSs layer on each of the sponge surface. On Day 21, the phosphate removal efficiency was climbed up to 9.23 %, which is higher than Day 16 and 18 (refer to Figure 6.3). Therefore, the result proven that the presence of EPSs layer will affect the performance of DHS reactor on phosphate removal. However, the removal of phosphate dropped to 7.91 % on Day 23 that may cause by the reaccumulating of EPSs layer.

Besides, the removal of nitrogen was deteriorated during the Phase 2 of experiment. It can be observed that the nitrification efficiency dropped from 6.91 – 39.43 % in Phase 1 to 5.28 – 21.72 % in Phase 2. The decreased in nitrification performance was attributed to the increase in COD loading rates. By referring to Figure 5.9 and 6.4, the COD concentration in the influent of Phase 2 was constantly stayed high at above 138 mg/L while in Phase 1 the value was mostly fluctuated between 120 – 135 mg/L. Although the difference was not significant, but the effect on the nitrification activity was momentous. As discussed early, the high concentration of COD loading will lead to the competition of oxygen between heterotrophs and nitrifying bacteria, which in all time, the latter would defeat (Machdar, et al., 2000; El-Kamah, Mahmoud and Tawfik, 2011). Similar to Phase 1, the denitrification activity in Phase 2 remained high between 4.39 % and 97.42 % despite the drop in nitrification efficiency or high COD loading. In addition to the anaerobic zone deep inside the sponge, the formation of EPSs during the Phase 2 also contributed to the high denitrification activity. As illustrated in Figure 6.5, the EPSs formed a thick layer that covered the surface of the sponge media that would impede the diffusion of air into the sponge matrix. Moreover, the dissolved oxygen in the system became more deficient when the influent wastewater failed to permeate the EPSs layer and enter into the sponge. Therefore, an anaerobic environment that favours the denitrifying bacteria was created at the area covered by EPSs. This phenomenon also described the reduction in the efficiency of aerobic phosphorus uptake.

In short, the changing of phosphorus concentration in the influent of the DHS reactor did affect the performance on nutrient and organic matters removal.

Table 6.1 below summarizes the influent condition of DHS reactor in different phases and the subsequent performance. There are some conclusions can be obtained from the Table 6.1. First, the phosphate removal efficiency increased when influent phosphate concentration reduced. Second, the nitrification efficiency decreased when the influent OLR or COD increased. Third, the denitrification activity inside the DHS reactor was relatively unaffected by the changes in influent nutrient and organic loading concentration. Lastly, the COD removal efficiency increased as the operating time became longer. The results described that DHS reactor was able to achieve simultaneous nutrient and organic matters removal. However, in the case of nutrient removal, the efficiency on phosphorus removal was much lower as compared to the nitrogen removal. The results of phosphate removal were low and the removal might be due to phosphorus uptake by the microbial population in the system for cell growth and maintenance instead of luxury phosphorus uptake by the PAOs and denitrifying phosphorus accumulating organisms (DPAOs). It was clearly shown that the PAOs and DPAOs populations were outcompeted by the nitrifiers and denitrifiers, which described by the high nitrification and denitrification efficiencies over phosphate removal. The operating condition of the DHS reactor had somehow switched to the environment that favour the growth of nitrifiers and denitrifiers. For instance, the accumulation of EPSs created an anaerobic environment that was suitable for the growth denitrifying bacteria. Therefore, without the enrichment of PAOs and DPAOs, the removal of phosphorus nutrient in the DHS reactor appeared to be insignificant.

Table 6.1: Performance of DHS Reactor in Different Phases.

Parameter	**Phase 1**	**Phase 2**
Influent Phosphate (mg/L)	18.569 – 25.371	16.812 – 19.395
Influent COD (mg/L)	120 – 148	138 – 145
Average Influent COD (mg/L)	129	141
Phosphate removal (%)	2.78 – 10.68	4.70 – 12.68
Nitrification (%)	6.91 – 39.43	5.28 – 21.72
Denitrification (%)	20.62 – 98.56	25.75 – 97.42
COD removal (%)	28.69 – 92.57	86.23 – 94.24

6.3 Simultaneous Nitrogen and Phosphorus Removal

Throughout the operation of whole study period, the results showed that there was always nitrogen removal during the treatment of synthetic wastewater by DHS reactor, despite the introduction of ATU. In other words, the nitrate was invariably present during the EBPR process. Generally, the presence of nitrate is considered to be an inhibition to biological phosphorus removal by PAOs. This is because the nitrate will undergo denitrification process to convert into nitrogen gas in the anaerobic zone, whereby the process consumes the organic substrates available in the system. The denitrification process will reduce the available organic substrates for the uptake by PAOs that to be later used for luxury phosphorus uptake in the aerobic zone. Without sufficient uptake of organic substrates by PAOs under anaerobic condition, there will be insufficient PHB stored to produce energy for phosphorus uptake under aerobic condition, hence, the process is said to be inhibited. However, based on the investigations carried out by numerous researches, it was believed that the presence of nitrate is helpful for the phosphorus removal by PAOs instead of inhibition.

According to Xiao, et al. (2013); Kuba, et al. (1993); and Kerrn-Jespersen and Henze (1993), the PAOs can uptake phosphorus with different electron acceptor conditions: only O_2 presence, only NO_3^- presence, both O_2 and NO_3^- coexisting. As dicussed previously, the biological phosphorus uptake involved PAOs that release intracellular phosphate during anaerobic condition to obtain energy for the uptake of carbon source, and followed by the excess phosphorus uptake during aerobic condition. The study done by Xiao, et al. (2013) observed that the phosphorus uptake by PAOs could also happened under anoxic condition. Anoxic condition is a description of environment without any oxygen but with the presence of common electron acceptor such as nitrate, nitrite or sulfate. It is different from the anaerobic condition where there is no any common electron acceptor including oxygen. This study found out that there was at least one part of PAOs that can utilize nitrate as an electron acceptor to carry out phosphorus uptake under anoxic condition. This process is in fact similar to the aerobic phosphorus uptake, except nitrate is used as the electron acceptor rather than oxygen. The PAOs responsible for the phosphorus uptake using nitrate as the electron acceptor is known as DPAOs, whereas the process is called as denitrifying phosphorus uptake. The finding of these researches was the specific phosphorus uptake rates were higher under the anoxic condition (coexist of O_2 and NO_2^-) than those with only O_2 present. This finding indicated that there exists denitrifying phosphorus uptake by the DPAOs under anoxic condition, which utilized nitrate as the electron acceptor. Moreover, the organic carbon source require by the DPAOs was much lesser as compared to aerobic phosphorus removal by the PAOs, which is an advantage of DPAOs over PAOs (Li, et al., 2009; Peng, et al., 2011). In short, there were three phosphorus removal mechanisms being observed: the aerobic phosphorus uptake by PAOs, the aerobic denitrifying phosphorus uptake by DPAOs and the anoxic denitrifying phosphorus uptake by DPAOs.

Next, the study carried out by Meinhold, et al. (1998) shows the similar results as above study. The phosphorus uptake and release were observed simultaneously under the presence of nitrate under anoxic condition. Moreover, the measure of overall denitrification rate reflected that the rate was an addition of denitrification rate by common denitrifiers (non-PAOs) and the DPAOs. Therefore, nitrate was seen to be an advantage to the biological phosphorus removal process owing to the contribution by DPAOs. Under simultaneous nitrogen and phoshorus removal, the organic substrates in the wastewater are utilized effectively as DPAOs will uptake phosphate by using nitrate as electron acceptor. The usage of nitrate as an electorn acceptor is of significance as the common limiting factor in nutrient removal is often the availability of organic substrate. In addition, this also indicated that aeration and sludge production can be reduced as the DPAOs do not demand on oxygen as an electorn acceptor; they use nitrate instead. The study also stated that DPAOs will slow down the anoxic phosphorus uptake by using nitrate as an electron acceptor when the stored organic material inside the cell becomes limiting. In contrast, the PAOs will have their intracellular organic supply that stored under anaerobic phase intact and the level even rised during the anoxic period. In other words, under anoxic condition, DPAOs are responsible for anoxic phoshorus uptake and PHB utilization whereas the normal PAOs will release intracellular phosphorus and accumulate PHB at the same time. Whether there is a net phosphorus uptake or release and a net PHB accumulation or utilization are very much depending on the relative speed of both processess. During a higher rate of organic matters supply to the system, the anoxic phosphorus release and PHB accumulation by the PAOs will become dominant and eventualy disguise the phosphorus uptake by DPAOs.

Similarly, the results table in Appendix B showed a simultaneous nitrogen and phosphorus removal. The results obtained from this study were in agreement with the findings of the researchers discussed above. First, the nitrate concentration in Figure 5.2 and Figure 6.2 rose to a peak and then followed by a drop, which indicated that both nitrification and denitrification processes were existed. This meant that the DHS system composed of aerobic and anaerobic conditions as proposed by Araki, et al. (1999); Machdar, et al. (2000); and Machdar, et al., (1997) where the oxygen concentration will diminish when getting deeper into the centre of sponge media. Secondly, the phosphorus removal was observed together with the occurrence of nitrogen removal and this indicated that DPAOs were present in the mixed culture. The removal of phosphorus was achieved by the PAOs and DPAOs by using oxygen and nitrate as electron acceptor respectively. In the DHS reactor, PAOs will first release their intracellular phosphorus and uptake organic materials from the environment that will be later stored as PHB. Meanwhile, the nitrification process also takes place to oxidize the ammonium and produces nitrate. This was illustrated by the increasing trend of phosphate and nitrate concentration at the beginning of the cycle and this marked the presence of anoxic environment in the system. Next, the decreasing trend of phosphate was due to the luxury phosphorus uptake by PAOs under aerobic condition where oxygen was used as the electron acceptor. Besides, the DPAOs also utilized nitrate as an electron acceptor in anoxic environment to uptake phosphorus, which described the declining trend of nitrate. It is also true that the reduction in nitrate concentration was mostly contributed by the denitrification activities, but there was possibility that the nitrate was consumed by the DPAOs as supported by the findings of the above researchers. In short, the presence of two parts of PAOs (PAOs and DPAOs) was an advantage to the DHS reactor in achieving simultaneous nitrogen and phosphorus removal within a single unit or system. Normally, the removal of both nutrients does not coexist in a conventional biological sewage treatment. Aerobic and anaerobic environment are normally created using two separate systems in the conventional field. This phenomenon shows the advantage of DHS reactor over conventional sewage treatment system.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

In this study, the treatment of synthetic sewage using the DHS system was evaluated. The DHS reactor that employing third generation sponge module (sponge media encapsulated in support material) was operated at a working volume of 2 L per day. Based on the available results, the performance of the proposed system demonstrated a concurrent nutrient (nitrogen and phosphate) and organic matters removal. The range of phosphorus removal, COD removal, nitrification efficiency and denitrification efficiency achieved from DHS reactor were 2.78 – 12.68 %, 28.69 – 94.24 %, 5.28 – 39.43 %, 20.62 – 98.56 %, respectively. From the results, the DHS system showed an outstanding performance on organic removal with an average removal efficiency on phosphorus and nitrogen.

Next, few findings had been observed from the results of the experiment. First, the DHS system composed of three different environments that were aerobic, anaerobic and anoxic. The presence of aerobic zone was identified by the removal of phosphorus and increase in nitrate concentration due to nitrification, whereby the progress of both processes require oxygen. Furthermore, the reduction of nitrate concentration by denitrification was corresponded to the presence of anaerobic zone. Also, the combination of anaerobic zone and the presence of nitrate in the DHS system confirmed the existence of anoxic zone.

Second, PAOs that responsible for phosphorus removal would undergone EBPR process, which indicated by the increase in phosphate concentration that was higher than the influent. PAOs will release intracellular polyphosphate (result in increase in phosphate concentration) during anaerobic zone to assimilate PHB and then follow by the luxury phosphorus uptake under aerobic condition. On the other hand, there was also possible present of the DPAOs that utilized nitrate (rather than oxygen) as the electron acceptor to uptake phosphate because nitrate was always present due to nitrification activity by the nitrifiers.

Third, the increase and decrease of nitrate concentration indicated the existence of nitrifiers and denitrifiers respectively. It was an interesting finding that the DHS reactor could achieved simultaneous nitrogen and phosphorus removal within a single system. However, the DHS reactor environment in this study was more favoured to the nitrogen removal. The accumulation of EPSs layer coupled with the anaerobic zone present inside the middle of the sponge media had deteriorated the phosphorus removal efficiency due to limited dissolved oxygen concentration. The PAOs were said to be outcompeted by the nitrifiers in the competition over oxygen. In addition, the heterotrophs that responsible for organic removal would also consume oxygen and compete with the nitrifiers and PAOs. Without sufficient oxygen, the PAOs failed to uptake phosphate through EBPR process and eventually loss their activities, which can be observed from the poor phosphate removal percentage. In this study, the sequence of competitiveness over oxygen will be heterotrophs > nitrifiers > PAOs.

Throughout the study period, there was no mechanical aeration provided to the system, yet the aerobic nutrient removal such as nitrification and phosphate uptake by PAOs were still observed. Moreover, it was observed that there was almost zero sludge produced at the effluent of the system, which suggested that the sponge matrix is capable of trapping particulate or suspended solids. Due to this merit of the sponge media, the DHS system was able to keep the COD concentration in the effluent below 20 mg/L throughout the study period. Most importantly, the COD concentration in the final effluent was corresponded with the discharge standards of sewage treatment system that approved after October 2009, which must be lower than 120 mg/L for standard A discharge (refer to Table 2.5). Thus, the DHS system with the combination of aerobic, anaerobic and anoxic environment unambiguously ensures a low cost (with no aeration requirement), less generation of excess sludge and small area requirement with nutrient and organic matters removal capacity. Therefore, the DHS system could be a potential solution to overcome the prevalent problem of nutrient presence in the municipal sewage. The system could be combined with the existing sewage treatment plant in Malaysia to treat the effluent from the conventional activated sludge process due to its nutrient and organic removal capability.

7.2 Recommendations for Future Work

The operation of the DHS system should be changed to automatic dilution and injection system instead of operate it mechanically. During Phase 1 of the study, the phosphorus removal was not achieved in DHS reactor due to the slowing and stopping of influent flow. The influent to the system must be refilled from time to time to ensure the water level is sufficient to induce the flow onto the sponge media. Meanwhile, the flowrate of influent had to be adjusted mechanically whenever there is a slowing of flow, which contributed to the uncertainty that might affect the results accuracy. Therefore, the employment of an automatic dilution and injection system to control the flow of influent is highly recommended. For this automatic system, concentrated "P-water", concentrated feed and distilled water can be prepared separately and store inside containers. Next, a flow and pumping system can be applied to channel the synthetic wastewater onto the sponge surface. The dilution factor of the concentrated "P-water" and feed can be adjusted by controlling the flowrate of distilled water through flow meter. With this automatic dilution and injection system, the problem of slowing or stopping of flow due to water level and pressure inside the laboratory could be avoided. In addition, human error and possible contamination during dilution of synthetic wastewater could be minimized with the aid of automatic system.

Moving forward, the cleaning of glassware and apparatus by using detergent that containing phosphorus ingredient should be avoided. Since this study was focused on the nutrient removal that included phosphorus, the phosphate or phosphorus content in the detergent might adsorbed on the apparatus and glassware during washing. It is possible for the adsorbed phosphate to dissolve into the effluent and samples, hence, affect the accuracy of the results. It was recommended to apply phosphate-free detergent to clean the apparatus and glassware to ensure the source of phosphate is only originated from the chemicals added during preparation of synthetic wastewater.

On the other hand, the focuses of this study were on the establishment of DHS reactor for nutrient removal and effect of phosphorus concentration in the influent to the performance of DHS reactor. Besides the parameter studied in this experiment, there are several other important operational factors that will affect the efficiency of DHS system such as HRT and sponge size or volume. It is known that the retention time of wastewater in the sponge media is depending on the HRT and sponge volume. The variation in HRT and sponge volume would greatly affect the results because it is directly related to the contact time between the wastewater and microorganism. Hence, proper study on other operating factors should be conducted in the future.

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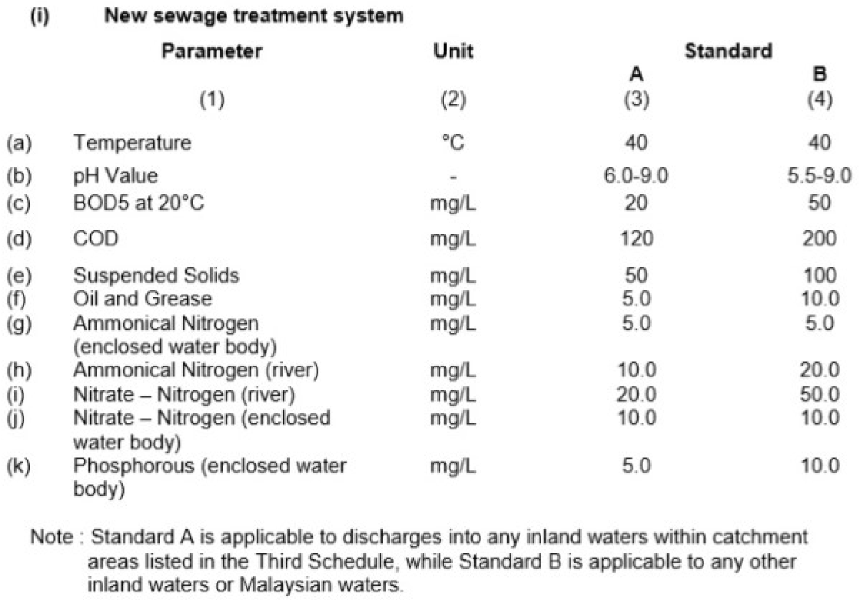
APPENDICES

APPENDIX A: Sewage Discharge of Standard A and B

The following figures show the sewage discharge of standard A and B. The information is extracted from Second Schedule (Regulation 7) that are recorded in Environmental Quality (Sewage) Regulations 2009 (PU (A) 432). There are three different sets of regulations on the acceptable conditions of sewage discharge of standards A and B for different types of sewage treatment plant: (a) New sewage treatment system (Figure A-1), (ii) Existing sewage treatment system that approved before January 1999 (Figure A-2), and (iii) Existing sewage treatment system that approved after January 1999 (Figure A-3).

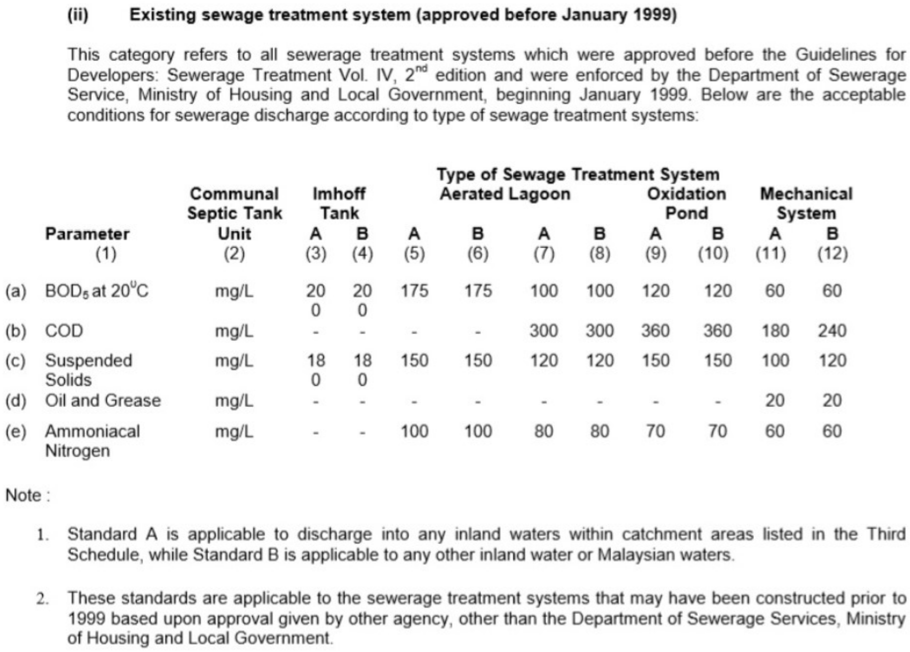


(i) New sewage treatment system

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

Note : Standard A is applicable to discharges into any inland waters within catchment areas listed in the Third Schedule, while Standard B is applicable to any other inland waters or Malaysian waters.

Figure A-1: Sewage Discharge Standards A and B for New Sewage Treatment System.



(ii) Existing sewage treatment system (approved before January 1999)

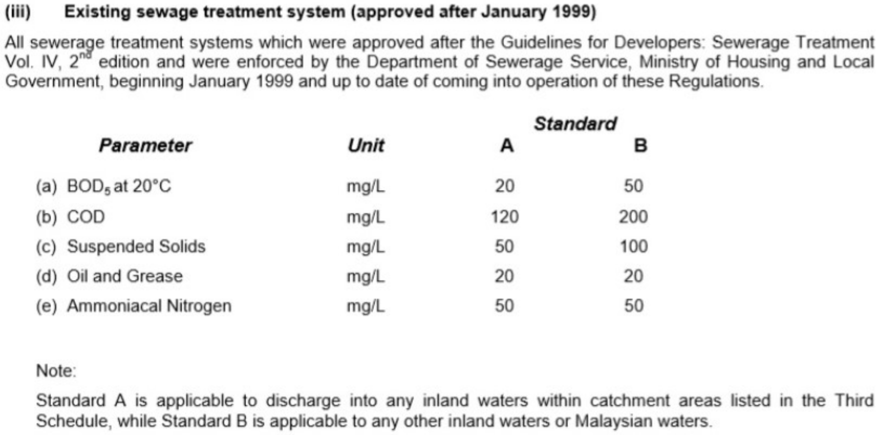
This category refers to all sewerage treatment systems which were approved before the Guidelines for Developers: Sewerage Treatment Vol. IV, 2nd edition and were enforced by the Department of Sewerage Service, Ministry of Housing and Local Government, beginning January 1999. Below are the acceptable conditions for sewerage discharge according to type of sewage treatment systems:

						Type of Sewage Treatment System						
		Communal Septic Tank	Imhoff Tank			Aerated Lagoon			Oxidation Pond		Mechanical System	
	Parameter (1)	**Unit** (2)	**A** (3)	**B** (4)	**A** (5)	**B** (6)	**A** (7)	**B** (8)	**A** (9)	**B** (10)	**A** (11)	**B** (12)
(a)	BOD$_5$ at 20^{0}C	mg/L	200	200	175	175	100	100	120	120	60	60
(b)	COD	mg/L	-	-	-	-	300	300	360	360	180	240
(c)	Suspended Solids	mg/L	180	180	150	150	120	120	150	150	100	120
(d)	Oil and Grease	mg/L	-	-	-	-	-	-	-	-	20	20
(e)	Ammoniacal Nitrogen	mg/L	-	-	100	100	80	80	70	70	60	60

Note :

1. Standard A is applicable to discharge into any inland waters within catchment areas listed in the Third Schedule, while Standard B is applicable to any other inland water or Malaysian waters.
2. These standards are applicable to the sewerage treatment systems that may have been constructed prior to 1999 based upon approval given by other agency, other than the Department of Sewerage Services, Ministry of Housing and Local Government.

Figure A-2: Sewage Discharge Standards A and B for Existing Sewage Treatment System That Approved Before January 1999.



(iii) Existing sewage treatment system (approved after January 1999)

All sewerage treatment systems which were approved after the Guidelines for Developers: Sewerage Treatment Vol. IV, 2nd edition and were enforced by the Department of Sewerage Service, Ministry of Housing and Local Government, beginning January 1999 and up to date of coming into operation of these Regulations.

		Standard	
Parameter	***Unit***	**A**	**B**
(a) BOD$_5$ at 20°C	mg/L	20	50
(b) COD	mg/L	120	200
(c) Suspended Solids	mg/L	50	100
(d) Oil and Grease	mg/L	20	20
(e) Ammoniacal Nitrogen	mg/L	50	50

Note:

Standard A is applicable to discharge into any inland waters within catchment areas listed in the Third Schedule, while Standard B is applicable to any other inland waters or Malaysian waters.

Figure A-3: Sewage Discharge Standards A and B for Existing Sewage Treatment System That Approved After January 1999.

APPENDIX B: Results Table

Table B-1: Physio-Chemical Parameters of DHS Reactor During Phase 1.

Day	Hour (h)	COD Concentration (mg/L)	Phosphate Concentration (mg/L)	Nitrate Concentration (mg/L)	Amount of N atom in Nitrate (mg/L)
0	0	122	18.569	1.140	0.258
	2	50	26.260	143.564	32.431
	4	106	19.611	16.195	3.658
	6	87	18.966	4.675	1.056
	8	87	18.576	2.065	0.466
1	0	132	15.761	1.136	0.257
	2	58	14.894	78.541	17.742
	4	24	15.129	5.363	1.212
	6	23	14.334	4.598	1.039
	8	20	13.128	4.491	1.015
3	0	121	15.724	1.099	0.248
	2	13	19.858	44.128	9.969
	4	11	18.040	22.619	5.110
	6	10	16.991	12.790	2.889
	8	10	16.798	16.831	3.802
6	0	130	20.936	0.000	0.000
	2	18	26.293	46.802	10.573
	4	16	24.342	25.815	5.832
	6	16	23.955	30.285	6.841
	8	13	23.752	18.602	4.202
8	0	120	20.993	0.000	0.000
	2	23	30.030	57.416	12.970
	4	21	25.115	29.658	6.700
	6	11	24.095	22.384	5.057
	8	16	23.123	20.112	4.543

Table B-1 (Continued)

13	0	148	24.892	1.274	0.288
	8	21	26.000	27.830	6.287
	24	11	24.199	21.087	4.764
16	0	145	25.371	0.000	0.000
	8	19	25.995	25.720	5.810
	24	12	23.592	15.570	3.517
18	0	125	18.582	0.000	0.000
	8	21	18.982	25.153	5.682
	24	12	16.598	19.967	4.511
21	0	121	17.524	0.000	0.000
	8	18	18.506	30.843	6.967
	24	15	19.417	10.374	2.343

Table B-2: Performance of DHS Reactor During Phase 1.

Day	**Percentage Removal (%)**			
	COD	**Phosphate**	**Nitrification**	**Denitrification**
0	28.69	-0.04	39.43	98.56
1	84.85	16.71	21.57	94.28
3	91.74	-6.83	12.12	61.86
6	90.00	-13.45	12.85	60.25
8	86.67	-10.15	15.77	64.97
13	92.57	2.78	7.64	24.23
16	91.72	7.01	7.06	39.46
18	90.40	10.68	6.91	20.62
21	87.60	-10.80	8.47	66.37

Table B-3: Physio-Chemical Parameters of DHS Reactor During Phase 2.

Day	**Hour (h)**	**COD Concentration (mg/L)**	**Phosphate Concentration (mg/L)**	**Nitrate Concentration (mg/L)**	**Amount of N atom in Nitrate (mg/L)**
0	0	138	18.528	2.385	0.539
	2	-	28.824	44.639	10.084
	4	-	21.138	13.983	3.159
	6	-	18.426	3.649	0.824
	8	19	16.748	1.152	0.260
1	0	142	16.812	0.000	0.000
	2	-	20.534	20.534	4.639
	4	-	16.380	14.178	3.203
	6	-	15.691	13.178	2.977
	8	14	15.549	12.533	2.831
3	0	139	17.247	0.000	0.000
	2	-	17.905	35.523	8.025
	8	13	16.257	8.333	1.882
6	0	139	16.997	0.000	0.000
	2	-	18.278	31.771	7.177
	8	8	14.841	6.132	1.385
8	0	140	17.698	0.000	0.000
	2	-	18.662	21.126	4.772
	8	12	16.478	4.452	1.006
13	0	141	18.607	0.000	0.000
	2	-	19.544	43.365	9.796
	8	13	17.733	14.997	3.388
16	0	138	17.462	0.000	0.000
	2	-	18.278	19.215	4.341
	8	10	16.064	10.029	2.266

Table B-3 (Continued)

18	0	145	19.395	0.000	0.000
	2	-	32.942	79.083	17.865
	8	15	18.157	21.608	4.881
21	0	138	18.351	3.494	0.789
	2	-	20.193	48.766	11.016
	8	9	16.658	31.222	7.053
23	0	148	17.852	2.765	0.625
	2	-	18.640	40.577	9.166
	8	11	16.440	30.128	6.806

Table B-4: Performance of DHS Reactor During Phase 1.

Day	**Percentage Removal (%)**			
	COD	**Phosphate**	**Nitrification**	**Denitrification**
0	86.23	9.61	12.26	97.42
1	90.14	7.51	5.64	38.96
3	90.65	7.74	9.76	76.54
6	94.24	12.68	8.73	80.7
8	91.43	6.89	5.80	78.93
13	90.78	4.70	11.91	65.42
16	92.75	8.01	5.28	47.81
18	89.66	6.38	21.72	72.68
21	93.48	9.23	13.39	35.98
23	92.57	7.91	11.14	25.75