STUDY ON THE FORMATION OF GRANULAR ALGAE

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2018

STUDY ON THE FORMATION OF GRANULAR ALGAE

by

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Thesis submitted in partial fulfilment of the requirement for the degree of

Bachelor of Chemical Engineering

June 2018

ACKNOWLEDGEMENT

First and foremost, I would like to take this opportunity to express my gratitude to my supervisor, Associate Professor Dr. Vel Murugan Vadivelu, for his guidance. Dr. Vel also willing to spent his spare time to provide advice, suggestion and encouragement throughout this project which enable me to accomplish my project successfully

Besides, I would like to extend my appreciation to Mr. G. Sivarajah a/l M. Ganesan, who had been providing guidance on laboratory and hands on skills. Helps from Ms. Lim Rong Er, Ms. Khoo Choon Gek and other postgraduate students are also important to me. Without their helps and guidance, it is impossible for me to complete my project smoothly

Last but not least, I would like to thank my family and friends who had given me moral support and useful suggestion when I faced problems in the project. Their spiritual and mental support motivate me to complete the project despite all the problems.

Tan Pei Chin

June 2018

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LIST OF ABBREVIATION

BBM	Bold’s Basal Medium
BCA	Bicinchoninic Acid
DI Water	Deionized Water
EPS	Extracellular Polymeric Substances
MLSS	Mixed Liquor Suspended Solids
MLVSS	Mixed Liquor Volatile Suspended Solids
SBR	Sequencing Batch Reactor

KAJIAN MENGENAI PEMBENTUKAN BUTIR-BUTIR ALGA

ABSTRAK

Mikroalga boleh digunakan dalam rawatan air sisa biologi kerana mikroalga dapat hidup dalam keadaan yang teruk. Mikroalga juga dikenal pasti sebagai sumber alternatif yang berpotensi untuk pengeluaran bahan api bio, tetapi terdapat kelemahan besar untuk kedua-dua aplikasi kerana kultur alga biasanya rendah dalam ketumpatan kultur dan kecil dalam saiz. Hal ini menyebabkan proses penuaian dan pemisahan mikroalga menjadi tenaga intensif. Oleh itu, kedua-dua aplikasi itu tidak dapat dilaksanakan secara besar-besaran. Mikroalga mampu mengeluarkan bahan-bahan polimer ekstraselular (EPS) yang penting untuk pembentukan butir-butir. Dengan menyediakan keadaan kultur yang sesuai bagi microalgae untuk membentuk butir-butir, penuaian dan pemisahan mikroalga akan menjadi lebih mudah kerana butir-butir tersebut mempunyai ketumpatan yang lebih tinggi dan dapat termendak dengan mudah. Dalam eksperimen ini, beberapa faktor yang mempengaruhi pengeluaran EPS dalam mikroalga diselidiki, antaranya termasuk kandungan nutrien dan fasa pertumbuhan mikroalga. MLSS, kandungan EPS dan analisis nutrien dijalankan secara berkala sepanjang eksperimen. Terdapat pembentukan butiran dalam mikroalga yang ditemui selepas pertumbuhan mikroalga mencapai fasa pegun. Masa termendap mikroalga menjadi lebih pendek dengan pembentukan butiran yang lebih besar dan lebih banyak.

STUDY ON THE FORMATION OF GRANULAR ALGAE

ABSTRACT

Microalgae can be used in biological wastewater treatment as it can survive harsh condition. Microalgae have also been identified as potential alternative resource for biofuel production. But there are significant disadvantages to both applications as the algae culture usually low in density and small in size. This causes the harvesting and separation processes of the microalgae to be energy-intensive and thus nonfeasibility of scale up. Microalgae are able to produce extracellular polymeric substances (EPS) which is crucial for the formation of granule. By providing cultural condition that is suitable for microalgae to form granule, harvesting and separation of microalgae will be much easier as granule has higher density and can settle down easily. In this experiment, several factors that influence the production of extracellular polymeric substances (EPS) in microalgae are investigated, which are the availability of nutrient and growth phase of the microalgae. MLSS, EPS content and nutrient analysis are carried out periodically throughout the experiment. There is formation of granular sludge in microalgae found after the growth of microalgae reach stationary phase. The settling time of the microalgae became shorter as bigger and more granule form.

CHAPTER ONE

INTRODUCTION

1.1 Introduction

Algae are simple organisms that are mainly aquatic and microscopic. Microalgae are unicellular photosynthetic micro-organisms, living in saline or freshwater environments that convert sunlight, water and carbon dioxide to algal biomass (Ozkurt, 2009). Algae are among the fastest growing plants in the world, and about 50% of their weight is lipid. Microalgae have faster growth-rates than terrestrial crops, with the per unit area yield of oil from algae is estimated to be from between 20,000 to 80,000 per acre, per year; this is 7–31 times greater than the next best crop, palm oil (Demirbas, 2010).

Microalgae have been identified as a potential alternative resource for biofuel production. Algae can be grown almost anywhere, even on sewage or salt water, and does not require fertile land or food crops, and processing requires less energy than the algae provides (Demirbas, 2010). But there are significant drawbacks to algae culture due to low density and small size of algae. Thus harvesting of algae is an energy-intensive process and is considered a major challenge for the commercial-scale production of algae biofuels. There are a few commonly used harvesting techniques such as gravitational sedimentation, centrifugation and filtration (Parmar et al., 2011). These techniques have a number of drawbacks, such as high cost, flocculants toxicity or nonfeasibility of scale-up, which increase the cost and reduce the quality of products (Bux, 2013).

There are many research works carried out on the formation of granule in bacteria for harvesting of bacteria in biological wastewater treatment. Granular bacteria are much denser than floc foaming and filamentous bacteria. The separation of granular bacteria from the treated water can be carried out in a short period of time as denser bacteria can settle faster in the settling tank. Granular bacteria form due to the production of extracellular polymeric substances (EPS). Microalgae are able to produce EPS too, but study of granulation in microalgae is still in progress as there is no viable solution to use at industrial scale has been recognized (Tiron et al., 2015). Therefore, this research focuses on the formation of granular algae by introducing stress to the algae during the culture process.

1.2 Problem Statement

There are several biomass harvesting method available for the recovery of algae from culture broth. However, no technique can be practically applied individually due to technical and economical limitations (Bux, 2013). By culturing microalgae under stress, microalgae can form granule, which is denser than the filamentous type. Granular algae can settle down easily, thus provide an energy efficient harvesting technique. In this work, formation of algae granule will be observed and sample will be taken from culture broth to test for production of EPS from algae.

1.3 Research Objectives

I. To study the production of extracellular polymeric substances (EPS) in microalgae.

II. To study the effect of nutrient content on the production of EPS.

III. To study the formation of granular sludge in microalgae.

CHAPTER TWO

LITERATURE REVIEW

2.1 Wastewater Treatment

Growing population especially in urban areas, has led to the need to treat the generated wastewater as untreated wastewater caused sanitary problems. Bacteria, viruses, and parasites are the types of pathogens in wastewater that are hazardous to humans. Harmful bacteria are responsible for several wastewater related diseases, including typhoid, paratyphoid, and Salmonellosis (Bryan, 1977). Nowadays, numerous methods and systems for wastewater treatment have emerged in order to treat the largest possible volumes of wastewater in an efficient way.

Besides causing sanitary problems, eutrophication of water bodies has also become a great problem. Research showed that excess nitrogen and phosphorus from various sources, including wastewater treatment, discharge to recipients was one of the causes (Garc á, 2017). The biological elimination of nutrients from wastewater has been developed over the year. There are numerous processes and different configurations for wastewater treatment to achieve better efficiency in nitrogen and phosphorus removal.

Wastewater is treated in different steps as shown in Figure 2.1. First it is subjected to a preliminary treatment where coarse materials and debris are removed. During the primary treatment, sedimentable suspended solids are removed in sedimentation tanks. Next, in the secondary treatment, the wastewater is transformed into biological reactor, where mainly dissolved organic matter, nutrients such as

nitrogen, phosphorus and pathogens are removed and the sludge is then separated from the treated water by sedimentation. Finally, in some wastewater treatment plants, the wastewater undergoes tertiary treatment for the elimination of pathogens, suspended solids and other compounds that have not been eliminated in the previous stage, by the application of physical and chemical treatments such as membrane filtration or chlorination.

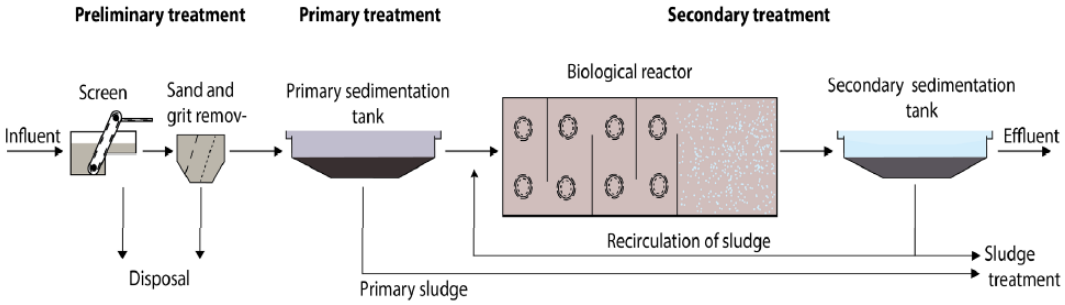

Figure 2.1: Schematic representation of a conventional wastewater treatment plant (Garc á, 2017).

2.2 Biological Wastewater Treatment

Biological treatment processes are considered as one of the most vital parts to wastewater treatment. The biological treatment of wastewater is based on natural microbiological processes. Different microorganism react differently to its surrounding. In order to removes the targeted contaminants from wastewater, different conditions were created in the biological reactors by applied different stress to the microorganism. Sludge purifies wastewater by removing organic matter through heterotrophic microorganisms that consist of mostly bacteria and occasionally fungi. Organic matter is broken down through either biological oxidation or biosynthesis. Biosynthesis combines colloidal and soluble organic matter to form particulate biomass that is then removed during the settling phase of treatment. Microorganisms consume organic and

inorganic matters to support their growth. A portion of the matter consumed is oxidized and the energy released from this reaction converts the remaining materials into new cell tissues that aggregate to form particulate biomass.

Generally, the biological wastewater treatment is classified into suspended growth and fixed films processes. They can also be classified into aerobic and/or anaerobic/anoxic. In the suspended growth processes, the microorganisms grow in suspension, in direct contact with the wastewater. Activated sludge, oxidation ponds, anaerobic digestion, sequencing batch reactors and membrane bioreactors are examples of suspended growth processes. Fixed film processes use an inert support material inside the biological reactor on which the microorganisms grow as biofilms and the substrates diffuse into the biofilm from the water. Trickling filters, rotating biological contactors, biological aerated filters, fluidized beds and moving bed biofilm reactors are fixed films processes.

2.3 Algae in Nutrient Recovery

Current methodologies applied for nutrients removal tend to be complex, expensive and energy demanding. Therefore, cultivation of microalgae has appeared as an emerging alternative for nutrients removal from wastewaters. Microalgae have been proven to be efficient in removing nitrogen, phosphorus, and toxic metals from a wide variety of wastewaters (Boelee et al., 2012, Sturm and Lamer, 2011, Zhou et al., 2012). Microalgae can be used in biofuel production as microalgae have high oil contents of 20–50% on a dry weight basis (Chisti, 2007). If key nutrients in the wastewater stream can be used to grow microalgae, this will significantly reducing the risk of harmful eutrophication while producing biofuel. After lipid extraction, the algae biomass residue

from biofuel production can be used as a nitrogen source, such as a protein-rich animal feed or fertilizer for crops (Spolaore et al., 2006). Microalgae cultivation from wastewater treatment has multiple applications, including biofuel production and carbon dioxide mitigation.

2.4 Challenges in Harvesting Microalgae

The harvesting of microalgae typically employs methods such as filtration, sedimentation, centrifugation, or flocculation, which act as a barrier to scaling-up microalgae-based technology designed for wastewater treatment (Cai et al., 2013). These typically applied harvesting techniques accounted for between 20 and 30% of total microalgae cultivation costs (Grima et al., 2003). This is due to the low settling velocity of the microalgae (lower than 0.0036 m/h) and their density, which is similar to that of water (Granados et al., 2012), with the microalgae species commonly used in wastewater treatment processes and the bioenergy production sector usually having a cell size of less than 30 μm (Wang et al., 2013). In recent years, other alternative of harvesting methods were proposed, such as bio-flocculation using auto-flocculating microalgae species as shown in Figure 2.2 (Salim et al., 2011), microalgae palletisation using fungi (Zhou et al., 2013) and microalgae separation using magnetic nanoparticles (Hu et al., 2014). Figure 2.3 shows magnetophoretic separation of algal biomass and recovery of magnetic nanoparticles from concentrated algae.

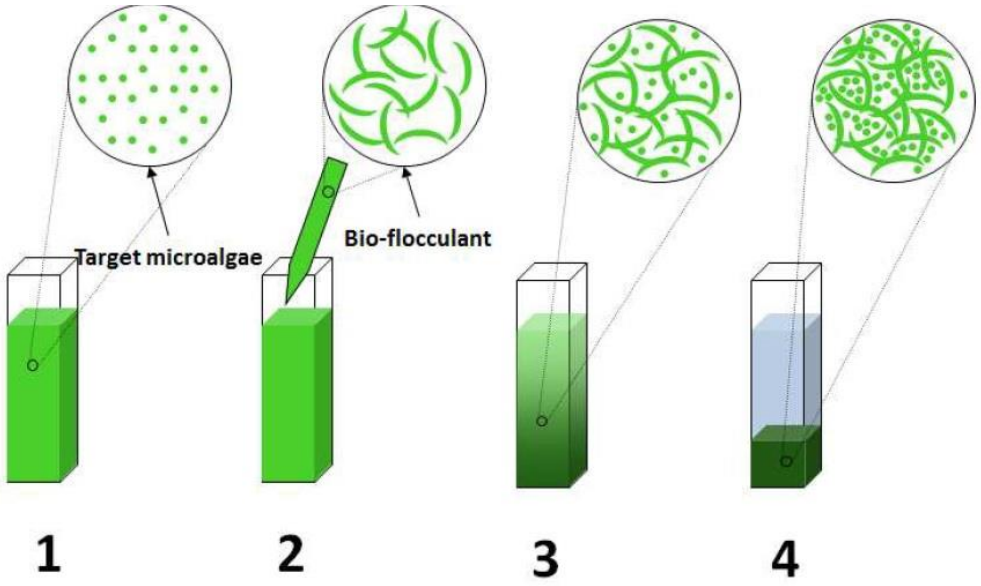

Figure 2.2: Schematic view of bio-flocculation (Salim et al., 2011).

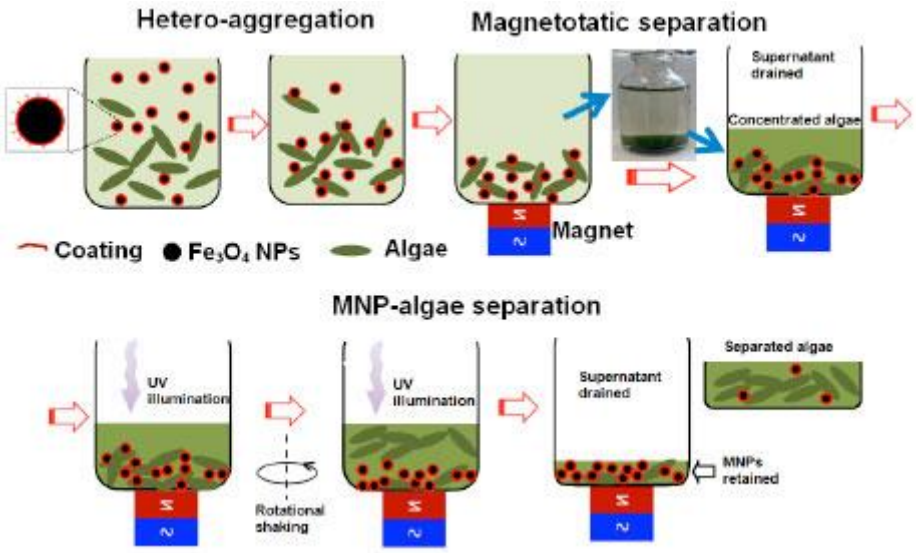

Figure 2.3: Magnetophoretic separation of algal biomass and recovery of magnetic nanoparticles from concentrated algae(Ge et al., 2015).

2.5 Aerobic Granulation

Aerobic granular sludge is a technology that has competitive advantages compared to activated sludge processes due to excellent settling properties, compact structure, smooth surface, regular morphology, high microbial densities and activities, ability to withstand high organic and nitrogen loadings, and tolerance to toxic

substances (Show et al., 2012). Numerous aspects that are understood to contribute to the granulation process include the use of specific self-aggregating cultures, selection by settling velocity, applied shear stress, growth rate of organisms, substrate gradients within the granules and the formation of extracellular polymeric substances (EPS). As the EPS molecules could form gel networks by means of chemical bonding as well as physical entanglement, amount of microorganisms were retained in the network, thus facilitated the accumulation of biomass and granulation of sludge. Aerobic granules are considered as suspended biofilms of microorganisms embedded in a matrix of EPS. Polymers of polysaccharides, proteins, humic acids, nucleic acids and lipids constitute the EPS and the distribution, proportion and chemical composition of these polymers determine the physical characteristics of granules (Seviour et al., 2010). Granular algae (Figure 2.4) have larger sizes than the individual algae, allowing harvesting process through simple filtration or sedimentation.

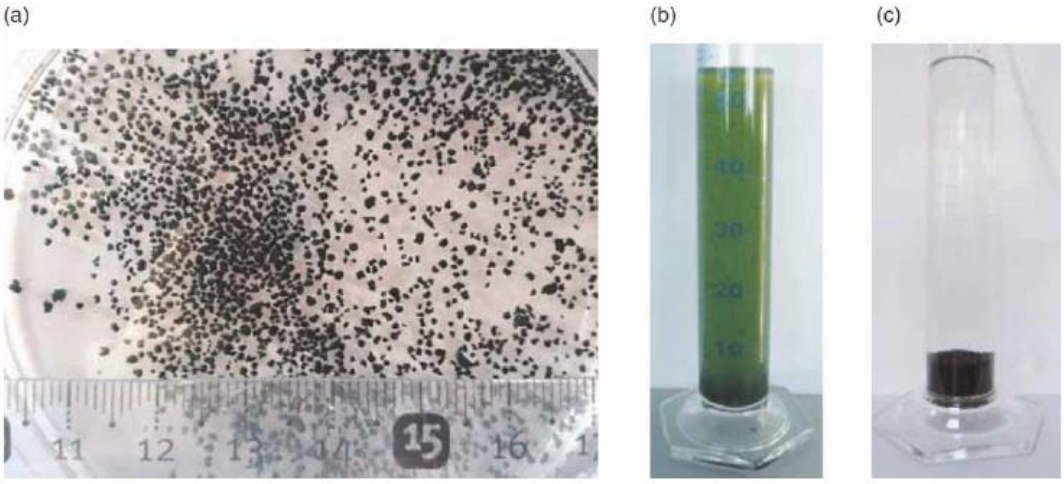

Figure 2.4: Images of the activated algae granules (a), settled activated algae flocs (note poor settleability of free Chlorella sp. cells remaining in the liquor) (b), and settled activated algae granules (note that clear effluent resulted after biomass settling) (c) (Tiron et al., 2015).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

There are several chemicals and other materials that are used during the experiment. The details of chemicals used are listed in Table 3.1 while the details of other materials are listed in Table 3.2.

Table 3.1: List of chemicals used in experiment.

Chemicals	Formula	Specification	Supplier
Di-potassium hydrogen orthophosphate	K_2HPO_4	≥99%	Merck
Potassium di-hydrogen orthophosphate	KH_2PO_4	99.50%	Merck
Magnesium sulphate	$MgSO_4.7H_2O$	99.50%	Merck
Sodium nitrate	$NaNO_3$	≥99%	Acros Organics
Calcium chloride	$CaCl_2.2H_2O$	≥98%	Merck
Sodium chloride	NaCl	≥99.5%	Merck
Ethylenediaminetetraacetic acid	EDTA	99.40%	Merck
Potassium hydroxide	KOH	96.25%	Fisher Scientific
Ferrous sulphate	$FeSO_4 \cdot 7H_2O$	≥99%	Bio Basic Canada Inc.
Sulphuric acid conc.	H_2SO_4	95%-98%	R&M Chemicals
Boric acid	H_3BO_3	99.50%	Merck
Zinc sulphate	$ZnSO_4 \cdot 7H_2O$	99.50%	Merck
Manganese chloride	$MnCl_2.4H_2O$	≥99%	Merck
Cupric sulphate	$CuSO_4.5H_2O$	≥99%	Merck
Cobalt chloride	$CoCl_2 \cdot 6H_2O$	99.00%	Merck
Sodium molybdate	$Na_2MoO_4 \cdot 2H_2O$	≥99.5%	Merck

Table 3.2: List of other materials used in the experiment.

Materials	Supplier
NitraVer 5 Nitrate Reagent Powder Pillow	Hach Company
Total Phosphorus Test 'N Tube Reagent Set	Hach Company
Novagen® BCA Protein Assay Kit	Merck

3.2 Methodology

3.2.1 Preparation of Culturing Medium

Bold's Basal Medium (BBM) (Connon, 2007) was used as culturing medium of the algae used in this experiment. 3 stock solutions were prepared based on the amount of chemicals listed in Table 3.3 using deionized (DI) water.

Table 3.3: List of stock solutions for BBM.

Stock Solution No.	Chemical name	Formula	Weight (g)	DI Water (mL)
1	Di-potassium hydrogen orthophosphate	K_2HPO_4	1.875	250
	Potassium di-hydrogen orthophosphate	KH_2PO_4	4.375	
	Magnesium sulphate	$MgSO_4.7H_2O$	1.875	
	Sodium nitrate	$NaNO_3$	6.250	
	Calcium chloride	$CaCl_2.2H_2O$	0.625	
	Sodium chloride	NaCl	0.625	
2	Ethylenediaminetetraacetic acid	EDTA	5.000	100
	Potassium hydroxide	KOH	3.100	
	Ferrous sulphate	$FeSO_4 \cdot 7H_2O$	0.498	
	Sulphuric acid conc. (wt per mL = 1.84g)	H_2SO_4	0.1mL	
	Boric acid	H_3BO_3	1.142	
3	Zinc sulphate	$ZnSO_4 \cdot 7H_2O$	0.706	50
	Manganese chloride	$MnCl_2.4H_2O$	0.116	
	Cupric sulphate	$CuSO_4.5H_2O$	0.126	
	Cobalt chloride	$CoCl_2 \cdot 6H_2O$	0.040	
	Sodium molybdate	$Na_2MoO_4 \cdot 2H_2O$	0.096	

10 mL of stock solution 1, 1 mL of stock solution 2 and 0.1 mL of stock solution 3 in Table 3.3 were added to a 1 L conical flask containing 900 mL tap water. After all stock solutions have been added, tap water is added to complete 1 L volume of medium.

3.2.2 SBR Operations

The experiment was performed in two cylindrical SBRs (R1, R2) with a working volume of 2 L each. The 2 L of algae culture was prepared by adding 40 mL of algae seed to 1960 mL of BBM. The SBRs were placed in an illuminated fluorescence light, which was set at a whole day photoperiod (24h light). Algae in both SBRs were cultured under aerobic condition. The aeration intensity of these two SBRs was kept at 2 L/min using an air pump. Solid and liquid samples were taken periodically for MLSS, MLVSS, EPS and nutrients test. Absorbance reading of the content of both reactors were tested daily. Formation of granular sludge of algae was observed. Figure 3.1 shows the experimental setup of this experiment. 20 mL of stock solution 1, 2 mL of stock solution 2 and 0.2 mL of stock solution 3 in Table 3.3 were added to both reactor whenever nutrient depletion was detected.

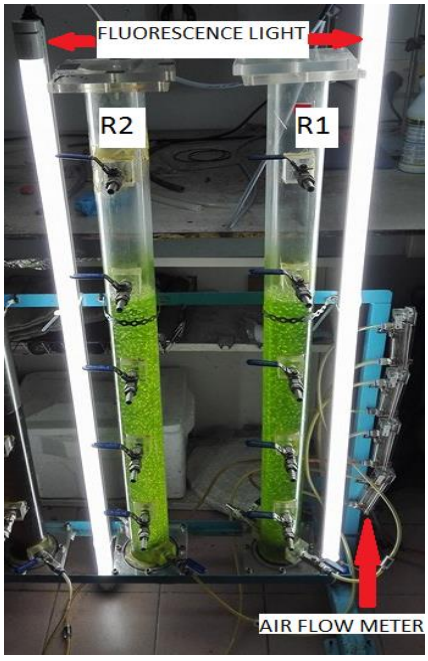

Figure 3.1: Experimental setup.

3.3 Analytical Methods

3.3.1 Biomass Analysis

Adequate amount of sample was taken out from the SBR. The absorbance of the sample is measured using spectrophotometer at wavelength of 540 nm. The weight of an empty filter paper was recorded before placing it on the filter holder of vacuum flask using a pair of tweezers. The sample was stirred to avoid sedimentation and get a good mixture for the experiment. Subsequently, 10 ml of sample was transferred into the filter holder using pipette and pipette bulb. The vacuum pump was turned on and run for 30 s. The vacuum pump was the switch off and the filter paper was removed from the filter holder and placed on the drying tray. This process was repeated as needed. All filters are placed into an oven at 105℃. The filters were weight periodically until constant weight were reached. The concentration of mixed liquor suspended solids (MLSS) in the sample were calculated using the following formula (APHA, 1998):

$$\text{MLSS, mg/L} = \frac{(A-B)x1000}{Sample\ volume\ (ml)} \tag{3.1}$$

where A = Sample and filter weight, mg

B = Empty filter weight, mg

The dry filters were then put into a muffle furnace for ignition at 550℃ to obtain the concentration of mixed liquor volatile suspended solids (MLVSS). The filters were let cool down before weighted. The concentration of MLVSS in the sample were calculated using the following formula (APHA, 1998):

$$\text{MLVSS, mg/L} = \frac{(A-B)x1000}{Sample\ volume\ (ml)} \tag{3.2}$$

where A = Sample and filter weight from MLSS test, mg

B = Sample and filter weight after ignition in furnace, mg

The reading of MLSS and MLVSS were taken of periodically. A graph of concentration of algae vs time (growth curve of algae) was then plotted.

3.3.2 Extracellular Polymeric Substances (EPS) Analysis

EPS was made up of carbohydrate and protein. Thus, to know EPS content in algae, protein and carbohydrate content was analysed. A sample containing algae was withdrawn and left to settle down for 20 min. The supernatant was discarded and 1.5 mL of the algae was transferred into a 1.5 mL microtube. This was conducted in triplicates. The microtube containing algae was centrifuged at 12500 rpm for 20 min. The supernatant was discarded and DI water was added to the microtube until 1.5 mL mark. The sedimentation was shaken until homogenous solution formed before further centrifuged at 12500 rpm for 20 min. The supernatant was discarded once again and DI water was added to the microtube until 1.5 mL mark. The sedimentation was shaken until homogenous solution formed before sonicating the sample for 10 min at 30^0C. The sample was then centrifuged again at 12500 rpm for 30 min. The supernatant after centrifuged is the sample for analysis.

For the analysis of protein content, 50 µL of the supernatant was added into a screw cap test tube. 1 mL of Novagen® bicinchoninic acid (BCA) was mixed with 20 µL of 4% copper sulphate to form BCA working solution. 1 mL of BCA working solution was added to the screw cap test tube containing the sample. The content was gently mixed before incubating at 37^{o}C water bath for 30 min for reaction to occur. The mixture was cool to room temperature before transferring into a cuvette. Another cuvette filled with DI water was used as a blank. UV-Vis spectrophotometer at 562 nm was used to analyse the sample (Frolund et al., 1995).

For carbohydrate analysis, phenol-sulphuric acid assay was used. 500 µL of the supernatant was added into a screw cap test tube followed by 0.5 mL of 4% phenol and 2.5 mL of concentrated sulphuric acid. UV-Vis spectrophotometer at 490 nm was used to analyse the resulting solution. Another cuvette filled with 0.5 mL of 4% phenol and 2.5 mL of concentrated sulphuric acid was used as a blank (Dubois et al., 1956). A standard calibration curve with known concentration was prepared earlier for the determination of protein and carbohydrate in the sample.

3.3.3 Nutrients Analysis

3.3.3.a Nitrate Content Analysis

The tested sample was collected in a clean beaker and filtered using filter syringe. The clear samples was then diluted to make sure that it falls into the range of the nitrate test kit. 10 mL of sample was added into a screw cap test tube with the contents of one NitraVer 5 Nitrate Reagent Powder Pillow. The cap of the test tube was screwed on and shaken vigorously for 1 minute. The sample was left to stand for 5 min for the reaction to occur.

A blank was prepared by adding with the contents of one NitraVer 5 Nitrate Reagent Powder Pillow to 10 mL of DI water. The blank sample was used to adjust the reading of DR2800 Spectrophotometer (Hach) to zero before the reacted sample was measured.

3.3.3.b Phosphate Content Analysis

A reactor was preheated to 150^0C before preparing the tested samples. A tested sample was collected in a clean beaker and filtered using a filter syringe. The clear samples was then diluted to make sure it fall into the range of the phosphate test kit. 5.0 mL of sample is added to the total phosphorus test vial followed by the contents of one potassium persulfate powder pillow for phosphonate. The vial was closed with the cap and then shake to dissolve the powder before inserted into the reactor for 30-minute reaction time. When the timer expires, the vial was removed from the reactor and set in a test tube rack to let it cool to room temperature. Then, 2 mL of 1.54 N sodium hydroxide standard solution is added to the vial. The cap was put backed on the vial and the vials was inverted to mix the content. The vials was cleaned before put into DR2800 Spectrophotometer (Hach) to adjust the reading of to zero. The contents of one PhosVer 3 Powder Pillow was added to the vial and shake to mix for 20–30 s. 2 min reaction time was given. The sample was measured within 8 min after the timer expires.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Growth of Microalgae

The growth of microalgae is observed throughout the experiment using two methods, (1) the estimated MLSS value from absorbance reading at 540 nm (using calibration curve) and (2) the MLSS from biomass analysis as mentioned in Section 3.3.1 previously. The results from methods (1) and (2) are as shown in Figure 4.1 and

Figure 4.2 respectively.

From both figures, the growth trend is similar for both reactors, R1 and R2. Even though on day 24th, there is nutrient addition in R1 as nitrate content is depleted in both reactors, the addition of nutrient did not help the growth of the microalgae in R1 as the MLSS reading continued to drop. On day 36th, nutrient was added to both reactors. The concentration of microalgae in R1 fluctuated slightly while concentration of microalgae in R2 maintained after the addition of nutrient, no obvious increment in concentration of microalgae was observed. This might due to the high concentration of microalgae in both reactors. The amount of nitrate and phosphorus added is only sufficient for microalgae to maintain their concentration and insufficient for the microalgae to reproduce. In other word, nutrient provided are used for maintenance energy instead of reproduction. Thus, concentrations of the microalgae in both reactors did not increase with addition of nutrient.

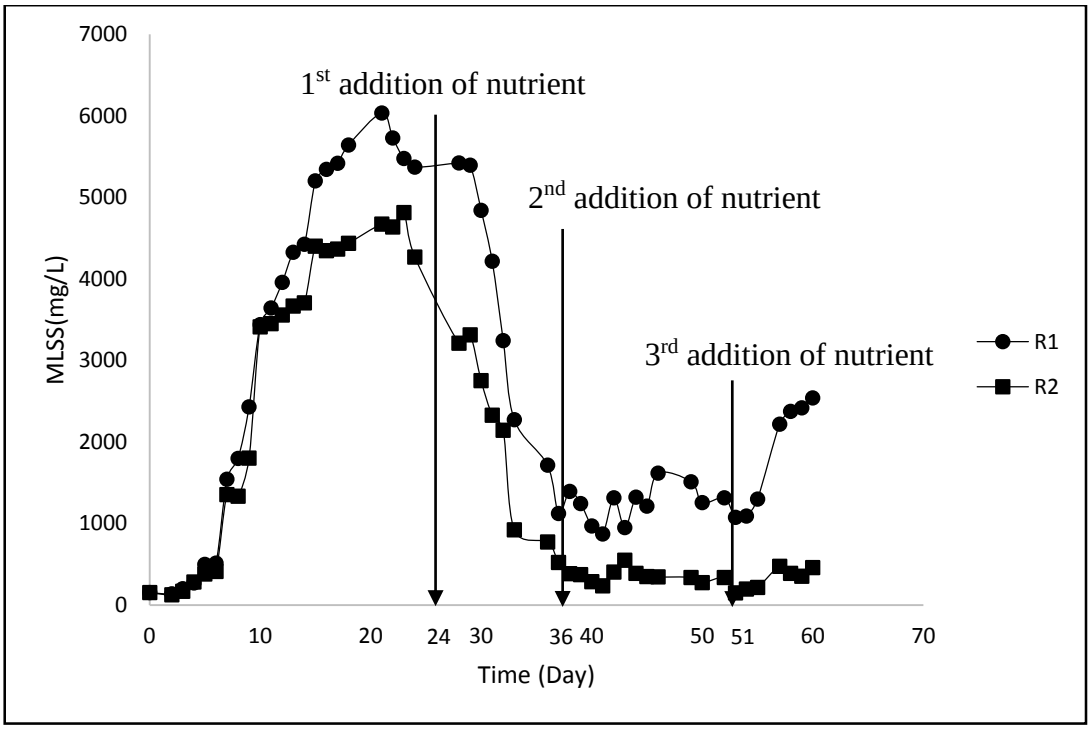

Figure 4.1: Growth curve based on MLSS value from absorbance reading of each reactor.

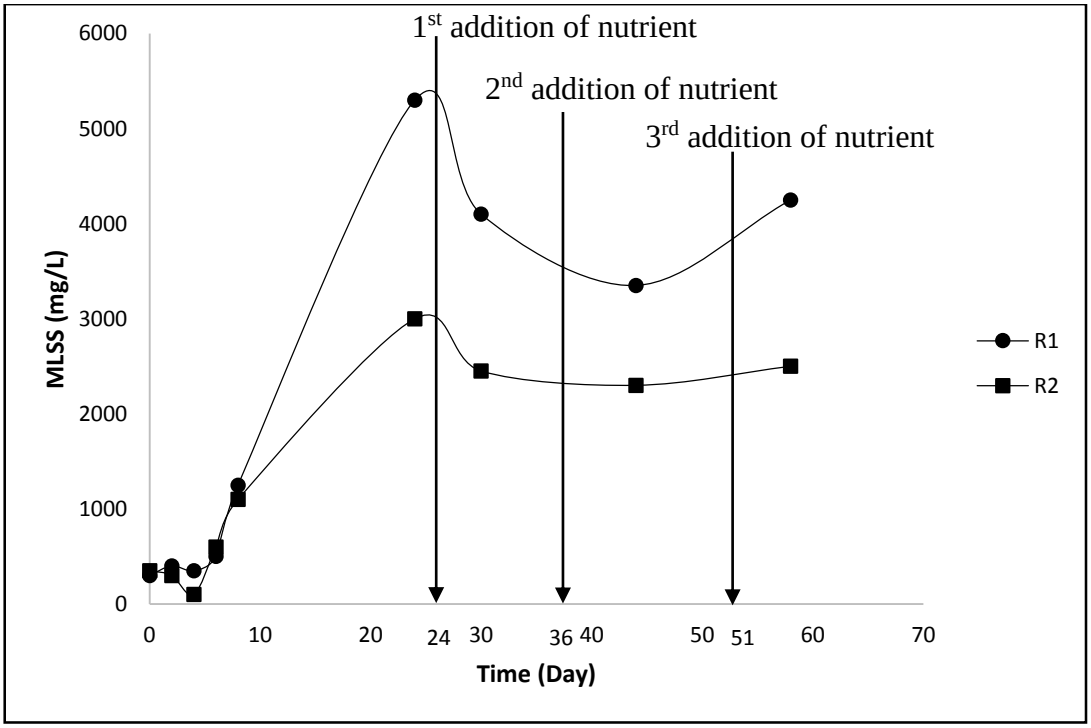

Figure 4.2: Growth curve based on MLSS value of each reactor.

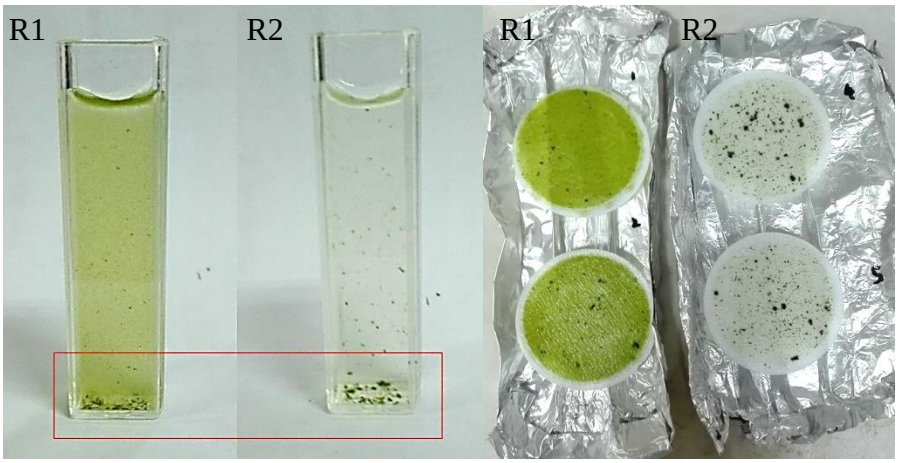

Figure 4.3: Sedimentation of microalgae biofilm and granular microalgae in a cuvette (left); Microalgae biofilm and granular microalgae that remain on the filter paper after filtration for MLSS reading (right).

The value of MLSS from

Figure 4.2 is much higher than in Figure 4.1 from day 30th onward. This is due to the formation of biofilm and granulation of microalgae. Spectrometer readings only reflect the microalgae that is well suspended in the sample. The reading did not include microalgae in the form of biofilm and granule as they can settle easily in the cuvette during the measurement of spectrometer as shown in Figure 4.3. Thus they did not contribute to the reading of MLSS. The readings in

Figure 4.2 on the other hand consist of microalgae in the form of biofilm and granule. Thus, it shows a higher reading. Readings from Figure 4.1 are only valid before the formation of granular microalgae as granular microalgae often do not contribute to the reading. Although readings from Figure 4.1 do not reflect the actual readings, but the trend obtained in Figure 4.1 is similar compared to Figure 4.2 from day 30th onwards. Readings from Figure 4.1 can be used to make quick decision whenever additional nutrient is needed. This is because absorbance reading can be obtained on the same day while readings from Figure 4.2 often require 1-2 days.

The formation of microalgae biofilm is due to the excessive addition of micronutrients. The BBM culturing medium contain micronutrients such as iron (Fe), magnesium (Mg), calcium (Ca) and other trace elements that will encourage the formation of biofilm when the concentration of micronutrients is high (Walach and Pirt, 1986). With each addition of nutrient, the concentration of these elements increased as the consumption of the micronutrients by the microalgae is less than the amount added, thus, causing the formation of biofilm.

4.2 Nutrient Analysis

The reading of concentration of nitrate and phosphorous were taken periodically to ensure there were no prolong starvation of microalgae. Figure 4.4 shows the concentration of nitrate in both reactors throughout the experiment while Figure 4.5 shows the concentration of phosphorus in both reactors. Nutrient (undiluted BBM stock solution) was added at day 24th in Reactor 1 (R1) only, day 36th and day 51st in both Reactor 1(R1) and Reactor 2 (R2) due to the depletion of nitrate in both reactors.

At growth phase (day 0th to day 20th) of microalgae, the nitrate consumption is relatively slower compared to stationary phase (day 30th onwards). This is due to the concentration of microalgae is still very low at this phase as compared to stationary phase. At stationary phase, reactor R1 took about 12 days to completely consume the nitrate available. The rate of nitrate consumption is lower at grow phase (lower concentration) compared to stationary phase (higher concentration). Fewer nitrate is needed for both maintenance and reproduction of cell at lower concentration of microalgae.

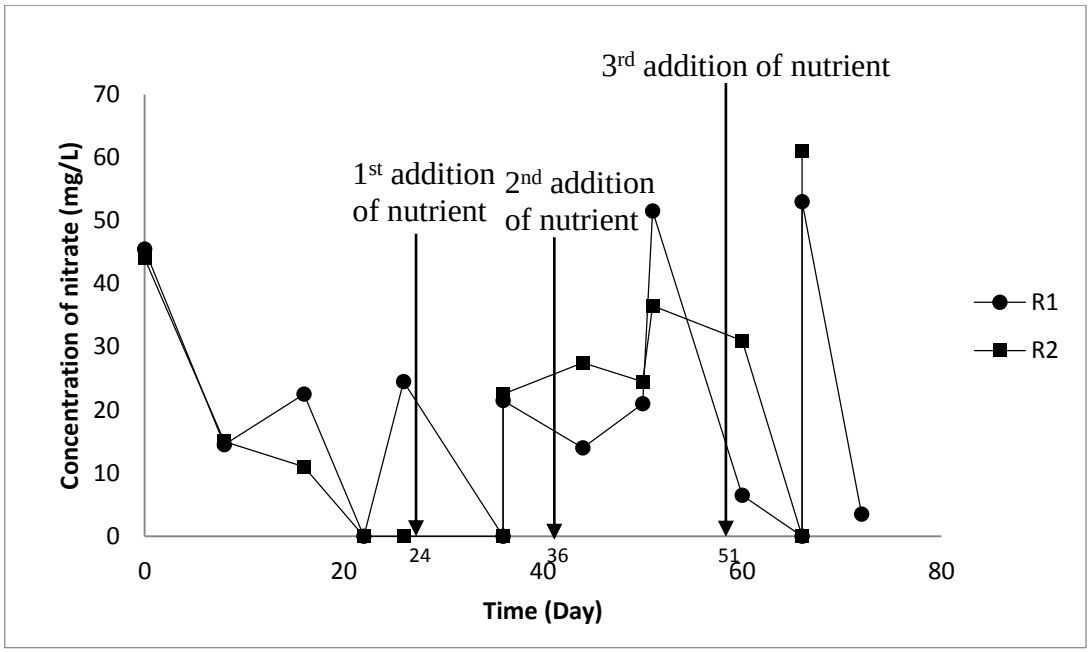

Figure 4.4: Nitrate content in both reactors throughout the experiment.

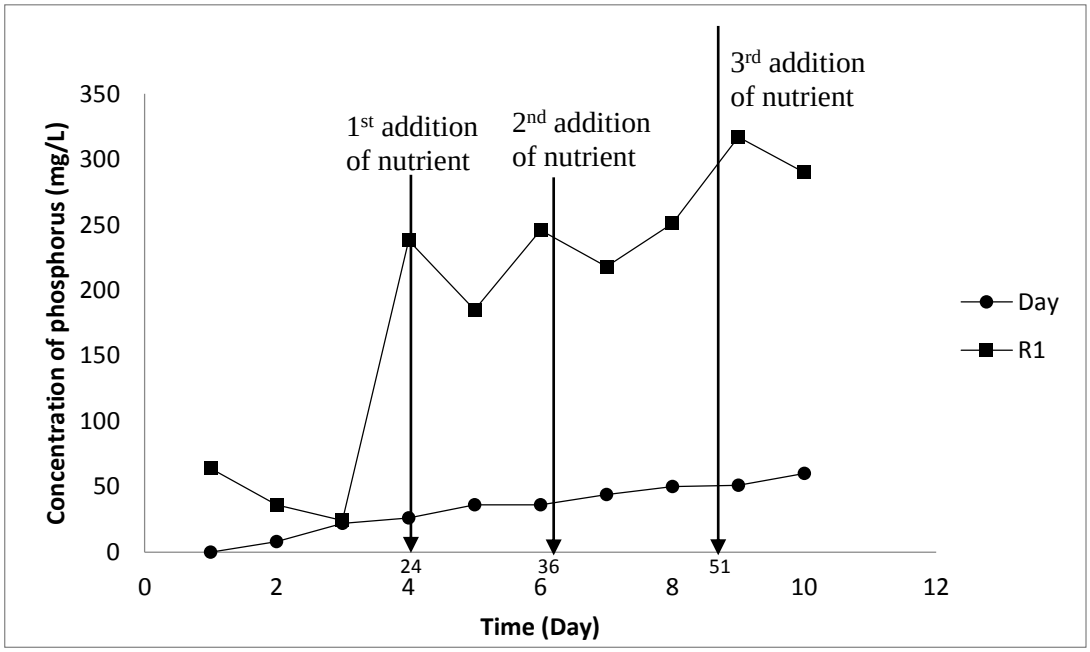

Figure 4.5: Phosphorus content in both reactors throughout the experiment.

As for concentration of phosphorus, there is a sudden increase in concentration of phosphorus at day 24th in R1 and day 50th in R2 after the addition of nutrient. Research showed that algae uptake phosphorus (Huang and Hong, 1999, Jansson, 1988). These sudden increase might be due to the releasing of phosphorus from

microalgae lysis or the ability of microalgae to release polyphosphate to form orthophosphate for energy use in reproduction or maintenance of cell. Further investigation is needed to find out the actual causes of these sudden increase in concentration of phosphorus.

4.3 EPS Production

EPS analysis of the microalgae is carried out on both reactors after the growth of microalgae reached stationary phase (after day 30th). Figure 4.6 shows the protein content in microalgae, Figure 4.7 shows the carbohydrate content and Figure 4.8 shows the EPS content in microalgae from day 32nd to day 60th. The EPS content in the microalgae seed is recorded at 121.5 mg/L (107.3 mg/L of protein and 14.2mg/L of carbohydrate).

The EPS content in day 32nd differs in two reactors most probably due to addition of nutrient in Reactor 1 (R1) only at day 24th. In Reactor 2 (R2), EPS might be degraded by microalgae as carbon and energy sources as there is a nitrate shortage. The EPS value for R2 increased to 136.7 mg/L on the second reading on day 36th as there is addition of nutrients. The EPS content of the sludge increased with an increase in food to microorganism ratio (Janga et al., 2007). Microalgae in the reactor started producing EPS instead of degraded it when there is no shortage of food. The EPS content is crucial in formation of granular microalgae used to “attach” individual microalgae to form granular microalgae.

For day 46th to day 58th, the readings of EPS in both reactors have almost similar value and are slightly increasing. This is because there is no prolong period of nutrient

deficiency in both reactor. Nutrient was added on day 36th and day 50th. The growth of microalgae in both reactors is around stationary phase during that period. A similar trend was also observed in a photosynthetic bacterial strain. The EPS content from a photosynthetic bacterial strain remained almost unchanged during the stationary phase (Sheng et al., 2006)

The protein concentration in all readings are higher than the carbohydrate value for both R1 and R2. The reason is that carbohydrate is used as primary source of energy instead of protein when starvation occur. Carbohydrate will be broken down into carbon and energy for maintenance of cell. As the reading of concentration of nutrient are not taken daily, short period of nutrient deficiency or starvation might occur before each addition of nutrients. Thus, the reading of carbohydrate remain low as compared to protein throughout the experiment.

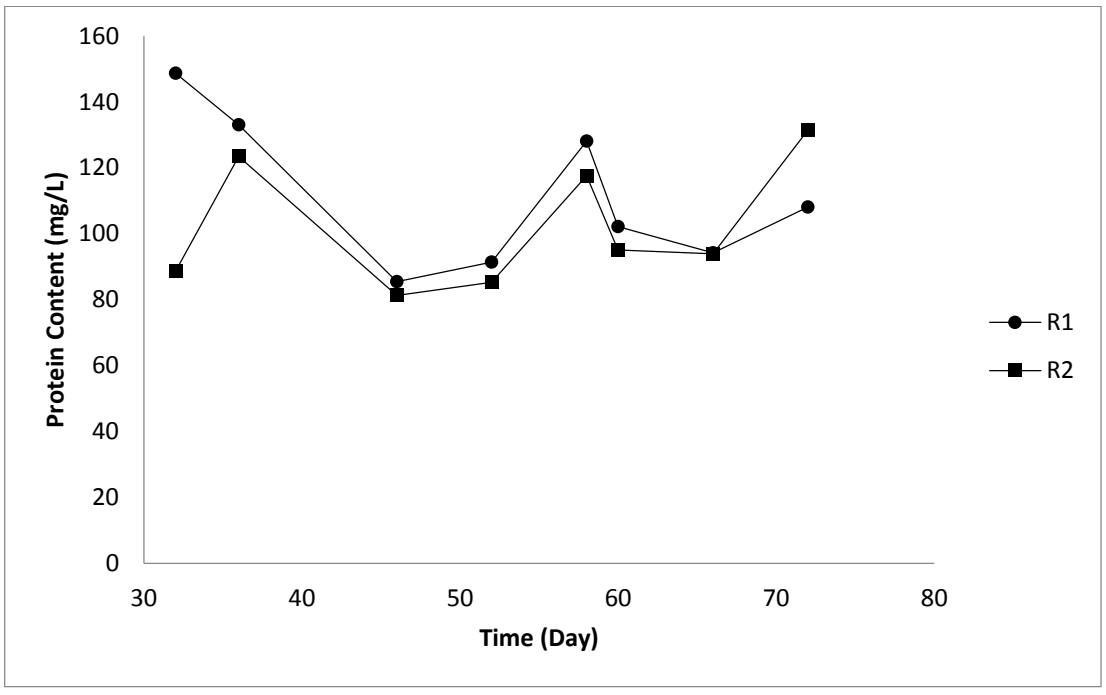

Figure 4.6: Protein content in both reactors from day 32nd to day 60th.

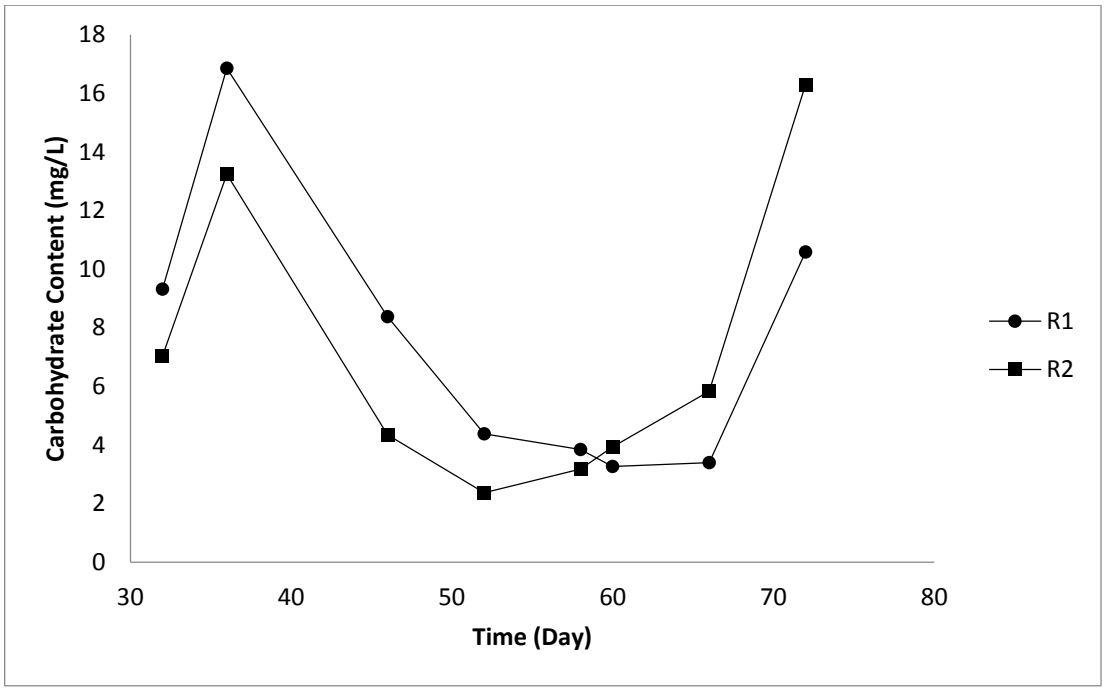

Figure 4.7: Carbohydrate content in both reactors from day 32nd to day 60th.

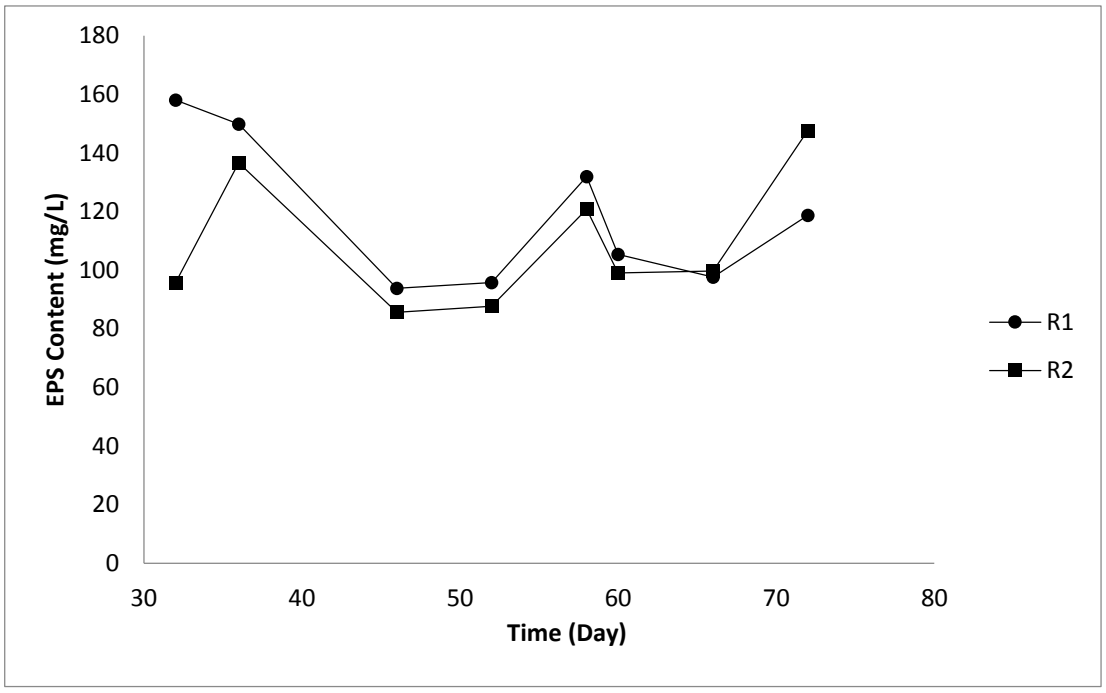

Figure 4.8: EPS content in both reactors from day 32nd to day 60th.

4.4 Settling Ability

The settling time of the microalgae from different cultivation period (seed, day 29th and day 43rd) are tested. Photo are taken periodically to compare the settling ability of each sample. The time taken for each sample to settle down completely are compared. The Seed took almost 48 h to form a clear supernatant. Sample from R1 at day 29th took 24 h while sample from R2 on the same day only took around 4 h to settle down. Another two sample at day 43rd from R1 and R2 took 4 h and 1 h only to form a clear supernatant.

Seed took longer to settle down as there is no granule or biofilm form, all microalgae are suspended in the sample. As for sample for R1 on day 29th, the sample settle down faster compared to seed as granule of algae and biofilm started to form. Granular algae and biofilm of algae are denser compared to suspended microalgae, thus shorter settling time is required. At day 43rd, more granule and biofilm formed, there is also microalgae attached to granules that formed previously as larger granules are observed, less suspended algae, thus, time taken to settle down is shorter. The same trend applied for sample from R2. Sample from R2 settled faster compared to R1 on the same day as the biomass concentration is lower in R2.