MICROALGAE GRANULATION:

CORRELATION BETWEEN MICROALGAE GROWTH AND EPS CONTENT

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UNIVERSITI SAINS MALAYSIA

2022

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by

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Thesis submitted in partial fulfilment of the requirement for the degree of Bachelor of Chemical Engineering

2022

ACKNOWLEDGEMENT

This report is for the fulfilment of the requirement for degree of Bachelor of Chemical Engineering, School of Chemical Engineering, USM. Support and commitments from several individuals have contributed to the success in completion of this report. Therefore, I would like to show my highest gratitude and appreciation to all the individuals involved for their contribution.

First and foremost, I would like to express my sincere gratitude to my supervisor, Assoc. Prof. Dr. Vel Murugan Vadivelu and postgraduate student, Syahirah Faraheen for their invaluable guidance and supervision to complete this report. Next, I would like to express my deepest gratitude to family and friends around for their endless support and encouragement throughout the time. All in all, I would like to thank all the people, including those who I might have missed out mentioning their names but have helped directly or indirectly in accomplishment of this project. Thank you very much.

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LIST OF ABBREVIATION

EPS	Extracellular Polymeric Substances
WWTPs	Wastewater treatment plants
SBR	Sequencing Batch Reactor
PBS	Photobioreactor
BBM	Bold's Basal Medium
DI Water	Deionized Water
MLSS	Mixed Liquor Suspended Solids
MVSS	Mixed Liquor Volatile Suspended Solids
BCA	Bicinchoninic Acid
PN	Protein
PS	Polysaccharide

GRANULASI MIKROALGA:

KORELASI ANTARA PERTUMBUHAN MIKROALGA DEMGAM

KANDUNGAN EPS

ABSTRAK

Mikroalga sering digunakan dalam rawatan air sisa biologi kerana ia boleh bertahan dalam keadaan yang teruk selain menjadi sumber alternatif yang berpotensi untuk pengeluaran biofuel. Walau bagaimanapun, terdapat sedikit kelemahan untuk kedua-dua aplikasi kerana kultur alga kebanyakannya berketumpatan rendah dan bersaiz kecil. Ini mengakibatkan proses pengasingan yang tegar, maka proses penuaian dan pengasingan yang lebih besar sahaja. Namun begitu, mikroalga mampu menghasilkan bahan polimer ekstraselular (EPS) yang penting untuk pembentukan granul. Dengan membekalkan keadaan kultur yang sesuai untuk mikroalga membentuk granul, proses penuaian dan pengasingan mikroalga akan menjadi lebih selamat kerana butiran mempunyai ketumpatan yang lebih tinggi dan boleh mendap dengan mudah. Dalam eksperimen ini, beberapa faktor yang mempengaruhi penghasilan bahan polimer ekstraselular (EPS) dalam mikroalga dikaji, iaitu ketersediaan nutrien dan fasa pertumbuhan mikroalga. MLSS, kandungan EPS dan analisis nutrien dijalankan secara berkala sepanjang eksperimen. Terdapat pembentukan enapcemar berbutir dalam mikroalga yang ditemui selepas pertumbuhan mikroalga mencapai fasa pegun.

ABSTRACT

Microalgae are often used in biological wastewater treatment as it can survive harsh condition aside from being a potential alternative resource for biofuel production. However, there are slight disadvantages to both applications as the algae culture are mostly low in density and small in size. This results in rigid separation process which requires greater cost in harvesting and separation processes. Nonetheless, microalgae are able to produce extracellular polymeric substances (EPS) which is significant for the granule formation. By supplying cultural condition that is suitable for microalgae to form granule, the process of harvesting and separation of microalgae will be much secure as granule has higher density and can settle down easily. In this experiment, several factors that influence the production of extracellular polymeric substances (EPS) by microalgae are studied, 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.

CHAPTER 1 INTRODUCTION

1.1 Background

As years pass by, the population grows where 9 billion people make up a family in 2050 according to the forecasts. Food and energy resources have become the biggest concern worldwide. Our planet is facing a food crisis alongside a population crisis, where more than 800 million people are undernourished although one third of the available food is gone to waste. To be able to ensure the food resources are available for future generations, some countries produce specific food including coffee, avocado, or even palm oil. This has then led to deforestation like in Indonesia, to provide space for growing crops. The second concern is on the energy sources whereby non-renewable energy sources such as oil, coal, gas is expected to run out sooner than expected. An alternative energy resource is highly in demand, and a renewable one is even more important, so this is where microalgae come handy due to being the great renewable energy resource. A microalgae is one cell so it's really small that it can only be seen under a microscope. Microalgae can be grown in any place be it in the desert or in a small lab. Microalgae is really known for its potential to produce lipids which then produce biofuel. Freshly produced microalgae is collected, then most of the water is removed, and lipids are extracted for the fuel (Gonçalves, 2019). Carbon dioxide (CO_2) is the most significant greenhouse gas that contributes to global warming. CO_2 concentrations have increased over the past years which leads to an increase of 50% of the global temperature. As microalgae grows, it removes CO_2 from the atmosphere by converting it to biomass and oxygen via photosynthesis. Not only that, but the rate of conversion is also relatively fast. Hence with several researches and methods on

microalgae cultivation, CO_2 concentration can be reduced significantly for the next few years (Polon, 2020).

1.2 Problem Statement

Based on previous studies, they mainly highlight how algae granules have great potential for phosphorus recovery and reuse. The operation cost on microalgae cultivation itself is slightly high just for algae separation. Due to its small size (3-30 μm) and negatively charged algal cells, it is difficult and costly to separate microalgae from their growth medium (Barros et al., 2015; Hu et al., 2017). Thus, making ways for the use of attached algae processes including granulation processes. By forming microalgae granules which has denser characteristics, it can settle down easily and thus reduce the separation cost. There are still limited research and studies on this method especially on a large scale. In microalgae granulation, it produces extracellular polymeric substances or EPS, where it plays a crucial role in granulation process. It is also helps in the maintenance of compact interior structure of granule. By knowing the EPS value, this might aid in observing the microalgae growth. Little to no info could be found on the correlation between EPS content and its growth.

1.3 Objective

The objective of this research:

i. To investigate the growth pattern of microalgae through biomass concentration.

ii. To study the correlation of EPS and microalgae growth.

CHAPTER 2 LITERATURE REVIEW

2.1 Wastewater Treatment

Over the past years, wastewater treatment has been one of the crucial processes underlying beneath both residential and commercial waste. There are various methods and systems in treating multiple volumes of wastewater efficiently. It is parted into several categories including chemical treatment, and biological treatment (Metcalf & Eddy et al., 2013).

Wastewater treatment is done in several steps starting from the preliminary treatment, primary treatment, secondary treatment, and tertiary treatment. In the preliminary treatment, debris and coarse materials are removed. During the primary treatment, sedimentable suspended solids are removed in sedimentation tanks. Then later in the secondary treatment, the wastewater is transferred into biological reactor to perform removal of dissolved organic matter, nutrients like nitrogen, phosphorus, and pathogens. In some cases, the wastewater undergoes tertiary treatment to eliminate pathogens, suspended solids and other compounds that have not been removed in the previous stage via physical and chemical treatments such as membrane filtration or chlorination. Conventional wastewater treatment plants (WWTPs) are now facing challenges, as sustainable economy principles impose rigid pollutant limits that are necessary before discharge and reuse of wastewater into the environment (Capodaglio, 2020). Other downside of conventional WWTPs are high energy consumption, greenhouse gas emissions, recyclable resource wastage, and excessive solid landfilling (Al-Jabri et al., 2021). These downsides strike a challenge to developing a sustainable wastewater treatment process. An alternative that could be beneficial is the use of microalgae to treat

wastewaters. It works by having the microalgae utilizing the direct sunlight onto the surface of wastewater as their energy requirement while simultaneously removing pollutants.

2.2 Microalgae

Algae is referred to plant-like organisms that are photosynthetic and aquatic without having true roots, stems, leaves, vascular tissue, and they have simple reproductive structures. They can be found in the sea, freshwater and in some cases on land. Most of them are microscopic but some may be enormous like the marine seaweeds. Algae have chlorophyll and can produce their own food via photosynthesis (Randrianarison & Ashraf, 2017). Microalgae are among potential and sustainable feedstock for the production of biofuels, fine chemicals, nutraceuticals, and cosmetics. This is highly due to their high lipid and carbohydrate content, fast growth, and rapid CO_2 capture ability. Aside from being able to convert into various fuels, microalgae are now widely being implemented in the wastewater treatment (Randrianarison & Ashraf, 2017).

2.2.1 Cultivation Methods

Some common methods on microalgae cultivation include open-ocean algal blooms, photobioreactors, and algal turf scrubbing. The most common microalgae cultivation method in wastewater is via suspended growth system, either in open or closed systems in a sequencing batch reactor (SBR) or a photobioreactor (PBR). Microalgae cultivation in PBR could minimize unwanted contamination, evaporation water loss, and loss of injected CO_2 (Al-Jabri et al., 2021). This method via PBR may cost slightly higher for wastewater treatment despite having advantages mentioned. Another method of microalgae cultivation is to immobilize or attach the microalgae cells to a support medium where the attached

cells contact wastewater, and the nutrients get absorbed and subsequently utilized by microalgae to produce biomass. The attached cells are placed on a static surface or mounted on a rotating paddle. Once the biomass growth on the surface has reached the desired thickness, the support media will be removed from the wastewater, and the biomass will be scraped out (Cai et al., 2019).

2.2.2 Harvesting Methods

Separation of microalgae biomass from the treated wastewater is one of the nit-picking processes of microalgae wastewater treatment. There are several methods or techniques for the separation of biomass from the bulk of the culture, and it depends on the cultivation system used. One of the techniques is via sedimentation. Cells with greater density volume and has no ability to stay afloat in the growth medium will sink to the bottom. This process is often optimized as it is operated at low cost and able to harvest in a large scale operation. Apart from sedimentation, several microalgae strains that are held together with the help of EPS that they produced will form flocs. Thus, this process is commonly called Auto-Flocculation. Factors that will affect the EPS productivity will affect the nature of flocculation. Other cultivation method is via filtration where a 100% cell recovery is possible, and it can be applied to most of the microalgae strains. Although it sounds promising, it has a relatively high cost and energy demand on its system (Cai et al., 2019).

2.2.3 Applications

Microalgae biomass has a promising line up of applications from energy conversion to being used as fertilizer. Most importantly, it has the potential to be a sustainable feedstock

rather than the feedstock generated by the typical treatment plant. Below shows the microalgae bioremediation of wastewater and the potential applications of the produced biomass based on Al-Jabri.

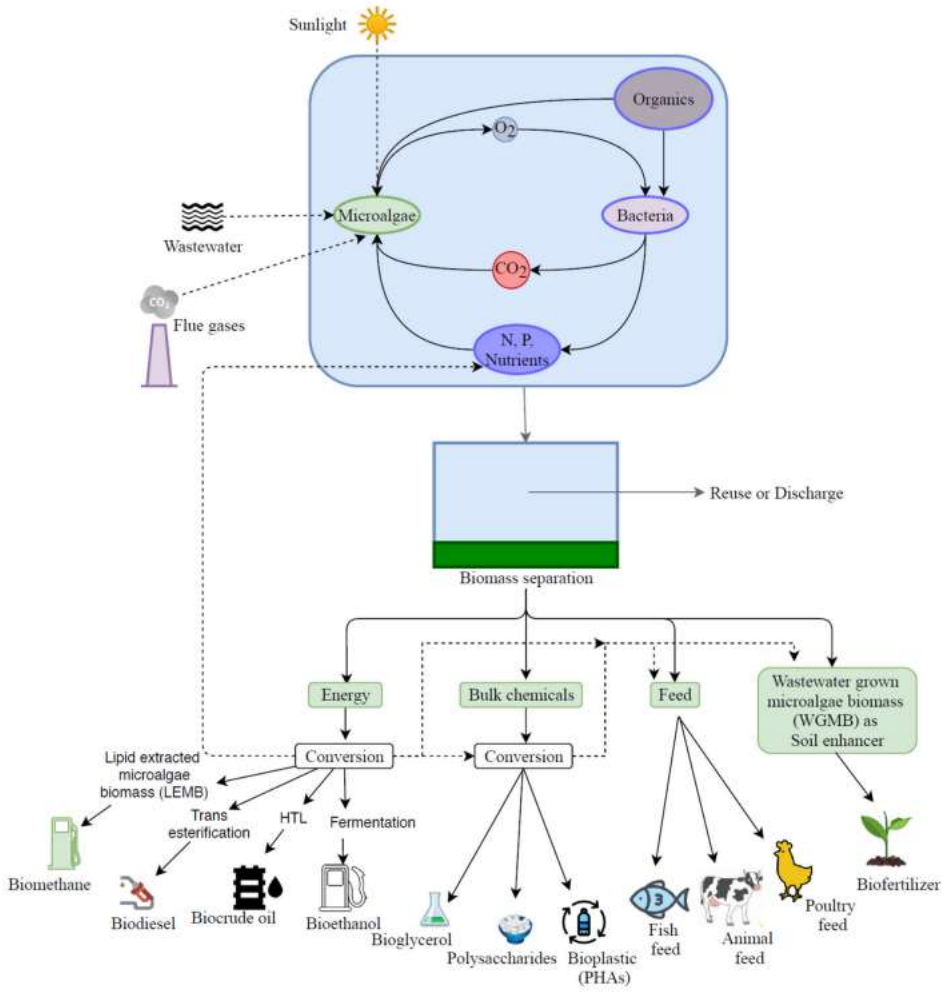

Figure 2-1 Microalgae bioremediation of wastewater and the potential applications of the produced biomass (Al-Jabri et al., 2021)

2.2.4 Microalgae Granulation

Despite its promising benefits, it is difficult and expensive to separate microalgae from their growth medium. The use of immobilised or attached algal processes were highlighted to solve this separation issue. One of the methods is using activated algae granules which show great settleability, high biomass content, dense and strong microbial structure with

successfully cultured via self-immobilisation. Granulation of algae is able to remove pollutants like dissolved organic carbon, phosphorus, nitrogen, and more.

The granules were characterised by their morphology, strength, relative hydrophobicity, and EPS content. In the process, the overall performance on the pollutants removal efficiencies were relatively high during the days of experiments. It is also reported that the phosphorus removal process is a success with more percentage in its recovery. From this study it can be seen that microalgae granulation is a promising method for the future sustainability. Hence, this paper involves in how does the EPS content correlates with the microalgae growth in microalgae granulation (Cai et al., 2019).

CHAPTER 3 MATERIALS AND METHOD

3.1 Materials

Chemicals that are used during the experiment are listed in Table 3-1.

Table 3-1 Chemicals used in the experiment

Chemical name	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	≥99.50%	Merck
Ethylenediaminetetraacetic acid	EDTA	99.40%	Merck
Potassium hydroxide	KOH	96.25%	Fisher Scientific
Ferrous sulphate	$FeSO_4.7H_2O$	≥99%	Bio Basic Canada Inc.

Table 3-1 continued.

Sulphuric acid conc.	H_2SO_4	95%-98%	R&M Chemicals
Boric acid	H_3BO	99.50%	Merck
Zinc sulphate	$ZnSO_4.7H_2O$	99.50%	Merck
Manganese chloride	$MnCl_2.4H_2O$	≥99%	Merck
Cupric sulphate	$CuSO_4.5H_2O$	≥99%	Merck
Cobalt chloride	$CoCl_2.6H_2O$	99.00%	Merck
Sodium molybdate	$Na_2MoO_4.2H_2O$	≥99.5%	Merck

The research activity is illustrated in Figure 3-1

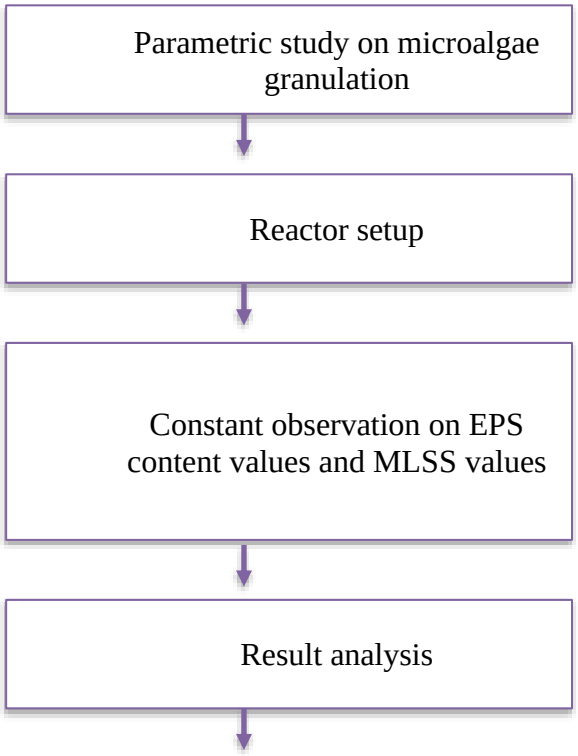

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Figure 3-1: Flow diagram of methodology

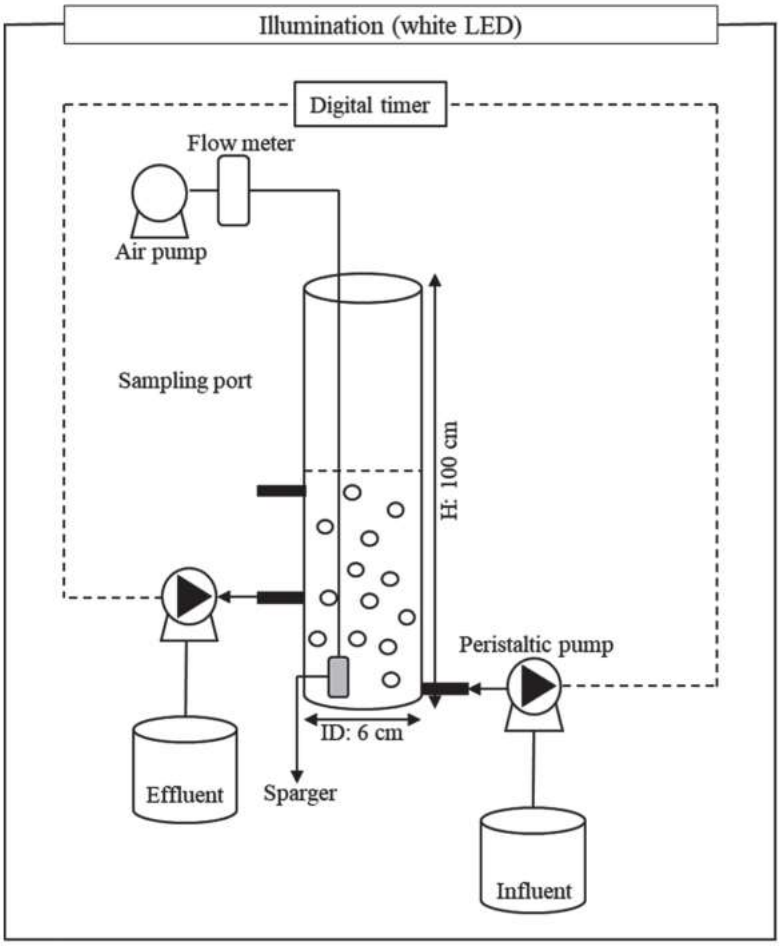

Figure 3-2: PSBR column diagram

This is an example of a photo-sequencing batch reactor (PSBR) column that is used in the experiment.

3.2 Methodology

3.2.1 Preparation of Culturing Medium

Bold's Basal Medium (BBM) 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 3-2 using deionized water (DI).

Table 3-2: List of stock solutions for BBM.

Stock Solution No.	Chemical name	Formula	Weight (g)	Distilled 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	$FeSO_4.7H_2O$	0.498	
	Sulphuric acid conc. (wt per mL = 1.84g)	H_2SO_4	0.1mL	

	Boric acid	H_3BO	1.142	
3	Zinc sulphate	$ZnSO_4.7H_2O$	0.706	50
	Manganese chloride	$MnCl_2.4H_2O$	0.116	
	Cupric sulphate	$CuSO_4.5H_2O$	0.126	
	Cobalt chloride	$CoCl_2.6H_2O$	0.040	
	Sodium molybdate	$Na_2MoO_4.2H_2O$	0.096	

3.2.2 SBR Operations

The experiment was performed in a cylindrical SBR with a working volume of 4 L. The 2 L of algae culture was prepared by adding 40 mL of algae seed to 1960mL of BBM. The SBR was placed in an illuminated fluorescence light, which was set at a whole day photoperiod (24h light). Algae in the SBR was cultured under aerobic condition. The aeration intensity of the SBR 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 the reactor was tested on a daily basis. Formation of granular sludge of algae was observed. Figure 3-3 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 3-2 were added to the reactor whenever nutrient depletion was detected.

Figure 3-3 Reactor setup

3.3 Analytical Methods

3.3.1 Biomass Analysis

An appropriate amount of sample, roughly 25 mL beaker was taken out from the SBR. The absorbance of the sample is measured using spectrophotometer at the 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 obtain a good mixture for the analysis. Then, 10 mL of sample was transferred into the filter holder using 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. All filters are placed into an oven at 105 °C. The filters were weight periodically until constant weight was reached. The concentration of mixed liquor suspended solids (MLSS) in the sample were calculated using the following formula:

$$MLSS, \frac{mg}{L} = \frac{(A - B) \times 1000}{Sample\ volume\ (mL)}$$

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 °C 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:

$$MLVSS, \frac{mg}{L} = \frac{(A - B) \times 1000}{Sample\ volume\ (mL)}$$

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

B = Empty filter weight after ignition in furnace, mg

The reading of MLSS and MLVSS were taken periodically.

3.3.2 Extracellular Polymeric Substances (EPS) Analysis

In this experiment, the content of EPS was analysed. To perform the extraction of EPS, 1.5 mL of the microalgae culture was transferred into a 1.5 mL centrifugal tube. This was conducted in triplicates. The samples were then centrifuged at 12500 rpm for about 25

minutes. The supernatant was discharged, and DI water was added until the 1.5 mL mark. The purpose of the above technique is to remove any excess chemicals and unwanted particulates from the sample. After that, the centrifugal tubes were sonicated at 20 kHz for about 20 minutes. Then, the solutions were centrifuged again at 12500 rpm for about 25 minutes before testing the samples for the presence of polysaccharide and protein content (Ganesan & Vadivelu, 2020). EPS was mainly consisting of carbohydrate and protein.

Figure 3-4 Triplicate samples in centrifugal tubes

Figure 3-5 Samples after first centrifugation

3.3.2.1 Analysis of Protein (PN) in EPS

For the analysis of protein content, the Lowry method was implemented. Firstly, 1 mL of Bicinchoninic Acid (BCA) protein assay solution and 20 μL of 4% Cupric Sulphate was added together to form the homogenous mixture of BCA working solution (Frølund et al., 1995). In the testing vial, for each 50 μL of supernatant, 1 mL of prepared BCA working solution was mixed together. The mixture was then placed in the water bath at 37 °C with reaction time of 30 min. Blank sample was also prepared using the DI water. When then reaction completed, the protein extracted samples were allowed to cool down at room temperature before transferring them into the cuvettes. UV-Vis Spectrophotometer was used with the wavelength of 562 nm to analyse the sample.

3.3.2.2 Analysis of Polysaccharide (PS) in EPS

The concentrated sulfuric acid and 4% (0.4M) phenol solution was used to analyse the polysaccharide content in the sample (Dubois et al., 1956). 500 μL of supernatant was filled in the screw cap test tube. 2.5 mL of 98% (18.8M) sulfuric acid was added into the test tube followed by 500 μL of 4% (0.4M) phenol solution. The phenol-sulfuric acid assay with the sample was allowed to react for about 5 min. Finally, the prepared sample was tested using the UV-Vis Spectrophotometer at wavelength of 490 nm.

CHAPTER 4 RESULTS AND DISCUSSION

4.1 Growth of Microalgae

The results of the EPS analysis and MLSS are as shown in Table 4-1.

Table 4-1 EPS and MLSS Concentration

	EPS Analysis	MLSS
Days	Conc (mg/L)	MLSS (mg/L)
0	10.62	
1		0.76
2		0.79
3	19.61	1.49
5	24.92	1.03
7		58
9	31.17	424
11	42.87	568
13	48.06	
15	49.61	
16		492
19	49.81	
21	52.11	
23		1371
25	54.09	
27	55.12	
30	59.03	2362
32	58.12	
34	58.82	
35	58.78	
37		2533
40	59.31	
42		2972
45	61.25	
49		2986
50	62.73	
55	59.19	
56		2981
60	63.99	
63		2978
64		2979
65	63.81	
66	62.91	2980

67		2983
68	63.03	2981
70	63.12	2978

A graph of EPS and MLSS over time is plotted as shown in Figure 4-1.

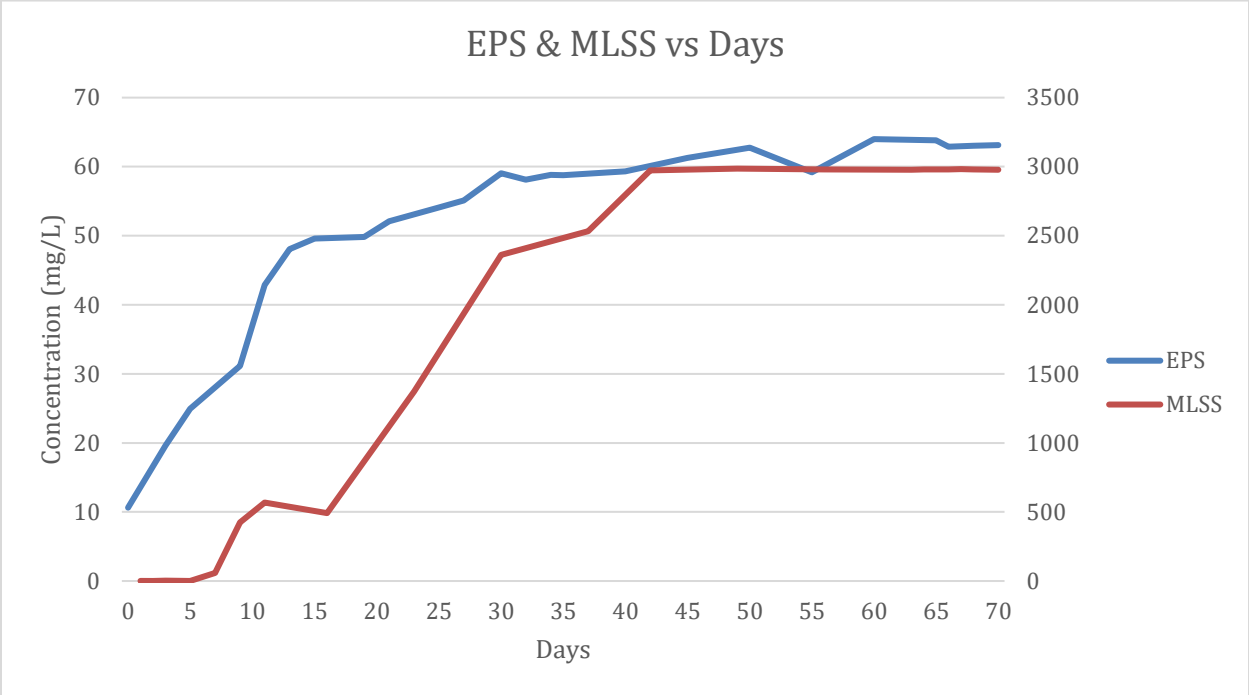

Figure 4-1 Graph of EPS & MLSS over time

The growth of microalgae is observed throughout the experiment using the estimated MLSS from biomass analysis performed. The results from the method are as shown in Figure 4-1. The growth trend is increasing from the start until 43rd day and remain constant afterwards until the 70th day. There is a slight decrease on day 12th until day 17th from 568 mg/L to 492 mg/L where it is probably due to nutrition addition on day 17th which then leads to tremendous increases in MLSS value until day 43rd, The MLSS value that remains constant from day 43rd till 70th at 2972 mg/L is most likely due to high concentration of microalgae in the reactor.

4.2 EPS Analysis

EPS analysis of the microalgae is done from the start. As shown in the figure, the EPS value increases over time. From day 0 to day 50th, the EPS content increased from 10.62 mg/L to 62.73 m/L during the operation. This describes the formation of a strong and sticky framework by PS, and the increase in PN content can enhance the linkage of adjacent microbial cells and attraction of organic and inorganic materials (Adav et al., 2008). High EPS content in granules is good for the formation and maintenance of granular algae structure. From the figure, the EPS content increased significantly heading towards being constant as per the MLSS value on the 43rd day till 70th day. The growth of microalgae in the reactor is approximately stationary phase during the final days. A similar trend was also observed in a photosynthetic bacterial strain. The EPS content from a photosynthetic bacterial strain remained almost similar during the stationary phase (Sheng et al., 2006).

CHAPTER 5 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

Based on the experiment conducted, it can be concluded that there are various factors that are affecting the production of EPS in microalgae, which is the availability of nutrients and growth phase of the algae itself. Microalgae in starvation mode will utilize EPS as energy source and will produce EPS when it is over supplied with food or nutrient. The growth of EPS production seems steady during the stationary phase. Not only that, greater value of EPS is good for formation & maintenance of granular algae structure. Thus, EPS content should be a parameter to be considered when performing microalgae granulation.

5.2 Recommendations

There are some recommendations to improve the study where the factors affecting the production of EPS should be done for instance the aeration rate and the photoperiod could be manipulated. This will aid in manipulating the EPS compositions and contents in granules while also improving the granules functions.

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