DOWN HANGING SPONGE (DHS) FOR NUTRIENT REMOVAL IN WASTEWATER 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 **"DOWN HANGING SPONGE (DHS) FOR NUTRIENT REMOVAL IN WASTEWATER TREATMENT"** was prepared by **TAN JIAN YU** 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

Water pollution has emerged to be a global issue for the past few decades that shows significant impacts to the environment and human health. Nutrients consist of nitrogen and phosphorus are the most common pollutant that contribute to the degradation of water quality. Conventional wastewater treatments such as activated sludge process and trickling filter are costly and unaffordable to the developing countries. Down-flow hanging sponge system (DHS) is proposed to be an innovative solution for the prevalent wastewater treatment problem due to its simplicity and affordable cost. In this study, the nutrients removal efficiency was investigated among the six types of DHS system. Several major factors influencing the performance of DHS system and microbial community in DHS reactor were also investigated. The review report commenced with collecting the published research papers followed by analyzing the data collected and lastly summarizing their findings to produce an overall review. From the findings, it was observed that DHS system exhibited satisfactory nutrients removal as well as excellent organic matter removal. Third generation and sixth generation DHS reactor showed better nutrients removal with more than 70 % ammonium nitrogen removal from most of the past research papers. Next, for the microbial community in DHS reactor, the compositions varied along with the height of the reactor. The bacteria involved in organic removal and nutrients removal was detected. Other functionality bacteria related to methane removal, sulfur removal and sludge degradation were also detected in the reactor. In addition, the performance of DHS reactor could be further enhanced by taking the parameters such as HRT, HLR, OLR, operating temperature, sponge size and ventilation system into account. In short, DHS system is recommended to replace conventional wastewater treatment for nutrients removal in the developing countries.

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LIST OF SYMBOLS / ABBREVIATIONS

ATU	allythiourea
ASP	activated sludge process
BOD	biochemical oxygen demand
COD	chemical oxygen demand
DHS	down-flow hanging sponge
DO	dissolved oxygen
DPAO	denitrifying polyphosphate accumulating organism
DRB 2000	digital reactor block
EBPR	enhanced biological phosphorus removal
HLR	hydraulic loading rate
HRT	hydraulic retention time
OLR	organic loading rate
PAO	polyphosphate-accumulating organism
PHB	poly-hydroxybutyrate
SCFA	short chain fatty acid
SMP	soluble microbial products
SRT	sludge residence time
SS	suspended solids
TBOD	total biochemical oxygen demand
TCOD	total chemical oxygen demand
TKN	total Kjeldahl nitrogen
TN	total nitrogen
TP	total phosphorus
TSS	total suspended solids
UASB	upflow anaerobic sludge blanket
USGS	United States Geological Survey
USHB	upflow submerged hanging bed

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CHAPTER 1

INTRODUCTION

1.1 Global Water Crisis and Water Pollution

Water is one of the basic needs for every living being on Earth. A person can live without food for a couple of weeks but cannot survive without water for just two days. In fact, one needs at least need 2 litres of drinking water every day for a healthy life. According to WHO, an average domestic use of water is at least 30 to 40 litres, which include daily activities such as bathing, cooking, and laundry. However, water is a finite resource and the demand for safe and clean water is increasing by day.

Global water crisis is a major growing issue, in which about 844 million people around the world have been struggling in water scarcity. Moreover, the human population is expected to increase to 40-50% in the next 50 years. This has brought water crisis issue into attention, as the most affected population are children and women. Without access to clean and safe water, children are easily infected with waterborne illness as they have weaker immune system. Indeed, about 3900 children suffered death from waterborne diseases every day. Furthermore, safe water access is one of the strategies to alleviate poverty (Harvey, 2008). In low-income countries, clean water access is very limited. To collect clean water, women and children are responsible to walk a long distance under blazing sun just to obtain few litres of clean water (Paul and Lama, 2020).

Today, 80% of the wastewater around the world is left untreated, thereby causing pollution to the rivers, seas, and lakes. With the growing sector of agricultural field and industrial revolution, water pollution is a serious issue to be taken of. Water pollution is the main cause of unsafe water, and it consists of pollutants, which are the main cause of the drastic reduction in water quality. Examples of pollutants include human wastes and toxic chemical discharges from industrials and agricultures field. Globally, nutrients are the most common pollutant that contribute to the reduction of water quality. Eutrophication is the result of high nutrients loading, which substantially degrade the water quality (UNESCO, 2017).

1.2 Wastewater

The main cause of water pollution is wastewater. As defined by the United States Geological Survey (USGS), wastewater is any kind of water that is polluted by human use. In other words, wastewater is defined as used water. It contains substances like human waste, food fragment, oil, detergents, and chemicals. From household wastes, it comprises water from domestic use such as sink water, toilets, and laundry wastewater. On the other hand, industrial wastewater also contributes to the pollution of water. Furthermore, storm runoff is also considered as wastewater. This is because it contains harmful substances from roads, rooftops and cars, which eventually flows to lakes and rivers, hence causing water pollution (USGS, n.d.).

Wastewater gives significant impacts not only to the environment, but also to human health. For instance, untreated wastewater harms animals and wildlife habitats, causes depletion of oxygen, leads to appearance of diseases, closure of beaches and many other restrictions, such as restrictions on fish harvesting. Common pollutants found in wastewater include organic matters, chlorine, metals such as lead and mercury, bacteria, and nutrients. Each pollutant has its own significant effects and therefore it must be carefully treated or effectively eliminated from the wastewater. The process of removal of pollutants from wastewater is known as wastewater treatment (USGS, n.d.).

1.3 Nutrients

Nutrients are one of the main pollutants in wastewater. It consists of mainly nitrogen and phosphorus which are naturally available in the aquatic ecosystem. Nitrogen and phosphorus help in the growth of aquatic plants and algae, which are food for fishes and smaller organisms living in the water (USEPA, 2019).

Excessive nitrogen and phosphorus in water will cause the dramatic increase in the growth of algae. Excessive growth of algae is known as algae bloom. Algae bloom will block the sunlight from entering the water, which leads to death of aquatic plants as they are unable to carry out photosynthesis without sunlight. Then, algae bloom will die eventually and sink into the bottom of the water. The bacteria will carry out decomposition to decompose the dead algae. However, decomposition requires oxygen for respiration. This will reduce the oxygen concentration in the water and therefore causing the aquatic life to suffocate to death. The water is no longer support any life or habitats. This process is known as eutrophication. Besides, some algae blooms are dangerous to humans. It releases toxins and bacteria that can cause diseases to humans if they are in contact with the contaminated water. For example, consuming the fishes captured from the water or drink the polluted water (USEPA, 2019).

1.4 Wastewater Treatment

Wastewater treatment is the process to remove contaminants in wastewater which convert them into cleaner and safer water that is fit for human use. The pollutants are either removed, broken down or converted to safer components during the process. In other words, it is a technology applied to improve the quality of wastewater. Common wastewater treatment includes three processes: physical, chemical and biological.

Physical process is a process where only physical phenomena are utilized to treat the wastewater. The most common physical processes are sedimentation, aeration, and filtration. Sedimentation applies gravity to settle the solid particles from the wastewater, and thus allowing the suspended solids (SS) to be separated and removed. Aeration supplies oxygen to the wastewater physically. Filtration consists of a filter medium to filter the solid particles from the wastewater (Toprak Wastewater Engineering, 2006).

Chemical process is a process that involves chemical reactions to enhance water quality, such as chlorination, neutralization, and coagulation. In the process of chlorination, chlorine is used as an oxidizing agent to kill germs and reduce the rate of decomposition of wastewater. On the other hand, neutralization is the process of adding acid or base to balance the pH value of the wastewater, while coagulation is the process of adding chemicals to coagulate the contaminants, allowing it to be removed from the wastewater easily (Toprak Wastewater Engineering, 2006).

Biological process in wastewater treatment utilises microorganisms such as bacteria to decompose the undesired wastes from wastewater into safer end products like carbon dioxide and water. It is divided into two categories: aerobic and anaerobic method. Aerobic method requires oxygen while anaerobic method does not require oxygen to treat the wastewater (Toprak Wastewater Engineering, 2006).

Generally, conventional wastewater treatment consists of three steps: primary, secondary and tertiary or advanced. In primary wastewater treatment, physical processes such as screening, comminution, grit disposal and sedimentation are used to remove most of the solids. It generally removes up to 35 % of Biological Oxygen Demand (BOD) and 60 % of total suspended solids (TSS). The effluent of primary treatment becomes the influent for secondary treatment. Secondary treatment utilizes the biological method, which involves bacteria to decompose the remaining contaminants, especially soluble organic matter. The common methods are trickling filters and activated sludge process. It can remove up to 85% of both BOD and TSS. Lastly, tertiary treatment is used to further remove the pollutants, mainly nutrients from the effluent of secondary treatment. It can remove up to 99% of the pollutants. However, it is seldom applied in wastewater treatment plants as the first two treatments are sufficient to achieve the desired water quality. Moreover, tertiary wastewater treatment requires high cost, which in fact cost up to almost double the price of secondary treatment. Thus, it is only utilized under certain circumstances (Encyclopaedia Britannica, 2019).

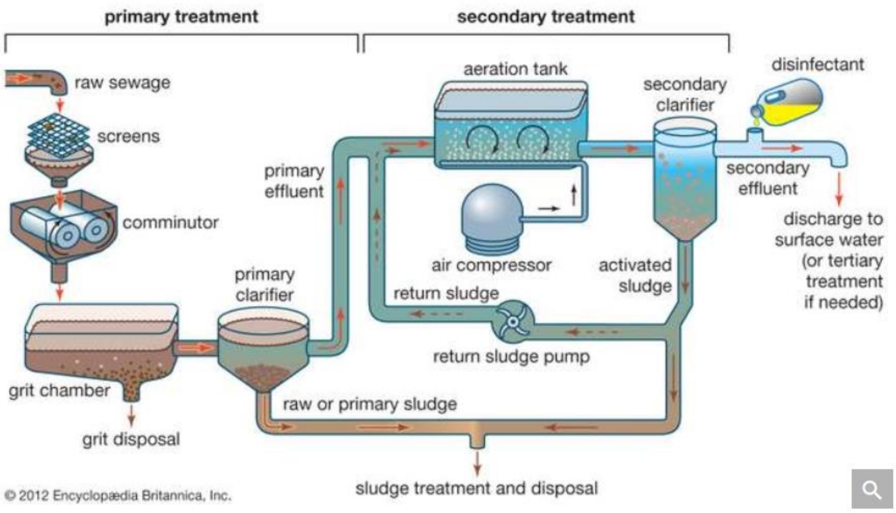

Figure 1.1: Wastewater Treatment Process (Encyclopaedia Britannica, 2019).

1.5 Down-flow Hanging Sponge (DHS) System

Down-Flow Hanging Sponge (DHS) is an aerobic treatment that has a similar principle to trickling filter, where the wastewater influent enters at the top of the supporting medium and flows down to the bottom of the reactor. However, the major difference between DHS and trickling filter system is that DHS uses polyurethane sponge instead of stones and plastic materials, this is because the sponge acts as a supporting material for the growth of microorganisms which will effectively oxidize the wastewater in the presence of oxygen, reducing the BOD of the wastewater (Nurmiyanto and Ohashi, 2019).

The sponges are not submerged in wastewater, exposing to the atmosphere and the high porosity of the sponge medium provides longer cell residence time. These allow oxygen to be diffused into the sponge easily. Therefore, it does not require any external forced aeration unlike the existing aerobic methods such as trickling filter and activated sludge process. Besides, the sponge has a void space of more than 90%, which ensure a perfect site for the growth and attachment of biomass. Thus, more biomass will be retained on the sponge, which causes significant increment in biomass concentration and sludge residence time (SRT). The longer SRT of DHS system provides sufficient time for the biomass to degrade, thus reducing the formation of excess sludge from the treatment. With the negligible sludge production, backwashing is not necessary for DHS system. Furthermore, the polyurethane sponge exhibits satisfactory water retention in the pores, which leads to longer hydraulic retention time (HRT). Hence, DHS can be developed at a smaller area compared to the conventional wastewater treatment system. The presence of anoxic zone in the DHS system shows that the aerobic and anaerobic can be carried out in a DHS system, which is suitable for nutrients removal. Moreover, the sponge is non-biodegradable, simple to be constructed, widely available and reasonable price. These advantages have proven DHS to be a potential alternative method to replace the costly conventional wastewater treatment system (Uemura and Harada, 2010).

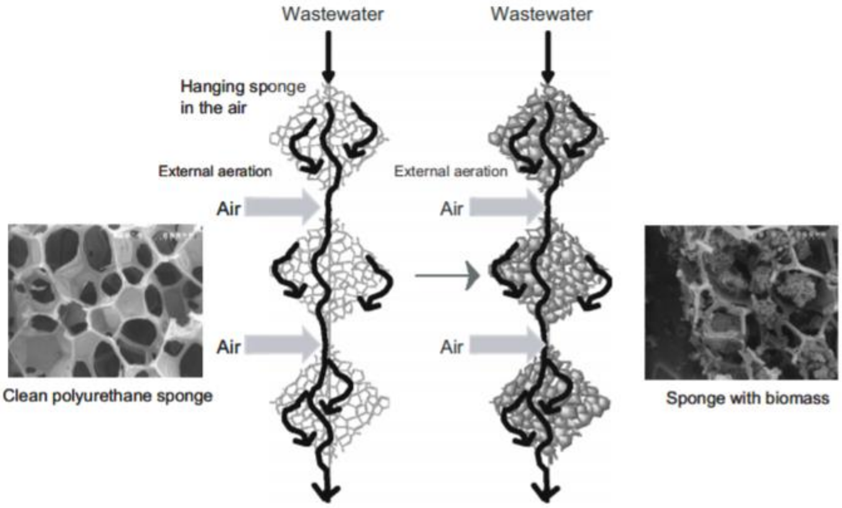

Figure 1.2: Principle of DHS (Uemura and Harada, 2010).

1.6 Problem Statement

Water pollution has emerged to be a serious issue in the past few decades. This is due to the increasing global development that leads to increasing number of industrial and agricultural activities. Chemical and toxic substances such as pesticides and asbestos that are released from industries and agricultures will contaminate water and decrease the water quality. Eutrophication contributes the most to the degradation of water quality. It is due to the excessive nutrients such as phosphorus and nitrogen. Therefore, it is crucial to treat the wastewater to remove nutrients or reduce the nutrient level to an acceptable level.

Besides, the conventional wastewater treatment such as activated sludge process and trickling filter system are not only costly and unaffordable to developing countries, but also hard to construct and operate. This is mainly due to the requirement for an external aeration system to provide oxygen for aerobic process. Therefore, DHS is a potential alternative to this problem due to its simplicity, low cost and high efficiency for removal of nutrients.

1.7 Aims and Objectives

DHS system is still an emerging wastewater treatment technology. Despite its advantages over conventional wastewater treatment, there are still inadequate information for the enhancement of performances in the DHS system. Not only that, DHS reactor has only been implemented by a few countries such as India and Thailand. Therefore, the objectives of this project are:

1) To review the performance of nutrient removal in different type of DHS system.
2) To review the parameters affecting the performance of DHS system.
3) To review the relationship of microbial population and the nutrient removal performance in DHS system.

1.8 Scope of the Study

A brief introduction about global water crisis and water pollution, wastewater, nutrients, wastewater treatment and downflow hanging sponge (DHS) system will be discussed in the first chapter. In Chapter 2, a review of DHS reactor including different types of configuration, comparison of nutrient removal mechanisms of both DHS and conventional wastewater treatment, and its applications such as municipal wastewater treatment, industrial wastewater treatment, cultivation bioreactor, rare metal recovery and methane recovery will be studied. On the following chapter, detail steps to conduct this study from the starting of search of literature and analysis to the write-up of this review report will be outlined. Next, Chapter 4 will focus on the comparison of works done by past researchers in evaluating the nutrients removal with various generations of DHS reactors as municipal wastewater treatment. On the next chapter, the factors affecting the performance of DHS reactor such as hydraulic retention time (HRT), organic loading rate (OLR), hydraulic loading rate (HLR), sponge size, ventilation and climate will be reviewed. In Chapter 6, the microbial community which helps to improve the performance of DHS reactor will be studied. On the last chapter, all the main findings from the results and discussions will be summarised to ensure the objectives of this report are achieved. Limitations and recommendations will also be discussed in this chapter.

CHAPTER 2

LITERATURE REVIEW

2.1 Development of DHS System

Upflow anaerobic sludge blanket (UASB) process can remove the organic substances effectively but not nutrients and pathogen. Therefore, UASB alone is inadequate to treat the wastewater. Aerobic treatment is applied after UASB as it can filter the remaining organic substances, nutrients and pathogens effectively. Previously, there were several post-treatments being suggested, such as trickling filter, series of batch reactors and activated sludge process (ASP). ASP is the most famous wastewater treatment process among other post-treatment methods due to its high efficiency in treating the wastewater. However, ASP is very expensive to construct, operate and maintain and larger area is required for construction. Thus, it is unsuitable especially for developing countries. DHS system was first proposed by a research team lead by Professor Hideki Harada in Japan as a replacement for post-treatment after pre-treatment UASB process. This is because DHS is simple to operate, cheap to construct and does not need any external forced aeration. In 1995, the first DHS prototype was developed and since then, it has been tested intensively through lab-scale and pilot-scale experiments in order to construct a more efficient, feasible and practical DHS reactor. DHS reactor evolved throughout these years in terms of configurations and sponge design. They were classified into six generations (Uemura and Harada, 2010).

The first generation of DHS reactor comprised of a series of polyurethane sponge cubes that hung diagonally using a nylon string. The size of the cubes and the height of the string were 1.5 cm and 2 m respectively (Agrawal et al., 1997). The first pilot scale experiment of first generation DHS was attempted to evaluate the performance in organic substances removal and nitrification without any external aeration. The reactor showed good performance in removing both organic substances and nitrogen. However, the sponge cubes and string were mechanically unstable when treating high rate of sewage, making this configuration incompatible for full scale operation. In other words, the wastewater distribution through the sponges was uniform, thus could only be carried out in lab scale operation (Nurmiyanto and Ohashi, 2019).

In order to overcome the drawbacks of the first generation DHS, second generation DHS was constructed with better sponge arrangement. The cube sponge was modified into long triangular sponge strips, with a length of 75 cm and triangular sides of 3 cm, attached on both sides of a 2 m height plastic sheet to form a curtain-like shape. Each successive strips consisted an interval of 0.9 cm (Machdar et al., 2000). The second generation DHS reactor was assessed to a pilot scale experiment with 1000 m^3/day wastewater capacity in Karnal, India. The performance was found to be comparable with the first generation (Tandukar et al., 2006). Nurmiyanto and Ohashi (2019) reported that second generation DHS were better in nitrogen removal compared to the previous generation. Nonetheless, there are still problems with the uniformity of sewage distribution, causing it to have difficulties in scaling up. Besides, the curtain type sponge media shows low efficiency in biomass attachment, thus increasing the formation of excess sludges.

The third generation DHS was constructed to overcome the small scales problem. The design was dissimilar with the previous two generations. It utilized the concept of trickling filter. Nevertheless, instead of the plastic or gravel used in a trickling filter, it used small polyurethane sponge pieces as filter media, putting inside a polypropylene net-like plastic as a support media. The sponge media was filled randomly with a height of 2.7 cm. The random packing of the sponge media caused the construction process to be simple and thus easier for the reactor to be scaled up. Tawfik, Ohashi and Harada (2010) reported that the third generation DHS reactor showed good performance of organic matters removal, nitrogen removal and even fecal coliform removal. However, the random packing of sponge materials caused lesser air transfer into the sponge. This led to lower activity of nitrification process and thus lower nitrogen removal (Nurmiyanto and Ohashi, 2019).

The fourth generation DHS was developed a year after the development of third generation DHS. It was developed to overcome the drawbacks from the previous generation. It used the same materials of sponge media and support media as the third generation, but with different sizes. DHS reactor was designed with long strips of sponge media that were located in a net-like cylindrical plastic to give rigidity. Fifteen sponges were arranged to make a row. The sponge rows were stacked on each other to produce a stack number of 20, with specific gap between each row. This design was known as module, with a total of 300 sponges. The sponge modules were stacked on each other to form a stack number of 4, with specific gap between each module. This configuration was developed to improve air transfer into sewage and also to reduce clogging in the reactor that were caused by washout from UASB. This DHS design showed excellent removal of organic matter, BOD, chemical oxygen demand (COD) and suspended solids (SS) but low nitrogen removal. Nonetheless, the long sponge strips design caused several drawbacks. The sponge strips were unable to support the high accumulation of biomass, causing sponge modules to deform after some time of operation (Tandukar et al., 2005).

After the development of fourth generation DHS system, the fifth generation DHS was developed in the year of 2014. It was constructed to improve the reactor configuration of second generation DHS. The curtains were allocated side by side in a rectangular cover, with a 4 cm gap between each successive curtain sheets. The plastic sheets were then fixed within the frame using a hanger. Twelve curtains were connected to develop a module. The modules were then stacked on each other to construct a fifth generation DHS reactor. The combination of UASB and fifth generation DHS reactor showed good performance in pollutants removal and the removal efficiency was comparable with the conventional wastewater treatment. However, fifth generation DHS reactor has the same drawback as the fourth generation, which is unable to withstand the high accumulation of biomass. The hanger has low strength and tend to bend after loading the biomass for few months. This causes the curtain sheets to tear off and unable to function (Nurmiyanto and Ohashi, 2019).

Finally, the latest design of DHS reactor, or can be known as sixth generation DHS reactor was developed in the similar concept as the third generation DHS reactor, which utilized the concept of trickling filter. However, this generation DHS did not employ soft polyurethane sponges. Instead, it used a rigid polyurethane sponge media which was harden by adding epoxy resin into the sponges. The rigidity characteristic of sponge media would not require a support media such as plastic cover and thus eased the construction of the reactor. The sponge surface area exposed to the wastewater increased due to the lack of support media. Hence, the interaction between the air and wastewater was improved. The combination of UASB and sixth generation DHS system in the pilot scale operation showed overwhelming results that were comparable with the previous five generations. Nonetheless, the usage of epoxy resin has decreased the void space of the sponge to 70 %. This causes lower biomass accumulation in the sponge and thus more sludge production (Onodera et al., 2014a). Figure 2.1 shows the evolution of DHS reactor.

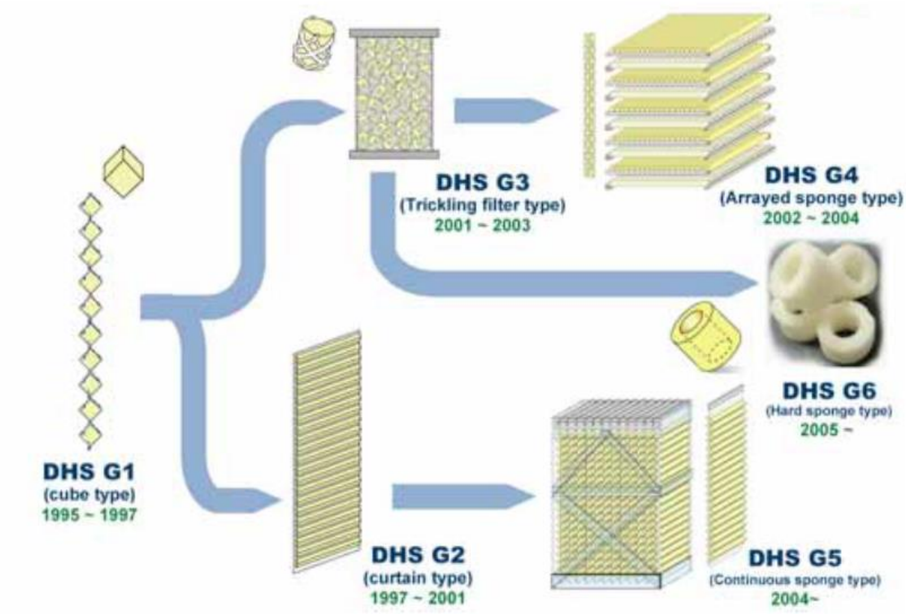

Figure 2.1: Evolution of DHS Reactor (Harada, 2008).

2.2 Nutrients Removal

Excess nutrients must be removed in order to maintain good water quality. Nutrients removal is divided into two categories: nitrogen removal and phosphorus removal.

2.2.1 Nitrogen Removal

Nitrogen can be removed chemically or biologically. The example of chemical practices such as ammonia stripping and ion exchange. Ammonia stripping had been used as one of the physicochemical treatment processes. It begins with a chemical treatment used to eliminate SS followed by nitrogen removal from ammonia stripping and lastly organic substances removal from activated carbon. However, this treatment was not applied into wastewater treatment as it had many drawbacks which outweighed benefits. For instance, this treatment produced a lot more sludge than biological methods and thus it was costly for sludge removal. Furthermore, the effluent produced by this treatment had lower quality compared to the biological method. Ion exchange is a water treatment process normally used for demineralization and softening of water. It showed great performance in nitrogen removal. However, it produced regenerants concentrated with ammonium ions that needed to be treated before discharging into the environment. One of the ammonium ions treatment is ammonia stripping and thus it is not a feasible method. Hence, chemical options are less widely used for nitrogen removal due to the significant drawbacks (Cooper, Day and Thomas, 1994).

Biological method utilizes a recycling pathway that consists of two main processes, in which begins with nitrification and followed by denitrification. Nitrification takes place under aerobic condition, where oxygen is present. In the presence of oxygen, the autotrophic bacteria or microorganisms will oxidize the ammonium (NH_4^+) present in the wastewater into nitrite (NO_2^-). The nitrite will further oxidize into nitrate (NO_3^-). Next, the nitrate is reduced into nitrogen gas through denitrification. The nitrogen gas will flow back into the atmosphere. Denitrification takes place under anaerobic or anoxic condition, where oxygen is absence. With the absence of dissolved oxygen (DO) in the wastewater, oxygen can only be obtained from nitrate. Heterotrophic bacteria that require oxygen and carbon to perform their mechanisms, will use nitrate as oxygen source and organic substances as carbon source. The common biological practices are activated sludge system and biofilter. Activated sludge system is a wastewater treatment that uses aeration and microorganisms to treat the wastewater. The activated sludge system normally begins with anoxic zone in order to utilize the carbonaceous matter from the settled wastewater after primary treatment. Biofilter system is a wastewater treatment process that uses bioreactor containing bacteria to capture and eliminate pollutants biologically. Nitrification begins first in biofilter followed by denitrification in a separate biofilter. An external synthetic carbon source is required in the denitrification process for biofilter. These two biological practices have shown good efficiency in nitrogen removal and they were well developed and widely used in wastewater treatment plant for the past 25 years (Cooper, Day and Thomas, 1994).

2.2.2 Phosphorus Removal

Domestic wastes, industrial wastes and fertilized land run-off contribute the most phosphorus concentration in wastewater. Phosphorus is normally present in several forms such as orthophosphates and condensed phosphates or phosphate salts. Large amount of phosphorus comes from cleaning agents such as detergents. Phosphorus can be removed by either chemical and biological methods in activated sludge system (Yeoman et al., 1988).

Chemical removal of phosphorus can be achieved through metal precipitation process, which is addition of metal salts such as calcium, iron salts and aluminium sulphates. The calcium salt commonly used to treat phosphate is lime (CaOH). Lime reacts with the phosphate ions to form a calcium phosphate crystal known as hydroxyapatite. Hydroxyapatite is insoluble in water and stable and thus easier to be separated. However, there are more than 22 kinds of precipitates that can be formed by using lime in wastewater. Thus, the competitive precipitation will increase the dosage of lime. Besides, the amount of lime added is strongly dependent on the alkalinity of the wastewater. Carbon dioxide is applied to reduce the water alkalinity as it is cheap and abundant. Nonetheless, lime will first react with carbon dioxide to form bicarbonate precipitates before precipitating phosphorus. Therefore, more alkalinity wastewater will lead to more lime requirement. Lime is seldom used in treatment now as it produces more sludges compared to other metal salts. Next, iron salt that is normally used in sewage treatment is iron(III) chloride ($FeCl_3$). Iron ions react with phosphate ion to form iron phosphate complexes. Other than phosphate ions, iron ions will also react with hydroxyl ions to form iron hydroxide. The competition between phosphate ion and hydroxyl ion will lead to the requirement for excess iron(III) salts for precipitations. Subsequently, the most common aluminium salt used for phosphorus removal is aluminium sulphate or alum ($Al_2(SO_4)_3$). The amount of alum used is very dependent on the concentration of phosphate in the wastewater. Alum will first react with orthophosphate to form phosphate precipitate, aluminium phosphate ($AlPO_4$), followed by other colloidal particles. The reason behind is that aluminium phosphate is kinetically favored compared to formation of aluminium hydroxide. At low concentration of phosphate ion, the precipitation will favor over the hydroxide formation instead of metal phosphate formation. In general, aluminium is the best metal salts, followed by iron and calcium (Yeoman et al., 1988).

The chemical salts can be added at various stages in activated sludge system, which are during the primary sedimentation tank, secondary sedimentation tank, aeration tank, or tertiary stage. Addition of metal salts in the primary sedimentation tank helps to remove a large portion of solid particles, causing a substantially decrease in the organic loading on the secondary treatment. This will result in reduction of aeration cost and excess sludge production in secondary treatment. However, it will result in higher usage of metal salts and increment of sludge production in the primary treatment. Another drawback is that the organically bound phosphorus in the primary treatment is hard to be precipitated, resulting inefficient phosphorus removal. Addition of metal salts in aeration tank or secondary sedimentation tank is more preferable in many wastewater treatment plants. This is because lesser metal salts are required for precipitation and organically bound phosphorus has been oxidized in the presence of oxygen. Thus, the phosphate ions can be precipitated easily. However, increment in the sludge production illustrates that the need for larger tank to withstand high sludge production. Addition of metal salts in tertiary treatment will give a better effluent quality with less chemicals used. Nonetheless, the requirement for an additional tertiary stage will increase the cost of the overall wastewater treatment plant (Cooper, Day and Thomas, 1994).

Biological phosphorus removal has a basic mechanism that requires an anaerobic condition consist of fermentation products such as short chain fatty acids (SCFAs). SCFAs are normally present in the wastewater. It must be added if they are absent in the wastewater. The anaerobic condition will allow the growth of certain strains of bacteria that are used for phosphorus removal. For example, polyphosphate-accumulating organisms (PAOs) and *Acinetobacter*. These bacteria uptake the SCFAs and keep them inside the bacterial cell as poly-hydroxybutyrate (PHB). The amount of energy required to consume SCFAs is provided by the hydrolysis of polyphosphate that are stored within the cells. The hydrolyzed polyphosphates are then released to the wastewater as orthophosphates. Next, the SCFAs are depleted during aerobic condition after being consumed as energy for oxidation. The PHB is oxidized to carbon dioxide by utilizing oxygen as electron acceptor. This results the phosphorus to be taken up by the bacteria as polyphosphate. The bacteria are then removed as sludges along with the phosphorus. This mechanism is known as enhanced biological phosphorus removal (EBPR) (Cooper, Day and Thomas, 1994). Besides, there are a small portion of PAOs that utilize nitrate instead of oxygen as electron acceptor. This special PAOs is known as denitrifying polyphosphate accumulating organisms (DPAOs). With the presence of DPAOs, denitrification and phosphorus removal can occur at the same time. Therefore, it will definitely enhance the nutrients removal. In addition, DPAOs are more reasonable as it does not require oxygen, thereby reducing the aeration cost. (Urdalen, 2013)

Nonetheless, biological treatment is less stable, leading to lower efficiency in phosphorus removal (Cooper, Day and Thomas, 1994). Furthermore, in the conventional biological phosphorus removal, the production of excess sludge occurs. Hence, the removal of excess sludge is required after the aerobic stage in order to remove the phosphorus. This leads to additional discharge costs (Kodera et al., 2013).

2.2.3 DHS for Nutrients Removal

DHS applies the biological mechanisms in removing both nitrogen and phosphorus. According to Machdar et al. (1997), dissolved oxygen (DO) was found to be sufficient at the outer part of the sponge cubes, proving that the presence of aerobic condition without the need of external aeration. Next, according to Onodera et al. (2016), the DO of the sponge decreased gradually towards the inner part of the sponge and eventually reached 0 mg/L at the center of the sponge. This examination has proven that both aerobic and anaerobic condition are present in DHS reactor. This is a special phenomenon that makes it to be better than trickling filter. Without the requirement of any external aeration, the capital cost, maintenance cost and operating cost will reduce and thus this solves the major drawback for conventional biological treatment, which is expensive.

With the presence of two zones, nitrification and denitrification can take place in DHS reactor. Nitrification takes place at the aerobic zone, that occurs at an approximate depth of 0.75 cm from the sponge surface while denitrification takes place at the inner anaerobic zone in the sponge (Araki et al., 1999). According to Tandukar, Ohashi and Harada (2007), the combination of UASB-DHS system in pilot-scale showed great nitrogen removal, with a total Kjeldahl nitrogen (TKN) removal of 72 % and ammonium-nitrogen (NH_4-N) removal of 60 % over the period of operation. It even reached a maximum of 90 % nitrogen removal under warmer condition. However, the nitrification and denitrification were observed to take place mostly in the lower portion of the reactor. Araki et al. (1999) also observed a similar phenomena. The investigation found that the organic loading at the upper part was higher and thus favoring the growth and activity of heterotrophic bacteria. This vanquished the activity of autotrophic bacteria to carry out nitrification. The organic matter was reduced before it reached the lower portion. This situation resulted the lower portion of the reactor to have a lower organic loading and hence enhanced the nitrification process (Tandukar, Ohashi and Harada, 2007). Furthermore, some researchers reported that most of the nitrification process took place at the upper portion while denitrification process took place at the bottom portion. This was due to the plug flow regime design of the reactor, which removed organic matter first followed by the nitrification process at the upper portion followed by denitrification at the lower portion. However, denitrification is limited for this configuration. This is due to the leak of carbon source at the lower portion of the reactor, causing the heterotrophic bacteria unable to carry out denitrification process. The limited denitrification increased the total nitrogen (TN) present in the effluent (Uemura and Harada, 2010; Onodera et al., 2016). Bundy et al. (2017) suggested bypassing the influent of DHS reactor to the lower portion. This increased the carbon source at the lower portion and reduced the DO level at the same time. This bypass design encouraged anoxia and eliminated the carbon issues, therefore promoting denitrification.

The biological mechanisms for phosphorus removal can also take place in the presence of aerobic and anoxic zone. The combination of UASB and DHS system is a cost-effective wastewater treatment process that is able to solve the excess sludge problem (Tandukar, Ohashi and Harada, 2007). Kodera et al.

(2013) proposed to recover phosphorus as concentrated solution by adding an improved version of trickling filter reactor with recirculation. The operation consists of three stages. Firstly, the aerobic stage where the effluent from UASB-DHS system is added from the top of the reactor. Air is supplied continuously to provide an aerobic condition. The phosphate is taken in by polyphosphate-accumulating organisms (PAOs) in the reactor and the treated water is discharged from the reactor. Next, the anaerobic stage where the recirculated water with organic substrates is added into the reactor in order to provide anoxic condition. The PAOs take in the organic substrates, releasing the accumulated phosphate into the solution. Lastly, the recovery stage where the solution is flowed to the recirculation tank, followed by recycling back to the aerobic period. The repeating steps have enhanced the proliferation of PAOs and increased the concentration of phosphate within the reactor, which eventually form a plateau. The plateau is then collected in the recirculation tank to be recovered. This system is able to remove phosphorus from the treat wastewater and recover the phosphorus by producing lesser sludge at the same time. The recovered phosphorus can be reused as fertilizer. From the operation, the phosphate concentrated solution was found to have about 125 mg P L^{-1}, that was 25 times higher than the typical treated wastewater (5 mg P L^{-1}). Nurmiyanto et al. (2017) proposed a similar mechanism for phosphorus recovery. The study was carried out in a pilot-scale system, consisting a total volume of 1206 L UASB-DHS reactor for 5 years. The performance was excellent and concentrated phosphate solution was found to contain up to 120 mg P L^{-1} under optimal condition. Therefore, the proposed operation system was feasible to be applied in a real wastewater treatment plant.

2.3 Application of DHS System

DHS System is very versatile compared to other conventional wastewater treatments. It consists of various applications other than treating wastewater. Several main applications are discussed in the coming subsections.

2.3.1 Municipal Wastewater Treatment

For the past twenty years, DHS system has been used as a post-treatment of UASB in treating municipal wastewater treatment. DHS system has shown good performance in treating the effluent of UASB in pilot-scale experiments (Machdar et al., 1997; Tawfik, Ohashi and Harada, 2006; Tandukar, Ohashi and Harada, 2007). These researchers strongly recommended DHS System as the post-treatment of UASB reactor. Recently, several studies have proven that the consistent performances of DHS system in full-scale operation. The full-scale application was carried out in developing countries such as India (Onodera et al., 2016) and Egypt (Mahmoud, Tawfik and El-Gohary, 2011). The local operators succeeded in operating DHS system due to its simple operations and maintenance. The simplicity of DHS system made it to be dependable and reasonable, which eased long-term operation.

2.3.2 Industrial Wastewater Treatment

Few trials were performed to investigate the performance of DHS system in treating different type of industrial wastewater. Dussadee, Reansuwan and Ramaraj (2014) applied a combination of UASB reactor and DHS reactor as post-treatment to treat molasses wastewater produced by a bioethanol plant. The UASB-DHS combination was mainly used to remove the organic substances from the effluent of anaerobic reactors. DHS reactor alone achieved over 99.9 % BOD removal despite its simple operation. Besides, molasses wastewater contains high concentration of sulfide, which will inhibit the activity of anaerobic microbes and hence reduces the performance of anaerobic reactors. DHS reactor was able to remove sulfide by converting them into sulfate. Therefore, the sulfide concentration was found to be negligible at the effluent of DHS reactor.

On the other hand, DHS system was investigated to decolorize the reactive dyes wastewater produced from textile industry through a bench scale experiment (Tawfik, Zaki and Zahran, 2014). The application of DHS system was able to remove almost 90 % of the color from the reactive dyes at optimum operating condition. The high color removal performance was mainly caused by the high retention time of biomass in the sponge, where the dyes adsorbed onto the sludge followed by biodegradation process. Various types of microorganisms were given adequate time to degrade different substrates within the reactor. The good color removal efficiency was also attributed to the presence of anaerobic and aerobic zone within the sponge. Reactive dyes were first decolorized and reduced to aromatic amines by co-metabolisms under the anaerobic zone inside the sponge. The amines formed moved towards the outer surface of the sponge where oxygen is present and biodegraded aerobically.

Furthermore, DHS system was applied to treat the landfill leachate wastewater with high concentration of organic components and ammonia (Ismail and Tawfik, 2016). Fenton reagent was added followed by DHS system showed high removal efficiency of total COD and ammonia. Fenton reagent as pre-treatment process helps to remove some organic components and also enhances the biodegradability of recalcitrant compounds before feeding into DHS system. The combination achieved 85 % total COD removal and 97 % ammonia removal. Thus, this further proves that the capability of DHS system in treating various wastewater other than domestic wastewater.

2.3.3 Cultivation Bioreactor

DHS reactor was employed by microbiologists as cultivation bioreactor to cultivate the methanogenic microbial community from methane-rich subseafloor sediments (Imachi et al., 2011). DHS reactor was made up of high porosity polyurethane sponges, thus providing greater surface area for sedimentary microbial habitats. Besides, the sponges were hanged freely in the atmosphere. Thus, the seawater inlet stream entered into the polyurethane sponges at the top of the DHS reactor and moved downwards by gravity, allowing the effective movement of the seawater on both the inside the sponges and surface of the sponges. Therefore, DHS system exhibited higher biomass accumulation which increased the cell residence time. This would further enhance the microbial cultivation. On the other hand, the flow of DHS reactor was set to be continuous in order to maintain the substrates at lower concentration, same as those that were found naturally in the environment. Continuous flow was able to remove metabolic products which might inhibit the growth of microorganisms. Imachi et al. (2011) concluded that DHS reactor has successfully enriched the methanogenic community from the sediments. It also produced ten different type of anaerobic microbes in pure culture. Four of them were methanogenic archeal species that could not be cultivated directly from the samples of subseafloor sediments.

2.3.4 Rare Metal Recovery

Rare metals are crucial in the current world with modern technologies. However, manufacturing of rare metals inevitably leads to small content of these metals being discharged into the wastewater. The metals will be hazardous to the environment, especially aquatic life even at low concentration. It will be a waste if these metals are eliminated through chemical reaction and therefore a recovery method is necessary to recover the rare metals from wastewater. One of the possible recovery methods is biosorption by biogenic manganese oxides (bio-MnO_2). Bio-MnO_2 has a special structure features in such a way that it can adsorb remarkable amount of rare metals on it. In order to produce bio-MnO_2, heterotrophic manganese oxidizing bacteria (MnOB) is cultivated from bioreactor system and these bacteria will oxidize manganese(II) (Mn(II)) into bio-MnO_2. Cao et al. (2015) studied that MnOB could be produced under a system with nitrification process. It was due to the presence of nitrifiers containing soluble microbial products (SMP) which could be used as substrates for MnOB production. A bench scale experiment was carried out by applying DHS system to cultivate MnOB with sufficient supply of ammonium (NH_4^+) and Mn(II). DHS system was favorable to the low growth rate nitrifiers and therefore suitable for MnOB cultivation. Moreover, the usage of sponges were able to cause the bio-MnO_2 particulates produced to fall onto the bottom of the system, where the particulates were collected easily for rare metal recovery. In long-term operation, oxidation of Mn(II) was successful to produce bio-MnO_2 at the bottom of the DHS reactor. The rare metals such as nickel(II) (Ni(II)) and cobalt(II) (Co(II)) adsorbed onto the bio-MnO_2 were removed along with it. The molar ratio of Co(II) to Mn(II) and Ni(II) to Mn(II) removed was found to be 45 % and 9 % respectively.

2.3.5 Methane Recovery

Methane is a greenhouse gas that commonly found in the wastewater. Methane gas emits into the environment will lead to global warming. Anaerobic treatment such as UASB was first applied to recover methane as useful burnable biogas. However, the methane was unable to be recovered completely as some portions of methane was dissolved in the wastewater. Matsuura et al. (2015) utilized two stages of closed DHS systems as a post-treatment to recover methane, thereby preventing it from emitting to the environment. At the first stage, air was supplied to the bottom of the reactor. Methane gas was transferred upwards by the supplied air, which then collected at the top of the reactor. The efficiency of methane recovery was observed to be high, ranging from 57 to 88 %. The effluent containing residual dissolved methane was proceeded to the second DHS reactor. At the second stage, air was supplied at the top of the reactor in order to oxidize the remaining dissolved methane in the wastewater. The dissolved methane was found to be almost fully oxidized in the second closed DHS reactor, with a removal efficiency of more than 90 %. The combination of two stages DHS reactor showed outstanding performances, achieving 99 % removal efficiency of dissolve methane.

2.4 Summary

In summary, today, there are a total of six generation of DHS reactors with different arrangement and configurations. As nothing is perfect, all six generations have their own benefits and drawbacks. Besides, the presence of aerobic and anaerobic condition in the DHS reactor proves the capability of the DHS reactor for nutrients removal. Furthermore, the versatility of DHS reactor is remarkable as it can be used to treat municipal wastewater and industrial wastewater, act as cultivation bioreactor for specific microorganisms and even recover rare metal and methane from the wastewater.

CHAPTER 3

REVIEW METHODOLOGY

3.1 Introduction

In this chapter, it will provide readers a brief glance into the preparation of this review report followed by write-up of this research report. Since the report is review-based, there will be no hands-on work or practical work. Instead, the results are all obtained through literatures and journals. A good review report requires precise evaluation and judgement on the previous research papers, which summarises the important data and point out several recommendations to the future researchers.

3.2 Literature Searching and Analysis

In the commencement of this project, the main focus was to search for journals that were related to the title of this project. The search of literature was time consuming and required the patience in seeking for relevant journals. Several keywords such as "nutrients" and "DHS" were applied to ease the searching process. The relevance of a journal could be identified through its title. However, some journals required licenses to be read, which could be solved by using e-library from university. The journals were downloaded and stored in a folder for further evaluation.

After collecting the journals, selection of journals was conducted to choose the suitable journals for review writing. Firstly, the journals were first scanned through using several keywords related to the objectives of this report. The unsuitable journals were deleted from the folder while the remaining journals would be brought forward to the second selection stage. In the second stage, the abstracts from the remaining articles would be read through in order to have better and clearer ideas on the whole journals. The journals that were qualified from this evaluation would be kept and brought to the next step. Finally, the remaining journals would be read through in detail to have a complete understanding on the paper. The useful information would be highlighted to save time when referring back the articles. As a tactic to acquire more related journals, the references used from the journals were searched and looked through to seek for more useful information. Besides, the authors of the useful journals would have their other papers searched through to see if any journal that was applicable to the research topic.

Literature analysis was conducted after confirming the relevant journals. The useful data was extracted from the journals and sorted out accordingly to several sections based on the research objectives. For example, data related to parameters affecting DHS system were arranged together. However, it may be possible to have insufficient data or results to support certain findings. One result was inadequate to proof the rightness of a finding. Therefore, additional journals needed to be searched in order to strengthen the finding. Finally, write-up of the review report could be started after gathering all the necessary data.

3.3 Write-Up of Review Report

The purpose of the review report was to summarize the discrete results and data produced from the past research papers regarding this topic. Besides, this review report aimed to provide the latest updates performed by the past researchers on this topic. Therefore, it was crucial to understand the journals completely so that there would be no misinterpretation on the meaning conveyed by the past researchers. Otherwise, the future readers would receive wrong information from this review report. This might cause troubles to the future researchers, which eventually slowed down the progress of this research topic.

The writing style of this review report was designed to be straightforward and simple so that the readers could understand it easily, even without any knowledge on the research topic. English words in this research report were applied as simple as possible to aid the readers who were weak in English. The data and results were summarized in the table format so that the future readers would save time in seeking for useful information from this review report.

For a good quality report, it must be written strictly based on factual instead of theory and pure logic. The facts must be verified with sufficient evidence from the past researchers. In addition, further evaluation on the information were required to ensure the findings provided by the past researchers were not a fraud. By taking these terms into considerations, the future readers would be able to produce similar results by following the procedures from the past researchers. To further improve the quality of this review report, unique hypothesis and recommendations should be provided rather than merely rephrasing the points collected from the past researchers. Therefore, this review report would not be just a duplication of past research papers. Instead, it would be beneficial to every future reader which assisted them with their future researches on this topic.

3.4 Outline of Review Report

The review study is divided into three sections based on the three objectives of this review report. The first section will be Chapter 4, where the performance of nutrients removal from different generations of DHS reactor is reviewed and compared. The most suitable DHS system in removing nutrients will be determined among the six generations. Chapter 5 is the second section of the review study, where the parameters influencing the performance of DHS system are identified and studied. These factors need to be designed accordingly in order to improve the overall removal efficiency of the system. The last section will be Chapter 6, where the microorganisms involved in nutrients removal are studied. Since DHS system applies biological mechanisms to treat wastewater, microorganism plays an important role to decompose or remove pollutants from the wastewater. The bacteria responsible to remove nutrients are studied, as well as several important bacteria that contribute to the performance of DHS system.

CHAPTER 4

PERFORMACE OF DIFFERENT GENERATIONS DHS SYSTEMS

4.1 Type of DHS Systems

As mentioned in Chapter 2, there are a total of six generations of DHS system. The performance might be different because each type of DHS systems has their own configurations. Throughout these twenty years, plenty works have been carried out by different researchers around the world to investigate the performance of different generation DHS system in treating municipal wastewater. Those works will be compared and discussed in this chapter.

4.2 First Generation

The first generation of DHS reactor was studied by Agrawal et al. (1997). The researcher applied a combination of UASB reactor, cubic hanging sponge and upflow submerged hanging bed (USHB) bioreactor to treat sewage. Over two years of operations, DHS system was able to perform high organic removal and nitrification even at low temperature. However, it was suggested that low denitrification process in DHS system due to the requirement of post-denitrification system. Similar studies have been performed by Machdar et al. (1997) and Machdar et al. (2018b) for a duration of six months. The performance of DHS as post-treatment was satisfactory in terms of organic removal and also nitrogen removal in a long run. The removal efficiency of first generation DHS system is obtained from the three studies and tabulated in Table 4.1.

Table 4.1: Removal Efficiency of First Generation DHS system from Different Researchers.

Type of Reactor	G1 DHS	G1 DHS	G1 DHS
TCOD (%)	71	71	75
TBOD (%)	–	97	–
TSS (%)	–	100	70
NH_4-N (%)	–	78	46
TN (%)	–	17	9
References	(Agrawal et al., 1997)	(Machdar et al., 1997)	(Machdar et al., 2018b)

4.3 Second Generation

For second generation DHS reactor, Machdar et al. (2000) proposed the combination of UASB and curtain-type DHS system as anaerobic pre-treatment and aerobic post treatment respectively to treat the municipal wastewater. The pilot scale experiment was conducted for 550 days and the whole system achieved outstanding organic removal and nitrification. The difference of DO profile in the internal part and external part of the sponge medium and the changes in the total nitrogen (TN) concentration suggested that the presence of nitrification and denitrification in the DHS reactor. Thus, it was clear that second generation DHS was better in nitrogen removal compared to the previous generation.

Besides, Tandukar et al. (2006) conducted similar combination system for 3.5 years under ambient temperature to treat the domestic wastewater. The result obtained was similar with Machdar et al. (2000). Besides, it was also found that DHS reactor was capable to tolerate sudden shock loads. Despite the nitrification activity deteriorated during the shock loads, the activity was able to recover within a day.

Furthermore, Uemura et al. (2012) developed three curtain type DHS reactors with different sponge size to treat settled sewage directly. All three DHS reactors showed excellent organic and ammonium nitrogen removal while the DHS reactor with smaller sponge size was the best among them. The results obtained from the three studies are summarized and tabulated in Table 4.2.

Table 4.2: Removal Efficiency of Second Generation DHS system from Different Researchers.

Type of Reactor	G2 DHS	G2 DHS	G2 DHS
COD (%)	64	62	85.2
BOD (%)	85	83	–
SS (%)	39	39	70
NH_4-N (%)	70	61	94.7
TN (%)	40	31	–
References	(Tandukar et al., 2006)	(Machdar et al., 2000)	(Uemura et al., 2012)

4.4 Third Generation

Ample studies were found to have conducted experiments to determine the performance of third generation DHS reactor (Tawfik, Ohashi and Harada, 2006; Mahmoud, Tawfik and El-Gohary, 2010; Tawfik, Ohashi and Harada, 2010; Mahmoud, Tawfik and El-Gohary, 2011; Onodera et al., 2014b; Okubo et al., 2016; Nomoto et al., 2017; Machdar et al., 2018a). From these studies, the random packing polyurethane sponge reactor exhibited satisfactory organic matter removal and nutrients removal which are able to meet the regulatory standard of their respective countries. Mahmoud, Tawfik and El-Gohary (2010) observed that the third generation DHS system was able to remove 43 % of total phosphorus at a HRT of 6 hours. It was suggested that the removal of phosphorus was due to the simultaneous phosphorus uptake and denitrification by DPAOs in the inner part of the sponge medium where the anoxic condition located. Another possibility for phosphorus removal may be the phosphorus particulates attached or adsorbed onto the sponge media due to its high void volume feature. This further verified the capability of third generation DHS reactor in nutrients removal. The data from the researchers were collected and tabulated in Table 4.3.

Table 4.3: Removal Efficiency of Third Generation DHS system from Different Researchers.

Type of Reactor	G3 DHS	G3 DHS	G3 DHS	G3 DHS
TCOD (%)	69	34	72	80
TBOD (%)	72	80	96	-
TSS (%)	55	28	68	67
NH_4-N (%)	40	65	86	86.3
TKN (%)	–	–	–	71.2
TN (%)	–	–	17	–
TP (%)	–	–	–	–
References	(Nomoto et al., 2017)	(Machdar et al., 2018a)	(Tawfik, Ohashi and Harada, 2006)	(Tawfik, Ohashi and Harada, 2010)

Type of Reactor	G3 DHS	G3 DHS	G3 DHS	G3 DHS
TCOD (%)	75	80	49	89
TBOD (%)	94	78	76	97
TSS (%)	80	83	95	92
NH_4-N (%)	70	89	98	99
TKN (%)	–	–	–	71
TN (%)	20	61	21	–
TP (%)	–	–	–	43
References	(Okubo et al., 2016)	(Mahmoud, Tawfik and El-Gohary, 2011)	(Onodera et al., 2014b)	(Mahmoud, Tawfik and El-Gohary, 2010)

4.5 Fourth Generation

The fourth generation DHS reactor was studied by Tandukar et al. (2005). The aim of this study was to evaluate the performance of the combination of UASB and fourth generation DHS reactor in treating municipal wastewater. This DHS reactor was built to overcome a few shortcomings from the third generation DHS. The combined system was continuously operated at a HRT of 8 hours for over 600 days. The result showed excellent removal of organic matter, BOD, COD and TSS but low nitrogen removal. However, the nitrogen removal was claimed to be sufficient despite low removal efficiency of nitrogen. It might be due to the low ammonium nitrogen concentration at the influent, which is only 21 mg/L. Table 4.4 summarizes the result acquired from this study.

Table 4.4: Removal Efficiency of Fourth Generation DHS system.

Type of Reactor	G4 DHS
TCOD (%)	73
TBOD (%)	89
TSS (%)	74
NH_4-N (%)	28
TKN (%)	40
TN (%)	–
Reference	(Tandukar et al., 2005)

4.6 Fifth Generation

Fifth generation DHS reactor is an advance version of second generation. Tandukar, Ohashi and Harada (2007) conducted a pilot scale experiment to compare the removal efficiency of two different systems, which are the combination of UASB and fifth generation DHS reactor and activated sludge process (ASP) in treating domestic wastewater. Similar sewage influents were fed to both systems for over 300 days. The outcomes displayed that the removal efficiency of UASB+DHS system was comparable with ASP. The organic removal of both systems was equivalent while ASP achieved slightly higher in nitrogen removal. In contrast, UASB+DHS system achieved better pathogen removal. Furthermore, the excess sludge produced by UASB+DHS system was much lesser compared to ASP. In addition, there was no requirement of external aeration system for UASB+DHS system, making it a more economical wastewater treatment than ASP. The performance of fifth generation DHS system for this study was obtained and tabulated in Table 4.5.

Table 4.5: Removal Efficiency of Fifth Generation DHS system.

Type of Reactor	G5 DHS
TCOD (%)	73
TBOD (%)	88
TSS (%)	57
NH_4-N (%)	61
TKN (%)	61
TN (%)	38
Reference	(Tandukar, Ohashi and Harada, 2007)

4.7 Sixth Generation

Finally, the sixth generation DHS system is the modification of third generation. It was studied by Onodera et al. (2014a) to evaluate its removal efficiency as a municipal wastewater treatment. The system consisted of UASB and DHS system with rigid sponge media, was operated at a total HRT of 10.6 hours for over two years. This combined system exhibited good removal efficiency of organic matter and nitrogen, particularly on nitrification. Despite its low porosity of rigid sponge media, which led to low retention of biomass, this system gave higher performance and lesser production of excess sludge compared to previous generations.

Another study regarding sixth generation DHS system was carried out by Okubo et al. (2017). The objective of this study was to compare the performance of third generation and sixth generation DHS reactor. A DHS reactor was divided into two parts, with rigid sponge (G6) filled at one side and the other side filled with soft sponge (G3). The experiment was operated continuously for 390 days at different HRT. It was found that the performance was similar for both type of sponge media in terms of organic removal, ammonium nitrogen removal and fecal coliform removal. This proved that third generation DHS system exhibits the same performance as sixth generation DHS system. The results from both studies were summarized and tabulated in Table 4.6.

Table 4.6: Removal Efficiency of Sixth Generation DHS system from Different Researchers.

Type of Reactor	G6 DHS	G6 DHS
TCOD (%)	68	80
TBOD (%)	87	89
TSS (%)	51	–
NH_4-N (%)	83	77
TKN (%)	82	–
TN (%)	28	–
References	(Onodera et al., 2014a)	(Okubo et al., 2017)

4.8 Discussion

Based on the results produced from the above studies, it is clear that all generations of DHS reactor are capable to perform stable and satisfactory removal efficiency as a post treatment of UASB reactor such that the effluent is able to meet the water quality standard. Despite its satisfactory performance, denitrification is observed to be lower compared to nitrification. It is suggested that there may be insufficient time for denitrification to be carried out. Nitrification activity was normally observed at the bottom part of the reactor (Machdar et al., 2000; Tandukar et al., 2006). The nitrate produced might be discharged out of the reactor before being able to denitrify into nitrogen gas. Besides, there may be due to the leak of carbon source at the bottom portion as most of the organic matter are removed at the upper portion of the reactor, thereby limiting the denitrification process (Onodera et al., 2016).

Furthermore, it is observed that the data for phosphorus removal is limited. Among all the above studies, Mahmoud, Tawfik and El-Gohary (2010) is the sole researcher who analyzed the concentration of total phosphorus at both influent and effluent of the reactor. The ignorance of phosphorus concentration might be due to the low phosphorus concentration at the wastewater influent, hence making it to be undetectable and negligible. According to Mahmoud, Tawfik and El-Gohary (2010), the concentration of phosphorus and total Kjeldahl nitrogen were 3.47 mg/L and 34 mg/L respectively. This reveals that the concentration of phosphorus is about ten times lesser compared to TKN concentration. Moreover, another possible reason is that the removal efficiency of nitrogen and phosphorus may be exactly the same. Since nitrification and denitrification take place simultaneously in a single reactor, both aerobic and anaerobic condition are present in the reactor (Onodera et al., 2014a). The presence of aerobic and anoxic condition indicates that phosphorus removal process may be carried out at the same time with nitrogen removal. Therefore, nitrogen removal alone may be sufficient to prove the efficiency of nutrients removal by DHS reactor.

Among the six types of DHS reactor, third generation and sixth generation exhibit better nutrients removal with more than 70 % ammonium nitrogen removal from most of the research papers. There are more papers which utilize third generation reactor as wastewater treatment system compared to other generations. This showed that the third generation DHS system is more favorable by most of the researchers. On the other hand, it was observed that there was no difference in the removal efficiency between third generation DHS system and sixth generation DHS system (Okubo et al., 2017). Therefore, it is recommended that either third generation DHS system or sixth generation DHS system can be utilized to remove the nutrients in actual wastewater treatment plant.

To conclude, the comparison discussed in this chapter is based on the general view. It may not be accurate and precise as the operational conditions for all studies are not exactly identical. For instance, the experiments are conducted with different reactor volume, different concentration of wastewater influent, different climate, different HRT and influent flow rate. Therefore, additional tests are necessary to evaluate the performance of various types of DHS reactors under identical experimental conditions.

CHAPTER 5

INFLUENCE OF DIFFERENT FACTORS ON DHS SYSTEM

5.1 Factors Affecting Performance of DHS System

DHS system has a similar design as trickling filter, but with sponges as packing materials instead of stones or plastics. DHS reactor is able to overcome several major drawbacks of trickling filter. However, this causes some factors affecting the removal efficiency to be different with trickling filter. For instance, the microorganisms in the trickling filter attach only on the surface of the medium to form a biofilm or slime layer. Nevertheless, the microorganisms in the DHS reactor attach on both inner part and outer part of the high porosity sponges. This improves the biomass accumulation and provides a longer sludge residence time (SRT) that are normally more than 90 days. Subsequently, the long SRT allows the nitrification to be carried out completely and therefore reduce the production of excess sludges. In contrast, trickling filter requires lower organic loading rate (OLR) to carry out nitrification (Tawfik, Ohashi and Harada, 2006). There are several major factors that affect the performance of DHS system such as hydraulic retention time (HRT), hydraulic loading rate (HLR), OLR, sponge size and ventilation.

5.2 Hydraulic Retention Time (HRT)

Hydraulic retention time (HRT) is defined as the time taken for the wastewater to pass through a tank in activated sludge process (Gerardi, 2002). In the DHS system, the tank is replaced by DHS reactor. It is crucial for the wastewater to stay within the reactor for certain period of time such that it is sufficient to effectively treat the wastewater. Based on theory, the longer the period of time for the wastewater retained in the reactor, the longer contact time between the wastewater and the microorganisms retained on the sponge media and hence better removal efficiency of the reactor. HRT is very dependent on the influent flow rate, sponge volume and sponge porosity of the DHS reactor. A slower influent flow rate to the reactor will increase HRT. However, in contrast, a greater volume and higher porosity of sponge materials will result in longer HRT.

According to Mahmoud, Tawfik and El-Gohary (2010), the performance of DHS reactors in removing organic substances and nutrients from municipal wastewater was evaluated under three different HRTs (2 h, 4 h, 6 h). The performance was evaluated based on several parameters such as total biochemical oxygen demand (TBOD), total chemical oxygen demand (TCOD), TSS, NH_4-N, TKN and total phosphorus (TP). The result revealed that the removal efficiency of DHS reactor improved when the HRT increased from 2 h to 6 h as shown in Table 5.1. Besides, it was found that the accumulation of sludges on the sponge increased when the HRT decreased. This was due to the higher production rate of sludges and entrapment of SS at higher OLR and lower HRT.

Table 5.1: Removal Efficiency of Organic Substances and Nutrients at Different HRTs (Mahmoud, Tawfik and El-Gohary, 2010).

HRT (h)	**2**	**4**	**6**
TCOD (%)	56 ± 15	80 ± 4	89 ± 3
TBOD (%)	$-$	93 ± 4	97 ± 2
TSS (%)	$-$	91 ± 4	94 ± 2
NH_4-N (%)	72 ± 4	90 ± 8	99 ± 2.5
TKN (%)	$-$	64 ± 8	71 ± 11
TP (%)	35 ± 16	38 ± 11	43 ± 14

Besides, Machdar et al. (2018a) also observed that the organic matter and ammonia removal efficiency of a pilot scale DHS reactor for wastewater improved when the HRT increased from 3 h to 4 h. The researchers also found that DO concentration of the effluent was almost constant at different HRT. This proved that the flow rate of wastewater within the reactor has no effect on the DO uptake. The oxygen within the reactor was able to diffuse into the wastewater easily. However, Yoochatchaval et al. (2014) found that the removal efficiency of DHS reactor was high and similar at different HRTs ranging from

1 h to 4 h. This might be due to the good performance of DHS reactor which outweighed the bad effects of low HRT.

In short, longer HRT will improve the removal efficiency of the DHS reactor as it allows longer contact time between wastewater and microorganisms, leading to more microbial activity to decompose the pollutants in the wastewater. Nevertheless, it is not practical to have too long HRT in a wastewater treatment plant. This makes the operation to be slow and inefficient. Therefore, an optimum HRT that is sufficient to achieve an acceptable removal efficiency must be figured out. Nonetheless, the optimal HRT is different based on the situation. There are several parameters that might altered the optimal HRT such as influent and effluent flow rate and volume of the reactor. Hence, the best HRT can be obtained after taking those parameters into account.

5.3 Hydraulic Loading Rate (HLR)

Hydraulic loading rate (HLR) is defined as the volume of the wastewater applied per surface area of the process unit per unit time (Theobald, 2016). For DHS system, the process unit will be the sponge media. Lower HLR simply means lower influent flow rate into the reactor. Lower flow rate leads to higher HRT and therefore better the removal efficiency of DHS reactor.

According to Mahmoud, Tawfik and El-Gohary (2010), the removal efficiency of DHS reactor was studied at different HLR. The results indicated that HLR exhibited a significant impact in the removal of COD as shown in Figure 5.1. The greater the HLR, the lower the COD removal from the influent. This result was proportional to the theory.

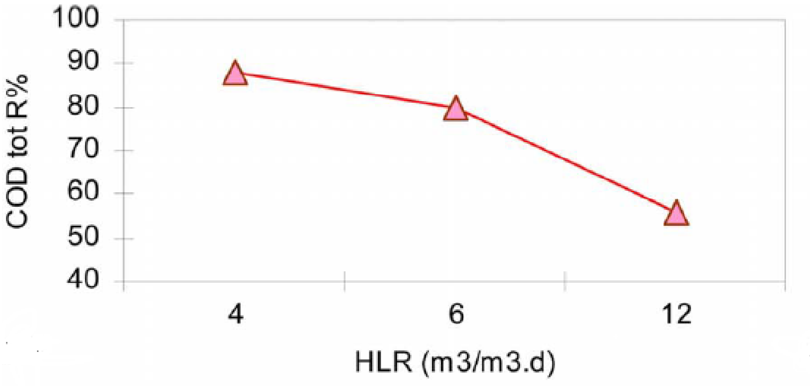

Figure 5.1: Total COD Removal Against HLR (Mahmoud, Tawfik and El-Gohary, 2010).

However, Tandukar et al. (2006) reported that the DHS reactor was able to tolerate the hydraulic shock loading, which was increase in the HLR from 12 m^3/m^3d to 24 m^3/m^3d , in a long run. The COD removal for the normal operating HLR was 22-29 mg/L, which was similar with the COD removal of 23-30 mg/L for increased HLR. The most affected process by altering the HLR was nitrification. The removal efficiency of ammonium was deteriorated from 73 % to 38.3 % by increasing the HLR. This was due to the contact time between nitrifiers and ammonia was too short at high HLR. Fortunately, the recovery of nitrification activity was immediate after the cancellation of hydraulic shock loads. It took about 2 hours for the nitrification activity to recover after changing back to normal HLR. This showed that the nitrifiers were still remained in the reactor, but they could not carry out nitrification because heterotroph bacteria were more dominant during the shock load with higher OLR. Besides, the SS was found to be abit higher at the DHS effluent. It could be clarified that high influent flow rate produces a higher shear force, hence causing a small portion of biomass attached onto the sponge to be washed out from the reactor.

In summary, high HLR will lead to higher flow rate of influent wastewater passing through the reactor. This induces lesser contact time between microorganisms and wastewater, causing lesser decomposition of nutrients and organic substances in the wastewater. Furthermore, the high flow rate of influent may detach the biomass on the sponge media, thereby reducing the quantity of microorganisms in the reactor. These possibilities will reduce the removal efficiency of DHS reactor. Therefore, the HLR should be set as low as possible to enhance the performance of DHS reactor. Similar to HRT, an optimum HLR need to be obtained as it varies based on the situation.

5.4 Organic Loading Rate (OLR)

Organic loading rate (OLR) can be defined as the amount of soluble and particulate organic matter applied per surface area of the process unit per unit time (Washington State Department of Health, 2002). In DHS system, the process unit will be the sponge media. OLR can be controlled by pre-treatment or primary treatment. This initial treatment may reduce the COD or TSS of the influent wastewater by removing more particulate matter, thus leading to lower OLR.

According to Mahmoud, Tawfik and El-Gohary (2010), apart from HLR, OLR also led to a significant impact to the COD removal efficiency. When the OLR increased from 1.8 kg COD / m^3d to 3.4 kg COD / m^3d, the total COD removal efficiency declined from 80 % to 56 % as shown in Figure 5.2. This was due to the escape of biosolid at high OLR as the microorganisms were unable to decompose all the organic matters. Another reason was the presence of non-biodegradable organic matters in the experimental wastewater which are unable to be removed by microorganisms. Besides, the NH_4-H removal also decreased when OLR increased. The reason behind was that high COD influent was more preferable for heterotrophic microorganisms compared to autotrophic microorganisms. Thus, nitrification decreased along with increased influent COD, causing higher NH_4-H concentration in the effluent.

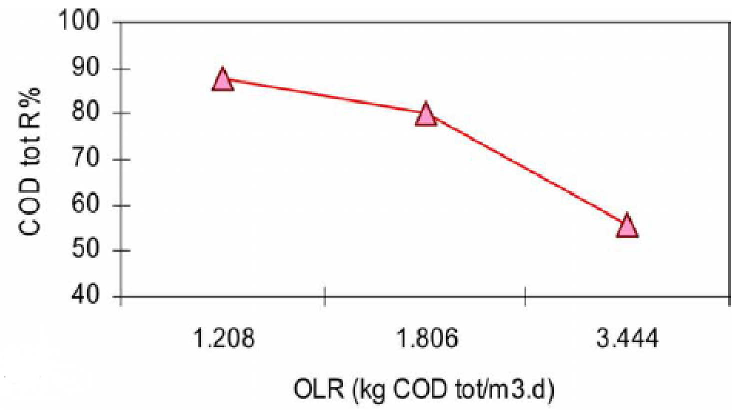

Figure 5.2: Total COD Removal Against OLR (Mahmoud, Tawfik and El-Gohary, 2010).

However, according to Tandukar et al. (2006), DHS system was capable to tolerate the high OLR. In this journal, the OLR of the DHS reactor increased from 2.03 kg COD / m^3d to 3.8 kg COD / m^3d and 7.6 kg COD / m^3d. Despite the COD at the effluent was higher at higher OLR, the COD removal was higher at higher OLR. This implied that the removal efficiency of COD was not much affected from the increasing OLR. Besides, similar with increasing HLR, nitrification was determined to deteriorate with increasing OLR. The NH_4−H concentration at the effluent increased from 12 mg NH_4-N / L to 20 mg NH_4-N / L and 25 mg NH_4-N / L, with the respective OLR. The recovery of nitrification activity was rather slow compared to HRT, taking about 2 to 5 hours after the termination of organic shock load. This could assume that the nitrifiers were still within the reactor. However, the heterotrophic bacteria overruled the nitrifiers and thus the nitrification activity was reduced. It was also indicated that the nitrifiers may not survive under organic shock loads for too long. It was predicted that longer period of high organic loading would wash out the nitrifiers from the reactor.

Nomoto et al. (2018) showed similar results with Tandukar et al. (2006). In this study, the DHS reactor was setup with four layers of sponge media. The DO profile was also investigated under different OLR. It was noticed that the DO concentration at the first layer or top layer was negligible at high OLR. This was due to the high microbial activity that consumed oxygen to decompose the organic matters. Thus, the oxygen consumption rate exceeded the oxygen supply rate, causing the DO concentration to be close to zero. As most of the organic matter was removed at the first layer, the following layers had lower organic matter and therefore the DO concentration was significant.

In conclusion, high OLR will lead to higher organic matter removal but lower nitrogen removal as the nitrifiers unable to carry out nitrification due to the dominance of heterotrophic bacteria in competing DO at high organic loading. Therefore, lower OLR is preferable to enhance the overall removal efficiency of DHS reactor. Similar to HRT and HLR, an optimum OLR needs to be obtained because denitrification process requires sufficient organic matter as carbon source to denitrify nitrate into nitrogen gas. The optimum OLR can be obtained through continuous experiments and trials.

5.5 Size of Sponge Media

Polyurethane sponge is used as a filter media to retain biomass that is crucial for the attached growth of microorganisms (Nurmiyanto and Ohashi, 2019). The size of sponge media is essential for pollutants removal in DHS reactor. The reduction in sponge size will increase the surface area exposed to the wastewater and thus enhance the contact between biomass and wastewater. This improves the removal efficiency.

The influence of sponge size on the performance of the DHS system was studied by Uemura et al. (2012). The experiment was carried out at three similar DHS reactors with different sponge sizes that formed a fixed sponge volume as shown in Table 5.2.

Table 5.2: Sponge Sizes for Three Different Reactors (Uemura et al., 2012).

Reactor No.	**1**	**2**	**3**
Sponge Volume (cm^3)	240	240	240
Number of Sponges	60	38	27
Surface area (cm^2)	480	430	384

The result indicated that all three reactors showed good performance in COD removal, with SS and COD concentration of less than 10 mg/L at the effluent. The reactor 1 with smaller sponge size showed a slightly greater COD removal compared to reactor 2 and reactor 3. Besides, similar results were found on the removal of ammonium nitrogen and fecal coliform as shown in Table 5.3. Furthermore, the DO profile was determined, with the highest DO at reactor 1 followed by reactor 2 and lastly reactor 3. This is because the surface area of the sponge is bigger when the sponge size is smaller. The smaller size of the sponge media will ease the oxygen uptake to by the sewage in the reactor and also improve the contact between biomass and wastewater. Besides, more sludges can be retained with smaller sponge size.

Table 5.3: Removal Efficiency of DHS reactor at Different Sponge Size (Uemura et al., 2012).

Reactor No.	**1**	**2**	**3**
COD (%)	85.2 ± 11.4	83.7 ± 12.4	82.9 ± 11.6
NH_4-N (%)	94.7 ± 9.2	91.1 ± 14.8	90.1 ± 13.1
Fecal Coliform (log_{10})	2.95	2.32	2.19

To conclude, smaller sponge size will increase the surface area of the sponge media. Large surface area will cause the oxygen to diffuse into the wastewater easily. The large surface area will also more contact between the wastewater and sludge retained on the sponge media. Therefore, more microbial activity will be carried out, thereby increasing the removal efficiency of DHS reactor.

5.6 Ventilation

Since oxygen can diffuse into the sponge media easily, there is no need for any external aeration. Natural ventilation is the only oxygen source in the DHS system (Nurmiyanto and Ohashi, 2019). Natural ventilation depends on the humidity and temperature of the air inside and outside of the reactor. The humidity and temperature differences are required to be large enough so that it produces the needed force that attract the air into the reactor, thus causing effective ventilation. Better ventilation will enhance the performance of DHS reactor as it provides oxygen for the microorganisms to carry out activities such as nitrification.

The theory was proven by Onodera et al. (2014a). The experiment to test the effect of ventilation on reactor performance was conducted from day 281 to day 313 under constant temperature. The ventilation was adjusted from the opening and closing of the windows at the three segments in the DHS reactor. The experiment was divided into three phases. All windows were closed in phase 1. Conversely, all windows were opened in phase 2. Lastly, the first window was opened while the remaining windows were closed in phase 3. The DO concentration was found to be lower in phase 1, followed by phase 3 and phase 2. The characteristic of the influents to the DHS reactor showed no substantial variation for all three phases. At the effluent, the removal efficiency at phase 2 is better than phase 1 and phase 3 as shown in Table 5.4. This result proved the importance of DO for nitrification process. The higher concentration of oxygen will allow more nitrifiers to carry out oxidation of ammonia, thus improving the removal efficiency. This study showed that better ventilation is crucial for better performance of DHS reactor.

Table 5.4: Removal Efficiency of DHS reactor at Different Phases (Onodera et al., 2014a).

Phase	1	2	3
TBOD (%)	78 ± 6	87 ± 2	78 ± 10
TKN (%)	32 ± 18	86 ± 6	45 ± 15
TN (%)	18 ± 23	32 ± 14	31 ± 21

In a nutshell, good ventilation is required to provide sufficient oxygen within the reactor so that microbial activity can be carried out by consuming the dissolved oxygen, therefore improving the removal efficiency. It is suggested to have windows or holes at the top of each segment of DHS reactor. This allows oxygen to diffuse into every segment of DHS reactor. In addition, natural ventilation is adequate to supply oxygen into DHS reactor. Hence, no external aeration system is required, which makes the development cost of DHS reactor to be cheaper.

5.7 Climate

Climate or in other words, operating temperature is another crucial factor that will have a significant effect to the performance of DHS reactor. The operating temperature does not really need to be adjusted as it usually depends on the weather of the respective country. The temperature is definitely lower when the system is applied in colder countries such as Mongolia. Theoretically, microorgansims are very sensitive to the climate. Lower temperature will reduce the microbial activity and thereby reducing the removal efficiency of DHS reactor.

Several studies have obtained similar results with the theory. According to Tandukar, Ohashi and Harada (2007) and Onodera et al. (2016), the removal efficiency of DHS system was found to be higher at warmer temperature ($20\ ℃ - 32\ ℃$). The low organic matter and ammonia concentration at the effluent were due to high nitrifying activity and metabolic rates under high warmer condition. The researchers suggested that the system is more suitable for developing countries with subtropical or tropical climates.

However, Nomoto et al. (2018) showed different results with the previous studies. The DHS reactor was setup with four layers of sponge media in this research study. The influence of temperature on ammonia nitrogen removal was studied at 489 days with an influent temperature of $26\ ℃$ and 565 days with an influent temperature of $17\ ℃$. Despite the temperature at 565 days was lower compared to 489 days, the ammonia nitrogen removal at 565 days was found to be higher than 489 days. The authors suggested that the organic matter and DO concentration has higher influences on the performance of DHS reactor compared to temperature. The organic matter was found to be lower at 565 days compared to 489 days. Besides, the DO concentration at the first layer was found to be higher at low temperature (565 days). There are three possibilities leading to high DO concentration. Firstly, the microbial activity was lower at low temperature, thus leading to lower oxygen consumption. Secondly, the saturated dissolved oxygen was higher at low temperature. Lastly, the difference in temperature at the internal and external of the reactor was larger at 565 days. Large temperature differences will force oxygen into the reactor. The low organic matter and high DO at 565 days allowed more nitrifying activity to be carried out, hence improving the net ammonia-nitrogen removal.

In conclusion, DHS reactor that has a higher operating temperature will improve the microbial activity and therefore enhancing the performance of DHS reactor. It is more preferable to build DHS reactor in developing countries with a warmer temperature ranging from 20 ℃ to 32 ℃ . For instance, India and Thailand.

5.8 Summary

There are several major factors that will influence the performance of the DHS system. In order to improve the removal efficiency, the DHS reactor must operate at a higher HRT, lower HLR and OLR, higher operating temperature and with smaller sponge size and better natural ventilation. The factors affecting the performance of DHS reactor is summarized in Table 5.5.

Table 5.5: Influence of Factors on DHS System.

Factors	Summary	References
Hydraulic Retention Time (HRT)	Longer HRT improves the contact time between microorganisms and wastewater.	(Mahmoud, Tawfik and El-Gohary, 2010; Yoochatchaval et al., 2014; Machdar et al., 2018a)
Hydraulic Loading Rate (HLR)	Lower HRT enhance the contact time between microorganisms and wastewater and reduce the biomass detachment from the sponge medium.	(Tandukar et al., 2006; Mahmoud, Tawfik and El-Gohary, 2010)
Organic Loading Rate (OLR)	Lower OLR allows sufficient organic removal and improve nitrification activity.	(Tandukar et al., 2006; Mahmoud, Tawfik and El-Gohary, 2010; Nomoto et al., 2018)
Sponge Size	Smaller sponge size increases the specific surface area which improve the diffusion of oxygen into wastewater.	(Uemura et al., 2012)
Ventilation	Good natural ventilation is essential to ensure high DO within the reactor.	(Onodera et al., 2014a)
Climate	Warmer temperature improves microbial activity in the reactor.	(Tandukar, Ohashi and Harada, 2007; Onodera et al., 2016)

CHAPTER 6

MICROBIAL COMMUNITY IN DHS SYSTEM

6.1 Microbial Community Analysis

The special feature of DHS reactor allows higher accumulation of biomass in the sponge media and hence providing an excellent environment for the development of microbial habitats. However, the microbial community structure is not similar throughout the whole reactor. It differs along the height of the reactor. This is because the pollutants concentration of the wastewater varies from the top segment to the bottom segments of the reactor, indicating different microbial activities have been carried out at the top segment and the bottom segment. Besides, the redox potential which varies from the external layer to the inner layer of the sponge media stipulates the presence of aerobic and anaerobic microorganisms in the sludge (Hatamoto et al., 2018).

Kubota et al. (2014) was the first research team to investigate the composition of microbial community in DHS reactor. Third generation DHS reactor with a HRT of 3.2 hours was applied in this study. The sludge samples were collected at the upper segment, middle segment and bottom segment during two periods: Period 1 (day 283) and Period 2 (day 441). Microbial community analysis was performed on the collected sludges based on 16S rRNA gene sequence technique. The clone libraries were developed and tabulated in Table 6.1.

Table 6.1: Microbial Community Compositions in G3 DHS Reactor (Kubota et al., 2014).

Period	**1**			**2**		
Reactor Part	**Upper**	**Middle**	**Bottom**	**Upper**	**Middle**	**Bottom**
Alphaproteobacteria	9.7	4.0	8.3	6.3	4.8	10.0
Betaproteobacteria	49.5	45.5	20.8	22.8	20.0	6.7
Gammaproteobacteria	25.8	16.2	30.2	37.8	29.1	14.2
Deltaproteobacteria	3.2	3.0	1.0	0.8	2.4	9.2
Bacteroidetes	5.4	12.1	9.4	7.1	5.5	9.2
Verrucomicrobia	NA	5.1	3.1	1.6	NA	0.8
Gemmatimonadetes	NA	2.0	4.2	NA	NA	2.5
Acidobacteria	NA	7.1	9.4	0.8	21.2	37.5
Nitrospira	NA	NA	6.3	NA	1.8	1.7
Firmicutes	3.2	1.0	4.2	18.1	3.0	NA

Based on the composition of microbial community, it was observed that the phylum *Proteobacteria* was superior in all samples, with more than 40 % at every segment in the reactor. Among the class of the *Proteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria* were the most dominant classes. Other than *Proteobacteria*, phylum *Bacteroidetes* was found with significant amount at all segment of the reactor. Furthermore, phylum *Acidobacteria* was discovered to be significant at the middle segment and bottom segment, especially during period 2. This analysis revealed that the composition of microbial community varies along the height of the reactor.

6.2 Bacteria Involved in Organic Removal

According to Kubota et al. (2014), it was observed that most of the organic matter were removed at the upper segment of the reactor. Therefore, bacteria involved in removing organic matter should be abundant at the top of the reactor. During Period 1, *Dechloromonas* was found to be the most dominant genus which constituted about 40 % of the total clones at the upper segment but the amount reduced to 14 % and 3 % at the middle and bottom segment respectively. This result inferred that this genus might has a significant contribution to the removal of organic matter. Horn et al. (2005) stated that *Dechloromonas* possessed the ability of consuming volatile fatty acids as carbon sources to carry out its microbial activities. Volatile fatty acids were supplied from the anaerobic zone of the sponge medium. It was suggested that certain amount of organic matter was degraded anaerobically using volatile fatty acids as carbon source. During Period 2, the number of *Dechloromonas* clones deteriorated at the upper segment, which stood only 4 % of the total clones. However, several genera such as *Xanthomonas axonopodis*, *Clostridiales*, *Comamonadaceae* and *Xanthomonadales* were found to be dominant at the top of the reactor. Besides, the number of clones for phylum *Firmicutes* increased to 18.1 % during this period. It was suggested that these bacteria may play a crucial role in the organic matter removal.

6.3 Bacteria Involved in Nutrients Removal

According to Kubota et al. (2014), clones of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria were present at the middle and bottom segment of the reactor, where the concentration of organic matter was low. During Period 1, genus *Nitrosomonas oligotropha* lineage from class *Betaproteobacteria* was the sole ammonia-oxidizing identified in the middle and bottom segment while genus *Nitrospira* was found to be the nitrite-oxidizing bacteria in the bottom segment. During Period 2, no clones of ammonia-oxidizing bacteria was detected while *Nitrospira* was present at both middle and bottom segment. For denitrification process, clones for denitrifying bacteria such as *Dechloromonas* were found to be abundant on both periods. A type of *Dechloromonas* can reduce nitrite and nitrate to nitrogen monoxide (Horn et al., 2005).

Tanikawa et al. (2019) conducted a lab scale experiment to investigate the nitrogen removal efficiency of DHS reactor in a natural rubber wastewater treatment system. Sodium acetate was added to the reactor as carbon source to enhance denitrification process. The microbial community related to nitrogen removal was analyzed. For nitrification, the most dominant nitrifying bacteria was observed to be *Nitrosovibrio*, *Nitrospira* and *Brocadia*. *Nitrosovibrio* oxidizes the ammonia to nitrite, which will be further consumed by *Nitrospira* and *Brocadia* to form nitrate. For denitrification, the dominant denitrifying bacteria was *Amaricoccus*, *Hylemonella* and *Pseudoxanthomonas*. *Amaricoccus* first reduces the nitrate to nitrite by consuming acetate, *Pseudoxanthomonas* then reduces the nitrite to nitrous oxide by consuming the oxygen as electron acceptor and finally *Hylemonella* converts the nitrous oxide to nitrogen gas. Besides, genus *Xanthobacter*, nitrogen fixing bacteria was observed to be abundant in the reactor. This implied that this genus may play an important role in nitrogen removal.

Likewise. Tanikawa et al. (2020) studied the microbial community composition for denitrification in DHS reactor. From the analysis, the dominant denitrifying bacteria in the biomass was found to be *Alicycliphilus*, *Acidovorax*, *Azospira*, *Thauera* and *Luteimonas*. *Alicycliphilus* was the main denitrifier that convert nitrite or nitrate to nitrogen gas. Besides, certain nitrates were reduced to nitrite, nitrous oxide and lastly nitrogen gas by cooperation of several genera such as *Acidovorax*, *Azospira*, *Thauera* and *Luteimonas*. In addition, *Xanthobacter* and *Azohydromonas* were the dominant nitrogen fixing bacteria in the reactor. These genera transformed some of the atmospheric nitrogen gas to fixed nitrogen.

As for phosphorus, there are no reports that study the microbial community for phosphorus removal in DHS reactor. It is suggested that phylum *Gemmatimonadetes* may consist of PAOs (Zhang et al., 2003). Furthermore, another possible phosphate removal bacteria may be *Candidatus Accumulibacter phosphatis* under *Betaproteobacteria* class. This genus was observed to be dominant in the anaerobic and anoxic sequencing batch reactor which functioned to further remove phosphorus from the effluent of combined UASB-DHS system (Hatamoto et al., 2015).

6.4 Other Important Bacteria

There are several important bacteria which have their respective functions in the reactor. According to Kubota et al. (2014), methanotrophs were found to be present in the DHS reactor. It oxidized the remaining dissolved methane from the UASB effluent. Therefore, low methane concentration was observed in the DHS reactor as the oxidizied methane would immediately vaporized and diffused into the air. Small amount of methanotrophs were found at the upper segment of the reactor, which consisted of genus *Methylomonas*, *Methylobacter* and *Methylosarcina*. Those methanotrophs were type I methanotrophs under the class *Gammaproteobacteria*. This indicated that the oxidation of methane was most likely took place at the top of the reactor.

Furthermore, small amount of sulfur oxidizer and sulfate reducer were present at the upper portion of the reactor (Kubota et al., 2014). The sulfur oxidizer belonged to genus *Thiothrix* under class *Gammaproteobacteria* while the sulfur reducer belonged to genus *Desulfobulbus* under class *Deltaproteobacteria*. *Thiothrix* first oxidized the dissolved sulfide from the UASB effluent to sulfate. Then, the sulfate would be uptaken by *Desulfobulbus*. Therefore, the composition of bacterial community showed the presence of simultaneous sulfur removal and organic matter removal at the upper segment of the reactor.

In addition, few clones associated to genus *Lysobacter* under *Gammaproteobacteria* were determined at all parts of the reactor (Kubota et al., 2014). These bacteria were capable to lyse a wide range of microorganisms. It was suggested that these bacteria might be in charged for self-degradation of biomass attached on the sponge medium within the reactor. Therefore, production of excess sludge was negligible in DHS reactor.

6.5 Concluding Remark

In short, the microbial community structure varies along the height of DHS reactor. Phylum *Proteobacteria* is the most dominant microorganisms in DHS reactor. The bacteria involved in organic removal is abundant at upper portion of the reactor while nitrifying bacteria is abundant at the middle and bottom portion of the reactor. Therefore, organic matter is mostly removed at the top of the reactor whereas the nitrogen is eliminated at the middle or bottom segment of the reactor. Besides, denitrifying bacteria is also detected, thus verifying the presence of simultaneous nitrification and denitrification in the reactor. Furthermore, few important bacteria such as methanotrophs, sulfur oxidizer bacteria, sulfate reducer bacteria and *Lysobacter* were found in the reactor. These bacteria have their respective functions in the reactor (Kubota et al., 2014). Nonetheless, microbial community for phosphorus removal bacteria has not been studied by any researcher yet. It is suggested that these bacteria should be present as the total phosphorus removal is observed in the reactor (Mahmoud, Tawfik and El-Gohary, 2010).

CHAPTER 7

CONCLUSIONS AND RECOMMEMNDATIONS

7.1 Summary of Review Study

In this study, it has provided clear evidence that the DHS system is capable to perform satisfactory nutrients removal as well as excellent organic matter removal to the municipal wastewater. The effluent quality has satisfied the water discharged standard for most of the countries. Based on the results from the past researchers, third generation and sixth generation DHS reactor exhibit better nutrients removal among the six generations of DHS reactor. Hence, third generation and sixth generation are more preferable to be applied as aerobic post-treatment along with UASB as anaerobic pre-treatment to remove nutrients from the municipal wastewater.

Moving forward, several major parameters such as HRT, OLR, HLR, sponge size, ventilation and climate have shown significant effects on the performance of DHS reactor. The system should operate under higher HRT, lower OLR, lower HLR and warmer temperature and with smaller sponge size and good ventilation system. However, optimum HRT shall be obtained through continuous experimental work as it is impractical to have too low HRT. Similar situations for OLR, HLR and sponge size.

Next, the composition of microbial community determines the type of pollutants that are able to be removed within the reactor. Microbial community structure varies along the height of the reactor. Heterotrophic bacteria involved in organic matter removal dominates the upper segment while the nitrifying bacteria involved in nitrification dominates the middle and bottom segment of the reactor. Furthermore, denitrifying bacteria involved in denitrification is present in the reactor. This finding is significant because it verified the capability of DHS reactor in removing organic matter and nutrients. Other important bacteria related to methane removal, sulfur removal and sludge degradation are also present in the reactor.

In short, DHS reactor is undoubtedly a simple, robust and reasonable wastewater treatment which able to perform stable nutrients removal and organic matter removal in municipal wastewater.

7.2 Limitations and Recommendations for Future Work

One limitation of this study is the comparison for the removal efficiency of different generations DHS reactors from various researchers may not be accurate. The experimental conditions or parameters set by the researchers were not taken into account. As mentioned from the results, the performance of the system will alter by having different factors such as HRT and sponge size. To address this limitation, future research should attempt to investigate the performance of all six types of DHS reactor under the exact same experimental conditions.

On the other hand, the phosphorus removal efficiency of DHS reactor is still remained unclear, as well as the microbial community that involved in phosphorus removal. This is due to lack of phosphorus related data for DHS system as almost all of the past researchers focus on nitrogen removal rather than phosphorus removal. It is crucial to take phosphorus into account as excess phosphorus will also increase the algae growth which lead to eutrophication, thereby reducing the water quality. To address this limitation, total phosphorus in the reactor influent and effluent should be considered in the future work. Besides, further research is needed to investigate the microbial community composition for phosphorus removal in DHS reactor.

Finally, despite having satisfactory denitrification, it is recommended to add carbon source such as sodium acetate to every segment of DHS reactor, especially to the middle and bottom segments where nitrification and denitrification happen frequently. Denitrifying bacteria will be able to perform its activites by having sufficient carbon source. This will further enhance denitrification and therefore improving the nitrogen removal.

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APPENDICES