FLOCCULATION TECHNOLOGY: DOUBLE LAYER FLOCCULATION TO ENHANCE THE SEDIMENTATION OF FRESHWATER MICROALGAE

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A project report submitted in partial fulfilment of the requirements for the award of Bachelor of Engineering (Hons.) Petrochemical 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

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ABSTRACT

Microalgae is one of the potential renewable energy feedstock to substitute petroleum fuels as high lipid content biofuel feedstock. It is a promising alternative solution to overcome critical global environment problem because it is clean, environmentally safe and produces lower or negligible levels of greenhouse gases. Furthermore, harvesting by flocculation is the lowest cost due to low energy consumption. It is also the simplest method and economic way to harvest the microalgae. However, the slow settling rate of microalgae by flocculation tends to deteriorate the quality of biomass. In this study, chitosan is used for first layer flocculation while polyethylene glycol (PEG) is used for second layer flocculation. In addition, PEG is non-ionic polymer that improves the sedimentation rate of microalgae by forming hydrogen bonding with chitosan. As a result, the optimum dosage of high molecular weight chitosan (HMWC) and low molecular weight chitosan (LMWC) is at 5 mg/L respectively, whereby the efficiency can reach to 99.04 ± 1.00% and 62.71 ± 17.76 % respectively. The size of the microalgae cell flocs formed by using optimum dosage of LMWC and HMWC can reach up to 29.46 μm and 24.80 μm. From the result, LMWC at 5 mg/L outperformed HMWC and promoted fastest sedimentation at 28.18 ± 4.71 cm/h to form clear medium. The addition of second layer flocculant, PEG, has improved the performance of LMWC significantly in term of sedimentation rate. The cell separation efficiency for the case of LMWC was still high, at 99.15 ± 0.54%. However, the microalgae sedimentation rate for the case of LMWC became 4.29 times faster, at 120.91 ±53.08 cm/h, due to the floc size was increased up to 82.76 μm that is 2.80 times larger in size.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

d	Cell diameter, m
η	Fluid dynamic viscosity, kg/m·s
g	Gravity force, m/s^2
ρ	Density, kg/m^3

LIST OF ABBREVIATIONS

CO_2	Carbon Dioxide
O_2	Oxygen
Alum	Aluminium Sulphate
PAC	Polyaluminium Chloride
TSS	Total Suspended Solid
DCW	Dry Cell Weight
LMWC	Low Molecular Weight Chitosan
HMWC	High Molecular Weight Chitosan
PEG	Polyethylene Glycol
PSA	Particle Size Analyzer

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of Study

The energy source can be categorized as non-renewable and renewable energy. There are over 80% of the non-renewable energy used in the world are from petroleum, coal and natural gas. The combustion of fossil fuel has contributed to about 98% of total carbon emissions (Demirbas and Demirbas, 2011). Therefore, the usage of fossil fuels should be reduced in order to eliminate the emission of carbon dioxide. Besides that, the energy availability of non-renewable energy is limited and the energy system is unsustainable due to equity issues includes environmental, economic, and geopolitical concerns that have far reaching implications (Demirbas and Demirbas, 2011). Currently, climate change is most critical global environmental problem. The global warming results in deteriorate effect include a potential increase in sea level and subsequent submerging of lowlands, deltas and islands, as well as changing of weather patterns (Ahmad et al., 2011). Moreover, the world is confronted with an energy crisis of world petroleum depletion (Demirbas, 2010). Hence, the non-renewable fossil fuels should be replaced by renewable and clean energy source in order to overcome the problem mentioned above.

Biodiesel is one of the renewable energy that is a promising alternative solution to replace the fossil energy. It is clean, environmentally safe and produces lower or negligible levels of greenhouse gases (Ahmad et al., 2011). Its ability of sequestering atmospheric carbon dioxide (CO_2) tends to achieve environmental and economic sustainability. Compared to traditional fuel, which is fossil fuel, the biofuel has greater

energy security and able to reduce environmental impact, foreign exchange savings and socioeconomic issues (Demirbas, 2010). In 2008, there are three largest producers of biodiesel fuel among 28 countries in the world, which are Germany (21 %), USA (17 %), and France (13 %). In addition, the world production of biodiesel fuel was about 13.9 million ton (Demirbas, 2011).

From literature review, microalgae is one of the potential renewable energy feedstock to produce biofuel. It is a promising source of biofuel feedstock and called as oil-algae with its high lipid content, at 30 – 75 % of lipid by dry basis (Demirbas, 2011). Microalgae is predicted can be used as substitute for petroleum fuels (Demirbas, 2010). Besides that, the cultivation of microalgae tend to assimilate the CO_2 from fossil fuel combustion process or industrial waste streams through photosynthesis. The cultivation of microalgae in wastewater also tends to remove the excess nutrients and contaminants from wastewater in order to sustain their growth (Griffiths et al., 2011; Trent. et al., 2012). The water is being treated once the microalgae biomass is removed from the water body while the biomass can be collected for biofuel production purpose. Therefore, the available of a cell removal technique without direct annihilation of microalgae will be economically more attractive.

From literature review, the harvesting of unicellular microalgae is still remains a major challenge due to high cost and energy demand (Griffiths et al., 2011). Microalgae in micron size can be harvested through the method of flocculation, flotation, sedimentation, centrifugation and filtration. Flotation can harvest the algae faster and more effective than sedimentation and possible to combine with gaseous transfer, but there is limitation by the energy requirement of bubble production and specific to some algae species. In centrifugation, addition of chemicals is not required and can often be used in the secondary dewatering process. It also can harvest most algae types rapidly and efficiently, but it requires high capital and operational cost and pre-concentration of algae broth is needed to reduce energy demands for centrifugation and associated costs. Although filtration can available in wide filter and membrane types and can achieve almost complete retention of biomass, but it can cause clogging or fouling and suited to large algae cells. High pressure and liquid velocity is also required in the process. For sedimentation, it is in low cost and simple technique but it is only applicable for microalgae with size larger than 70 μm and dense non-motile

cells. Coagulants must be added on smaller microalgae that is about 5 - 20 µm to enhance the sedimentation. Thus, this method is slow and produce biomass in low final concentration.

Furthermore, the flocculation process followed by sedimentation is in lowest cost too compared to other harvesting methods due to low energy consumption (Coons, 2014). There is a wide range of coagulant/flocculant is available. However, the chemical contamination might happen in chemical flocculation process whereby the coagulant/flocculant should be collected before discharge the clear water to the environment (Vandamme et al., 2013; Milledge and Heaven, 2013). Therefore, the selection of flocculant is important to confirm the reliability of the flocculation process used to aid the sedimentation of microalgae cell.

1.2 Problem Statement

The flocculation is recognized as the simplest method and economic way to harvest microalgae from aqueous medium (Vandamme et al., 2013; Wan et al., 2014; Lee et al., 2009). Theoretically, flocculation is driven by the electrostatic attraction when charged coagulant/flocculant is used. The coagulant/flocculant is attached on the cell surface and cause charge neutralization on cell. Then the cells will lose their stability and flocculated together by bridging effect to promote sedimentation. So, the maximum cell separation efficiency will be achieved at an optimal dosage of flocculant.

From the literature review, chitosan is a well-known natural polymer that is usually used as coagulant in order to harvest the microalgae cells (Xu et al., 2013). Study showed that the low molecular weight of chitosan had resulted in the decrease of flocculation efficiency (Yang et al., 2014) while the higher molecular weight of chitosan can lead to a better flocculation (Renault et al., 2009). However, the performance of chitosan in different molecular weight was never being compared. Therefore, a study use to investigate the effect of chitosan when in different molecular weight toward the optimal dosage of chitosan and also the performance of flocculation and sedimentation of microalgae cells is indeed important.

From the study of Christenson and Sims (2011), they showed that the low settling rate of the flocculated biomass, at the range of 0.10 to 2.60 cm/h, tend to deteriorate the quality of biomass. It tends to cause the method of flocculation and sedimentation become not reliable. Therefore, we are interested to discover an approach in order to enhance the settling rate of the flocculated microalgae biomass.

In this study, the *Chlorella* sp. is employed as the study model because it is common spherical shaped microalgae with average cell diameter of 3.45 μm and it does not settle readily at a slow settling rate of 15 cm/h (Milledge and Heaven, 2013; Toh et al., 2014).

1.3 Research Objectives

The objectives of the research are:

1. To determine the optimum dosage of chitosan in order to achieve the maximum cell separation efficiency.
2. To study the effect of chitosan molecular weight toward the settling rate of *Chlorella* sp.
3. To enhance the settling rate of *Chlorella* sp. through second layer flocculation.

1.4 Outline of Study

In chapter one, background of microalgae and harvesting methods are introduced. There is also the problem statement about slow settling rate of microalgae promoted by flocculation. The objectives in this study are listed out.

In chapter two, characteristics of microalgae is reviewed. The application of microalgae in wastewater treatment and acting as biofuel feedstock is reviewed too. Besides that, there are explanation on various harvesting methods in details, which are centrifugation, flotation, sedimentation, filtration and flocculation. In this research,

chitosan and polyethylene glycol are used as coagulant/flocculant, whereby, their properties and usage are studied in details. Moreover, the theory of sedimentation rate is studied since it is the target in this research.

In chapter three, there is a flow diagram used to explain the flow of experiment, which is including the cultivation of microalgae until the step to enhancing the settling rate of microalgae. Materials and chemicals used in this research are also listed out. Furthermore, methodology in this research is explained in details in this chapter.

In chapter four, the optimum dosage of different molecular weight of chitosan was studied by comparing the cell separation efficiency and sedimentation rate. There are also included the discussion on cell separation efficiency, sedimentation rate and floc size. Furthermore, optimum dosage of polyethylene glycol for second layer flocculation is studied and discussed too.

In chapter five, the result and discussion of this study are concluded. Moreover, there are some recommendations are provided for further improvement.

CHAPTER 2

LITERATURE REVIEW

2.1 Microalgae

2.1.1 Characteristics of Microalgae

Algae can grow and be found from deserts to Arctic Ocean, which including both salt and fresh water. Microalgae are usually small that are about less than 2 μm in diameter. It cannot be seen by naked eye when without the aid of microscope (Griffiths et al., 2011). The diameters of most microalgae are below 30 μm and their density is slightly greater than that of the water (Milledge and Heaven, 2013; Chatsungnoen and Chisti, 2016). For example, the average diameter of *Chlorella* sp. is 5 μm (Milledge and Heaven, 2013).

Microalgae are microscopic photosynthetic organism (Demirbas, 2011; Demirbas, 2010). They can save us from the threat of global warming by consume carbon dioxide (CO_2) from the atmosphere and released oxygen (O_2). They then store the energy in the form of oils, carbohydrates and proteins (Demirbas, 2011). Microalgae are well known as the fastest growing photosynthesizing organisms (Demirbas, 2011; Demirbas, 2010). They can complete an entire growing cycle within few days. They are in highly productive and easier to cultivate compared to other plants (Ahmad et al., 2011). The main species of microalgae that used for cultivation are *Spirulina* sp., *Chlorella* sp., *Dunaliella* sp. and *Haematococcus* sp. because their annual global production can reach up to 10 000 metric tons (Griffiths et al., 2011). The three most important classes of microalgae that are abundance on earth are the diatoms

(Bacillariophyceae), the green algae (Chlorophyceae), and the golden algae (Chrysophyceae) (Demirbas, 2010).

2.2 Microalgae as Biofuel Feedstock

Microalgae are potential third generation biodiesel feedstock because of their large capacity of oil (Demirbas, 2011). The microalgal biomass can be converted to bio-oil, bioethanol, bio-hydrogen and biomethane by using thermochemical and biochemical methods (Demirbas, 2010). The microalgae are in simple unicellular structure where their entire cell surface can capture the sunlight and also allow the mass transfer of substrates or nutrients from the aquatic medium into the cells. The high rates of substrate uptake and photosynthetic efficiency are then lead to a higher annual oil yield per land area. Study shown that the annual oil year per land area of microalgae can reach up to 25 times higher than that of the palm, which is the current best oilseed crops, as shown in Table 2.1 (Griffiths et al., 2011). Besides that, study also proved that some species of microalgae could contain lipid up to 70 % of their dry weight (Demirbas, 2011).

Table 2.1: **Average Productivities of Some Common Oil Seed Crops Compared to Algae** (MJ Griffiths et al., 2011).

Oil Source	Yield ($L/m^2 \cdot yr$)
Algae	4.7 to 14
Palm	0.54
Jatropha	0.19
Rapeseed	0.12
Sunflower	0.09
Soya	0.04

From literature review, there are some benefits from using microalgae as biofuel feedstock. The microalgae biomass used as the biofuel feedstock can prevent the conflict between food and fuel. Besides that, microalgae can be grown up in various

types of environments and it does not require a large area of land for cultivation. After the oil extraction, the residual of biomass that contains protein and carbohydrate can be used to feed the livestock or as fertilizer. Moreover, the use of microalgae as biofuel feedstock able to achieve zero carbon cycle, where the emission of CO_2 from the consumption of biofuel will be utilized by the microalgae to sustain their growth through photosynthesis (Ahmad et al., 2011). Therefore, microalgae are the potential biofuel feedstock due to the high oil yield and zero carbon emission (Ahmad et al., 2011).

2.3 Microalgae Cultivation for Wastewater Treatment

The microalgae *Chlorella* sp. and *Dunaliella* sp. have been used for wastewater treatment and mass production for 75 years. Nowadays, USA, Australia, Thailand and Mexico are involved in developing wastewater treatment systems by using hyper-concentrated algae cultures that can remove nitrogen (N) and phosphorus (P) efficiently in less than 1 hour (Abdel-Raouf et al., 2012).

The typical wastewater is consisted of man-made compound and mixture of natural organic and inorganic materials. The carbohydrate, fat, protein, and amino acid are the organic carbon that can found in sewage, while the inorganic constituents found in sewage are calcium, potassium, magnesium, chlorine, sulphur, phosphate, bicarbonate, ammonium salts and heavy metals (Abdel-Raouf et al., 2012). The available of organic and inorganic compound, especially the phosphate ions, in the surface water system tend to cause harmful microalgae blooming problem and eutrophication (Abdel-Raouf et al., 2012).

When a specific species of microalgae are being cultivated in the nutrient-rich wastewater, it can eliminate the need of synthetic fertilizer for microalgae cultivation and also able to eliminate the energy needed for wastewater treatment (Trent et al., 2012; Chiu et al., 2015). Moreover, the cultivation of microalgae is very useful for tertiary and quandary treatment of wastewater because microalgae will consume the nutrients to sustain their growth. They can remove heavy metals and some toxic organic

compounds from the wastewater too (Abdel-Raouf et al., 2012). From literature review, microalgae were proven can treat the domestic wastewater by removing the P at an efficiency of 97.8 % (Abdel-Raouf et al., 2012).

Before the treated wastewater being discharge to the downstream water, the microalgae biomass should be harvested in order to avoid the secondary pollution cause by the decomposition of microalgae biomass in the downstream water system. Undeniably, the harvested microalgae biomass can be found in various usages. For example, the microalgae biomass harvested from wastewater treatment can be used for methane production, composting, production of liquid fuels, animal or aquatic feed and production of fine chemicals since the concentration of N and P in biomass is high (Abdel-Raouf et al., 2012). Therefore, the usage of microalgae has been optimized after used for wastewater treatment.

2.4 The Potential of *Chlorella* sp.

From literature review, the microalgae *Chlorella* sp. able to adopt themselves to grow in the municipal wastewater, where its biomass productivity can achieve up to 0.9 g/L·d (Chiu et al., 2015). They also can remove the pollutants, such as N and P, effectively from wastewater with a wide range of initial concentration. Study shows that the *Chlorella vulgaris* can remove inorganic N and inorganic P from water at an efficiency of 86 % and 78 % respectively (Abdel-Raouf et al., 2012).

Besides that, *Chlorella* sp. are readily an ideal biodiesel feedstock because they contain most fatty acids that use to form biodiesel. The fatty acids are linoleic acid (C18:2) and palmitic acid (C16:0), which contribute about 14.4 - 24.4 % and 15.2 - 19.1 % respectively from the total lipid (Chiu et al., 2015). In addition, the *Chlorella* sp. that cultivated in wastewater contained over 80 % of C16 - C18 from the total fatty acid methyl ester (Chiu et al., 2015). Therefore, *Chlorella* sp. is proven has the potential to treat the nutrient-rich wastewater and then the biomass can be harvested for biofuel production purpose (Chiu et al., 2015).

2.5 Microalgae Harvesting Method

There are various methods can be used to harvest the microalgae biomass from the medium. They are centrifugation, flotation, filtration, sedimentation and flocculation.

2.5.1 Centrifugation

Centrifugation is a method that uses centrifugal force to accelerate the rate of sedimentation of cells under a rotational force rather than gravity (Milledge & Heaven, 2013; Gerardo et al., 2015). Since the force driving separation can provide much greater force, so gravity is replaced in centrifugation process (Griffiths et al., 2011).

This method is reliable and highly efficient to separate mixture of cells found in different densities and also applicable for all types of microalgae (Griffiths et al., 2011; Milledge and Heaven, 2013). The efficiency of centrifuge is based on the size and density of the particles, the speed of the rotor, the time of centrifugation and the volume and density of the liquid (Griffiths et al., 2011). There are many different designs of centrifuges according to demands of size of solid particles as shown in Figure 2.1.

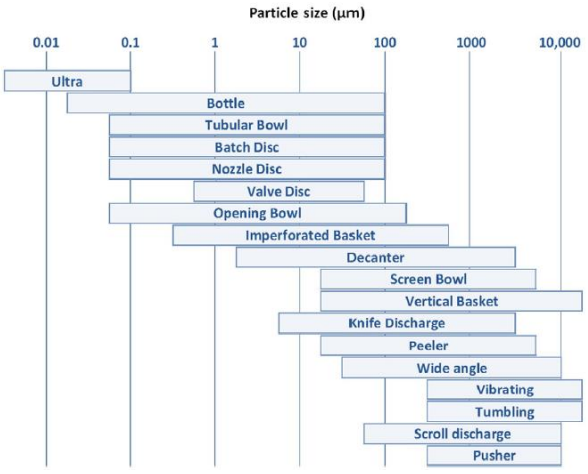

Figure 2.1: Classification of Centrifuges by the Size Range of the Solid Particles (Gerardo et al., 2015).

2.5.1(a) Disc Stack Centrifuge

The disc stack centrifuge is the centrifuge that can reduce the separation time by applying about 4000 to 14000 times of gravitational force. Disc stack centrifuges also produce high value algae products in commercial plants. The most ideal conditions for the centrifuges are for 3 - 30 µm particle size and 0.02 - 0.05 % of concentration of algae cells. This method is more preferable to the recovery of microalgae that grown on pig waste compared to solid bowl centrifuges.

From literature review, this method is in high energy consumption. For an instance, the maximum power of the motor of Westfalia HSB400 disc-bowl centrifuge equipped with intermittent self-cleaning bowl centrifugal clarifier is 75 kW and energy cost for separation is 1.4 kWh/m^3, but normal operating demand is around 50 kW. Study shows that the harvesting of microalgae by using this method tend to consume more operating energy, which is approximately four times more than the amount of energy that can produce by the microalgae biodiesel (Milledge and Heaven, 2013).

2.5.1(b) Decanter Centrifuges

Decanter centrifuges produce more concentrated output than that of the disc bowl centrifuges (Milledge and Heaven, 2013). It can discharge the product continuously in form of suspension with higher solid fraction. Study proves that it can concentrate the suspended solid with total suspended solid (TSS) of 2 % to about 22 % (Milledge and Heaven, 2013; Gerardo et al., 2015).

There are some limitations on this method. It is not suitable for all types of microalgae, such as the *Chlorella* sp. Its high gravitational and shear forces tend to damage the cell structure and then causing the valuable materials loss into the medium. Moreover, the capital cost of this equipment will increase with scale. The high maintenance costs and special materials is needed when harvest microalgae from saline environment (Gerardo et al., 2015). Besides that, it consumes more energy, at rate of 8

kWh/m^3, than that of the disc bowl centrifuges, at rate of 1.4 kWh/m^3 (Milledge and Heaven, 2013; ML Gerardo et al., 2015).

2.5.1(c) Hydro-cyclones

Hydro-cyclone is another type of centrifuge with lower capital cost and also lower energy consumption, at rate of 0.3 kWh/m^3. This method is only applicable for limited number of microalgae strains. Hydro-cyclone disrupted natural flocs of marine microalgae *Phaeocystis* sp. and flocs of microalgae might be broken up and so leads to high difficulty of subsequent harvesting (Milledge and Heaven, 2013).

2.5.2 Flotation

Flotation is another method used to harvest the microalgae biomass. This method is faster and more efficient compare to the method of sedimentation. The microalgae can be separated by introducing air bubbles through a solid-liquid mixture, where the bubble will attach to the cell surface and then the cell will float to the top of the liquid. Then, the biomass will skim from the water body (Griffiths et al., 2011). This method is only applicable for a number of microalgae species (Griffiths et al., 2011; Milledge and Heaven, 2013). Its performance will be affected by particle size, the probability of collision, the probability of adhesion and the attachment of air bubbles to cell (Gerardo et al., 2015). Study shows that the addition of chemical coagulants or flotation agents to change the surface property of cells become hydrophobic can help to solve the natural repulsion between negatively charged algal particles and air bubbles (Griffiths et al., 2011; Gerardo et al., 2015), but this technique is relying on the pH and ionic strength of the medium (Griffiths et al., 2011).

From literature review, floatation can be categorized to dissolves air flotation, electro-flotation, micro-flotation, and dispersed air flotation. They are discussed below.

2.5.2(a) Dissolved Air Flotation

For dissolved air flotation (DAP), the water and air, at pressure 25-90 psi, is supersaturated by compressor for 0.5 - 3.0 min in a pressure tank. After the water is released into a flotation tank at atmospheric pressure, small bubbles with mean size of 40 µm ranging from 10 - 100 µm are generated (Milledge and Heaven, 2013; Gerardo et al., 2015). DAF is an efficient flotation option to harvest the microalgae that grown on pig slurry. However, high dosage of alum is required that is about 0.3 g/L (Milledge and Heaven, 2013). Moreover, the energy requirement is very intensive, around 7.6 kWh/m^3, due to high pressure is needed to supersaturate the water and air in the dissolved air flotation (Gerardo et al., 2015).

2.5.2(b) Electro-flotation

For the electro-flotation, it is effective in a bench scale on a range of microalgae. Salt water is more useful in this method than freshwater. It is in high energy consumption because it is also equipped with DAP (Milledge and Heaven, 2013).

2.5.2(c) Micro-flotation

The micro bubbles are generated in micro-flotation by fluidic oscillation at a specific frequency. The bubbles are detached from the exiting pores in the diffuser (Gerardo et al., 2015). This method is effective in the recovery of algae biomass from growth medium (Milledge and Heaven, 2013). A study states that the bubbles, in radius of 34-100 µm, are produced by frequency of 70-200 kHz at pressure of 11.6 psi (Gerardo et al., 2015). It consumes less energy than that of traditional method. Besides that, the separation efficiency of the process can achieve up to 99% (Milledge and Heaven, 2013; Gerardo et al., 2015).

2.5.2(d) Dispersed Air Flotation

Dispersed air flotation is a method where the bubbles are produced at 15 psi and at energy consumption of 3 kWh/m^3 (Milledge and Heaven, 2013; Gerardo et al., 2015). Although this technique is similar to DAF, but it need the addition of surfactant and the energy intensive compressor that used in DAF has replaced by the low pressure sparger or agitator to generate bubbles and foam. The energy consumption can be as low as 0.015 kWh/m^3 to achieve maximum biomass concentration of 14 – 24 g dry cell weight (DCW)/L by using 10.2 L dispersed air flotation-foam fractionation. Besides that, maximum biomass concentration can be increased to 28 g DCW/L if combined with fluidic oscillation with energy consumption of 0.105 kWh/m^3 (Gerardo et al., 2015).

2.5.3 Filtration

The method of filtration uses semi-permeable filter as the barrier to harvest the microalgae by solid-liquid separation. Normally the selected pore size is between 10–20 times smaller than the cells in membrane filtration. The reducing of pore size will increase the operating energy (Gerardo et al., 2015; Milledge and Heaven, 2013). So, this method requires high energy input to form high pressure. Since there is accumulation of material on the surface of the membrane or slowing filtration, the fouling will form and tends to cause pore blocking, cake formation and the adsorption of gel foulants (Griffiths et al., 2011; Gerardo et al., 2015).

2.5.3(a) Ultra-filtration

Ultra-filtration could be alternative for the recovery of fragile cells, but the operating costs and maintenance costs are high. Therefore, it is generally not used in microalgae. In the case of *Spirulina* sp., the filtration faced clogging rapidly. In the semiconductor manufacturing plant, ultrafiltration is used to harvest microalgae by using the membrane in pore size of 0.03 µm and at energy consumption of 1 to 3 kWh/m^3

(Milledge and Heaven, 2013). However, there is no advantage to harvest the microalgae by ultra-filtration due to low fluxes and high operating pressure. It also cannot lead to better performance (Gerardo et al., 2015).

2.5.3(b) Rotary Vacuum Filters

Rotary Vacuum Filters is used in dewatering organic sludge from anaerobic digestion. This method is not recommended to use to recover the microalgae biomass although it can form microalgae cake with 18% dry weight solids. This is because high energy input is required and the filtration rate tend to fall rapidly. Study shows that the rotary vacuum filters can filter larger size of microalgae such as *Spirulina* sp. and *Micractinium* sp. by using pore diameter of 12 µm and lead to yield of 1 - 3% dry weight microalgae slurry, but this method is not so effective to smaller microalgae such as *Chlorella* sp. (Milledge and Heaven, 2013).

2.5.4 Sedimentation

Sedimentation is a solid-liquid separation by gravitational settling (Gerardo et al., 2015; Milledge and Heaven, 2013). When frictional force is equal to net gravitational force, the cells are suspended in the fluid at terminal falling velocity (Gerardo et al., 2015). The settling time can be reduced by flocculation method. The addition of flocculant can produce microalgae flocs with size larger than 70 µm or form higher density to induce settling (Milledge and Heaven, 2013). Other than that, it can combine other technologies such as DAF and centrifugation so that high solid concentration can be produced (Gerardo et al., 2015).

Settling rates are influenced by light intensity, nutrient deficiency and sinking rate. Older cells can promote settling rates by increasing sinking rate and the settlement rate is decreased by nutrient deficiency. Besides that, high lipid microalgae leads to lower cell density and promote slower settlement rate (Milledge and Heaven, 2013).

Sedimentation often use in water treatment. The capital and operating cost of this method is low and large throughput volumes can be produced (Gerardo et al., 2015; Milledge and Heaven, 2013). This method consumes less energy too. For an instance, a total energy of 0.1 kWh/m^3 is used to produce microalgae biomass at output concentration of 0.1 - 1.5 % by using lamella separator (Griffiths et al., 2011). However, large land area is required for settling ponds and tanks (Gerardo et al., 2015). The energy requirement is too high if ultrasound is used for large scale application (Milledge and Heaven, 2013). Study also found that the sinking rate of microalgae with size of 4 – 5 µm in open-ocean is 'insignificantly small' (Griffiths et al., 2011). During harvesting process, high temperature environments tend deteriorate the biomass produced too (Gerardo et al., 2015).

2.5.5 Flocculation

Natural sedimentation rate of microalgae is very slow because of the mutual repulsion between the negatively charged microalgae cells and they are in small particle size (Gerardo et al., 2015). When flocculation takes place, the repulsion between cells will be reduced by adding positively charged ions or polymers, followed by aggregation of algae cells to increase the size and the settling rate (Griffiths et al., 2011; Milledge and Heaven, 2013). Large quantities of microalgae suspension and a wide range of microalgae can be handled by flocculation (Vandamme et al., 2013).

The performance of flocculation depends on some factors, which are on pH, temperature, density, hydrophobicity, surface charge and culture age. Coagulants such as aluminium sulphate (alum), ferric chloride and polyaluminium chloride (PAC), which are in positively charge, are added to neutralize the charge of cells (Milledge and Heaven, 2013; Vandamme et al., 2013). Then, the cells will coagulate to form larger flocs and hence settle down easily. Besides that, flocculation can be categorized into chemical flocculation, auto-flocculation, bio-flocculation, electro-flocculation and electrolytic flocculation. They are discussed in following section.

2.5.5(a) Chemical Flocculation

Chemical flocculation can be carried out by adding organic and inorganic chemical (Milledge and Heaven, 2013; Gerardo et al., 2015). Ferric chloride, a form of inorganic chemical coagulants, can cause high concentration of metals found in the harvested biomass. The metals contamination in the biomass residue will interfere with animal feed (Vandamme et al., 2013). Synthetic polyacrylamide polymers from organic flocculants contain traces of toxic acrylamide. It also tends to contaminate the microalgae biomass. Therefore, safer alternate, such as using the biopolymer, is needed to promote flocculation (Vandamme et al., 2013). Study shows that the chitosan, a natural biopolymer, is a very effective flocculants for microalgae harvesting purpose (Vandamme et al., 2013). It also can reach high efficiency of 99.3± 0.7 % to remove the microalgae cells by using 10 mg/L of chitosan (Ahmad et al., 2011).

Chemical flocculation is easy and in low cost but the efficiency can be affected by pH, microorganisms' characteristic, water salinity, dose applied and biomass concentration (Gerardo et al., 2015). High dosage and pH correction are required for certain chemical coagulants. Moreover, chemical contamination can cause the media cannot be recycled unless the chemical is removed (Milledge and Heaven, 2013; Gerardo et al., 2015). It is also hard to ensure the optimum polymer dosing when this method is applied in the environmental water system due to the fluctuation of cell density and water condition. Besides that, flocs are easily broken up due to weak bridging if less polymer dosing. However, the electrostatic or static hindering can reduce the bridging potential between the cells if the dosage of flocculant is too high (Gerardo et al., 2015). Therefore, more study on this method is desired.

2.5.5(b) Auto-flocculation

Auto-flocculation of microalgae can occurs spontaneously at above pH 9 by the consumption of dissolved carbon dioxide without addition of supplementary chemicals (Milledge and Heaven, 2013; Gerardo et al., 2015; Griffiths et al., 2011; Vandamme et

al., 2013). For certain species of microalgae, the value of pH greater than 10 can lead to rapid aggregation too. More robust structure of flocs is formed and promotes faster settlement with efficiency of 97 ± 2 % (Gerardo et al., 2015).

Besides that, environmental stress also can cause auto-flocculation of microalgae. When the air or CO_2 supply has been stopped, the pH of the medium will increase and then causes the super-saturation of calcium and magnesium with the phosphate ions and form positively charged calcium or magnesium phosphate precipitate. Hence, it leads to neutralization of the negatively charged algae cells and cause sedimentation (Gerardo et al., 2015; Vandamme et al., 2013). The drawback of this method is the harvested biomass might contaminate with high concentration of minerals (Vandamme et al., 2013).

Auto-flocculation is not applicable for all species of microalgae. For an instance, *Chlorella* sp. can be flocculated at pH 11 - 12 while the addition of alkali cannot induce flocculation of *Chlamydomonas* sp. Furthermore, extreme pH may also damage the cell and lead to cell death (Milledge and Heaven, 2013). Moreover, auto-flocculation is slow, unreliable and uneconomic for microalgae harvesting purpose (Milledge and Heaven, 2013; Gerardo et al., 2015).

2.5.5(c) Bio-flocculation

Bio-flocculation occurs spontaneously with the aid of extracellular polymer substances or proteins from microalgae for flocs forming (Vandamme et al., 2013; Gerardo et al., 2015). Some fungi or bacteria in positively charged can interact with negatively charged microalgae cells too. Moreover, carbon source in wastewater also allows co-cultivation of microalgae and bacteria and the bacteria tend to aid the harvesting of microalgae at the same time (Vandamme et al., 2013). This method is used in wastewater treatment successfully and able to prevent chemical contamination of biomass, but is microbial contamination. Another issue is the risks of competition for nutrient sources between the microalgae and bacteria, antagonistic interactions and significant changes to the

final biomass obtained (Gerardo et al., 2015). However, the underlying mechanism for bio-flocculation is still poorly understood (Vandamme et al., 2013; Gerardo et al., 2015)

2.5.5(d) Electro-flocculation

Electro-flocculation uses sacrificial electrodes, such as iron and aluminium, release metal cations to induce flocculation but there is metal contamination in the algae concentrate. Besides that, there are some disadvantages such as cathode fouling and maintenance, temperature increase of the medium, influence of mixing changes in pH, electrode design and arrangement (Gerardo et al., 2015). However, the power consumption of electro-flocculation, between 0.3 and 2 kWh/kg, is the lowest energy consumption in salt water. Although it is low power consumption but the power consumption is greatly influenced by the distance between electrodes (Milledge and Heaven, 2013).

2.5.5(e) Electrolytic flocculation

Electrolytic flocculation is a physical technique without addition of flocculants. Non-sacrificial anodes are used to attract the negatively charged microalgae cell so that the cells lose their surface charge and forming flocs (Milledge and Heaven, 2013; Gerardo et al., 2015). There is limited research on this method. Study shows that the separation efficiency of microalgae can achieved up to 96 % at 0.3 kWh/m^3 in 75 minutes. The biomass recovered is suitable for animal feed or food due to no flocculant contamination (Gerardo et al., 2015).

2.6 Chitosan as Coagulant

From literature review, chemical flocculation is the easiest and effective compared to other flocculation methods. The chitosan is a natural biopolymer that is a very effective flocculants for microalgae harvesting purpose through chemical flocculation (Vandamme et al., 2013). Therefore, it is further reviewed as below.

2.6.1 Properties of Chitosan

Chitosan is produced by the deacetylation of chitin. It is linear polymer made by the copolymer of of D-glucosamine and N-acetyl-D-glucosamine. Due to the presence of primary amino groups, chitosan has high nitrogen content compared to cellulose. It is a biopolymer that is low cost, non-toxic, biodegradable, renewable, non-corrosive and environmentally friendly. Moreover, the use of chitosan for microalgae flocculation does not lead to secondary pollution or biomass contamination. Lower concentration of chitosan is needed compared to that of metal salts (Renault et al., 2009). Therefore, chitosan coagulant is more preferred compare to the metal salts.

2.6.2 Flocculation Mechanisms of Chitosan-based Flocculants

There are four simple mechanisms in flocculation. First of all, there is simple charge neutralization between coagulants and microalgae cells and then followed by charge patching (Yang et al., 2016). Surface of suspending cells will absorb the positive charged chitosan-based coagulants immediately and then cause the cell destabilization (Yang et al., 2016; Renault et al., 2009). Then, the large molecular weight flocculants will bridge the cells together to form primary flocs (Yang et al., 2016). The small flocs are then aggregated into large flocs or form polymeric precipitates by enmeshing and entrapping of small flocs (Yang et al., 2016; Renault et al., 2009). Hence, the large and heavier flocs are readily to settle down from medium.

2.6.3 Effect on Molecular Weight and Degree of Deacetylation

In the case of flocculation by using the chitosan as coagulant, the molecular weight and degree of deacetylation of chitosan are important parameter on the performance of flocculation. The chitosan in longer chain, which is in higher molecular weight, is more effective than the shorter chain (Renault et al., 2009). This is because the low molecular weight chitosan does not show obvious 'bridging effect' (Yang et al., 2016). However, the molecular weight of chitosan does not affect the flocculation when humic acid is available. This is due to the more complex and flexible structure of humic acid (Renault et al., 2009).

Study shows that the performance of chitosan is better when in high degree of deacetylation and at low pH condition (Renault et al., 2009). Moreover, the dosage of chitosan needed for microalgae flocculation is lower when the chitosan is in higher charge density (Renault et al., 2009; Ahmad et al., 2006). Since the degree of deacetylation is directly proportional to the charge density of chitosan, so the degree of deacetylation of the chitosan is an important factor to be considered (Renault et al., 2009). Therefore, the molecular weight and degree of deacetylation of the chitosan are the important factor on the performance of cell flocculation.

2.6.4 Comparison with Aluminium Sulphate and Polyaluminium Chloride

Aluminium sulphate (alum) is a common coagulant that is used widely in conventional water and wastewater treatment. It is cheap, easy to use and wide availability but its efficiency is fully depends on the pH and not consistent in cold water (Renault et al., 2009). For the polyaluminium chloride (PAC), it is less pH dependent and able to produce lower volume of sludge compared to that of alum. It is also efficient at low temperature compare to that of alum (Renault et al., 2009). However, alum and PAC are toxic when in high concentration of aluminium and metals, so they are hazardous to human health (Renault et al., 2009; Ahmad et al., 2006). For an instance, Alzheimer's disease can be caused by large amount intake of aluminium salt (Ahmad et al., 2006).

Compared to the alum and PAC, chitosan is a promising substitute for them because it does not cause any health threats. Furthermore, chitosan can produce larger flocs with better quality and reduce the settling time by faster settling rate (Renault et al., 2009; Ahmad et al., 2006). It can also be cost-effective compared to them in the water treatment process. The required amount of chitosan to achieve same level of turbidity removal is half of that of PAC and also the chitosan can reduce the volume of sludge compared to alum (Renault et al., 2009). From the study of Ahmad et al. (2006), the performance of chitosan is dependent on the dosage, mixing time and pH and its performance is better than alum and PAC. Therefore, chitosan is more preferred than alum and PAC.

2.7 Polyethylene Glycol as Flocculant

2.7.1 Properties of Polyethylene Glycol

Polyethylene glycol (PEG) is a linear chain polymer which contains neutral charge (Şentürk et al., 2011; Hansson et al., 2005). It is an environmentally safe macromolecular material, user-friendly, non-toxic, chemically stable and good wettability (Şentürk et al., 2011; Zhang et al., 2016). PEG has high hydrophilicity to improve surface segregation (Fan et al., 2016). PEG can play dual-role which are surface modifier and pore forming agent. It undergoes the solidification of membrane bulk materials to separate polymer/water interface by forming a hydrophilic membrane surface (Fan et al., 2016). Blending with PEG can improve the adsorption of chitosan. For PEG/Chitosan composites, average pore width is 16.238 nm which is higher than PVA/Chitosan composites, 15.52 nm in the removal of nitrate (Rajeswari et al., 2016).

Furthermore, PEG/chitosan membrane is used to remove iron and manganese ions from aqueous solutions. The maximum adsorption capacities by using chitosan/PEG (2:1) can reach 80 mg/g and 35 mg/g for iron and manganese ions respectively (Salehi et al., 2016). PEG is rich in oxyethylene groups, resulting in potentially improving chalcopyrite leaching (Zhang et al., 2016). Besides that, PEG graft polymer can resistant the adsorption and adhesion from protein and platelet

because PEG has high hydrophilicity and high surface water retaining capacity (Abednejad et al., 2014; Hansson et al., 2005). From other study, protein adsorption from blood serum can be reduced to less than 5 ng/cm^2 (Hansson et al., 2005). In general, PEG can be used in many applications, which are ultraviolet radiation, plasma treatment and chemically induced grafting (Fan et al., 2016).

2.8 Sedimentation Rate

Flocculation is important to accelerate the rate of sedimentation of microalgal cells. Sedimentation rate can be described by Stokes' Law and applicable to spherical shaped microalgae such as *Chlorella* sp. (Milledge and Heaven, 2013; Gerardo et al., 2015). The sinking velocity is directly proportional to the square of the diameter of cell and also the density difference between the microalgae and medium while is inversely proportional to the fluid dynamic viscosity. The formula is shown below:

$$\text{Sinking Velocity, } v = \frac{gd^2(p_S - p_1)}{18\mu} \qquad (2.1)$$

where

d =cell diameter, m

η = fluid dynamic viscosity, kg/m·s

g = gravity force, m/s^2

$p_s - p_l$ = the density of cell and medium kg/m^3

Therefore, the sedimentation rate of the cell flocs can be estimated by using this formula (Milledge and Heaven, 2013).

CHAPTER 3

MATERIALS AND METHODS

3.1 The Flow of Experiment

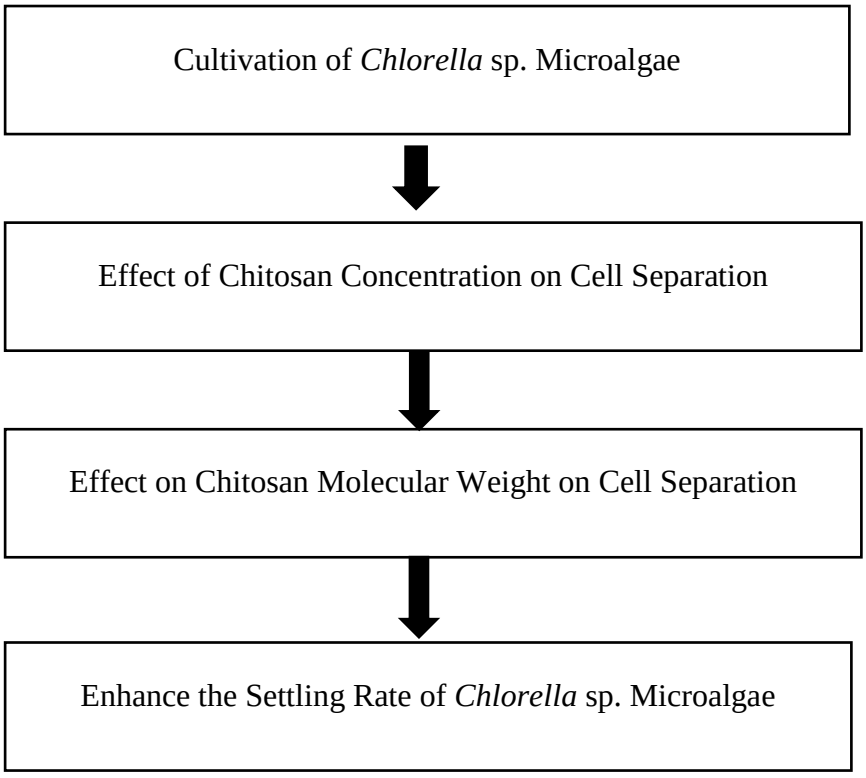

Figure 3.1: Schematic Diagram of Flow of the Experiment.

3.2 Materials and Chemicals

Material/ Chemical	Supplier/ Source
Bold's Basal Medium (BBM)	USM
Microalgae Strain	USM
Low Molecular Weight Chitosan (Average Molecular Weight: 190000-310000 g/mol)	Sigma-Aldrich
High Molecular Weight Chitosan (Average Molecular Weight: 310000-375000 g/mol)	Sigma-Aldrich
Acetic Acid (99.8 %)	R&M Chemicals
Polyethylene Glycol (Average Molecular Weight: 9000-12500 g/mol)	Merck

Equipment	Supplier/Source
Centrifuge	Ara Gemilang Saintifik Sdn. Bhd.
Autoclave	Himayama
Electronic Balance	BEC Engineer
Air Pump	Big Boy
Oven	Memmert
Magnetic Stirrer	2 Mag
Horizontal Laminar Flow Cabinet	ESCO
Particle Size Analyzer	Malvern Mastersizer

3.3 Culture and Characterization of *Chlorella* sp.

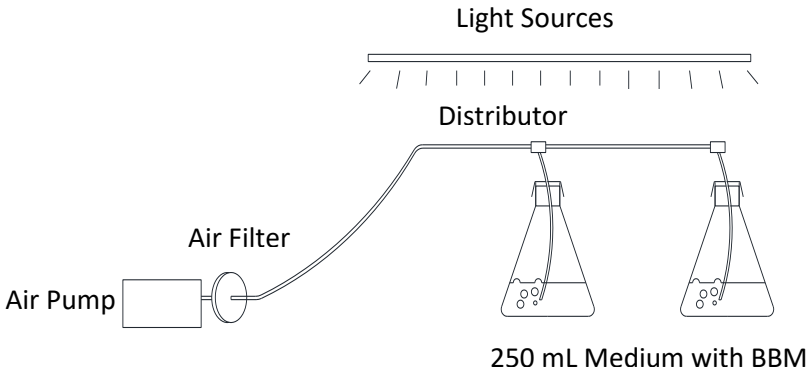

Figure 3.2: Setting Up for Cell Cultivation.

*Chlorell*a sp. strain was obtained from School of Biological Sciences, USM. The cells were cultivated in 500 mL conical flask that contained 250 mL Bold's Basal Medium (BBM) under light. The medium and conical flask were sterilized by autoclave at temperature of 121 °C for 15 minutes before cell cultivation. The inoculum size of *Chlorella* sp. is 1.9 x 10^5 cells/mL. Continuous aeration was provided for the culture medium throughout the cultivation period of 14 days. The counting of cell was conducted by haemocytometer. The desired cell density can be achieved with appropriate dilution using the supernatant of centrifuged medium.

3.4 Preparation of High and Low Molecular Weight Chitosan Solution

High molecular weight and low molecular weight of chitosan was obtained from Sigma-Aldrich, Inc. Acetic acid (99.8 %) was purchased from R&M Chemicals. The chitosan was dissolved into 1% acetic acid solution to form chitosan solution. The concentration of chitosan solution was prepared according to the Table 3.1 in order to achieve respective chitosan concentration in cell medium after 1 mL of chitosan solution was added into 15 mL cell medium. After the chitosan powder added into 1% acetic acid, the chitosan solution was stirred for 24 hours to make sure the chitosan was dissolved completely.

Table 3.1: Mass of Chitosan to Different Concentration of Chitosan Solution or PEG Solution

Concentration of chitosan in cell medium (mg/L)	Concentration of chitosan solution (mg/mL)
1	0.016
5	0.080
10	0.160
15	0.240
20	0.320
25	0.400
30	0.480
35	0.560
40	0.640
45	0.720
50	0.800
55	0.880
60	0.960

3.5 Preparation of Polyethylene Glycol Solution

Polyethylene Glycol (PEG) was obtained from Merck. The PEG solution was prepared in similar concentration of chitosan solution as shown in Table 3.1. PEG powder was solubilized in water and stirred for 24 hours to achieve complete dissolution.

3.6 First Layer Flocculation Test

The desired cell density of the cell medium, at 3 x 10^7 cell/mL, was achieved with appropriate dilution using the supernatant of centrifuged medium. A total of 1 mL

chitosan solution was added into 15 mL of the cell medium and then stirred at 120 rpm for 20 min at room temperature by using magnetic stirrer as shown in Figure 3.3. Then, the samples were left for 60 mins of sedimentation.

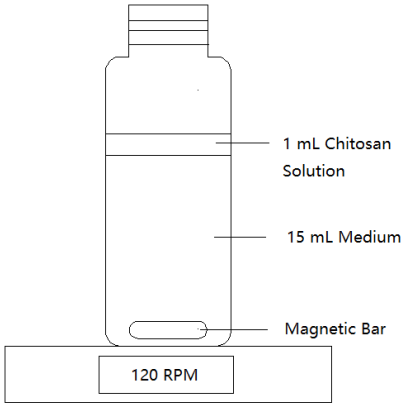

Figure 3.3 Setup of Flocculation Test.

3.7 Second Layer Flocculation Test

A total of 1 mL PEG solution was added into the mixture of chitosan solution and cell medium that has gone through the first layer flocculation processes described in section 3.6. Then, the mixture was stirred at 120 rpm for 20 min at room temperature by using magnetic stirrer. Then, the samples were left for 60 mins of sedimentation.

3.8 Determination of Cell Separation Efficiency

After sedimentation, a total of 4 mL of sample, that is about 1 mm distance below the liquid surface, was collected and the absorbance of the sample collected was measured by UV-Vis Spectrophotometer at wavelength of 660 μm. The cell separation efficiency can be calculated by using the formula below:

$$\text{Cell Separation Efficiency} = \frac{ABS_0 - ABS}{ABS_0 - ABS_{centrifuged}} \times 100\% \quad (3.1)$$

Where

ABS_0 = Absorbance Intensity of Medium

ABS = Absorbance Intensity of Sample

$ABS_{centrifuged}$ = Absorbance Intensity of Centrifuged Medium

3.9 Measurement of Sedimentation Rate

The rate of cell sedimentation was determined by measure the sedimentation distance of the cells in function of time. The sedimentation distance of the cells was determined quantitatively by measuring the distance between the green cell boundary layer to the water surface as shown in Figure 3.4. The duration for the sedimentation of cells, which do not show a clear green colour boundary layer as shown in Figure 3.5, within a distance was recorded by using stopwatch. Then, the sedimentation rate was obtained by dividing the distance of sedimentation with sedimentation duration.

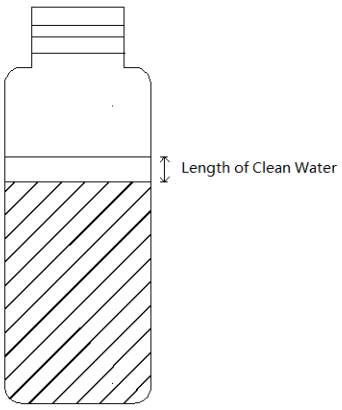

Figure 3.4: Way to Determine Length of Clean Water.

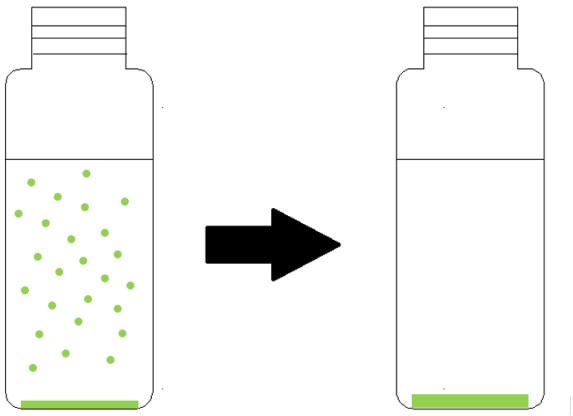

Figure 3.5: Way to Verify the Cleanliness of Sample.

3.10 Measurement of Floc Size

After flocculation process, floc size was evaluated by using Particle Size Analyzer (PSA), Malvern Mastersizer 2000. A cumulative curve shown the volume percent of different floc size was generated by PSA. In this study, refractive index, n value used was 1.08 (Green et al., 2001).

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 To Determine the Optimum Dosage of Chitosan

In this study, the optimum dosage of coagulants, the high molecular weight chitosan (HMWC) and low molecular weight chitosan (LMWC), were studied and observed. From the Figure 4.1, the cell separation efficiency promoted by HMWC when at dosage of 1 mg/L was very poor, where the cell separation efficiency reached to only 29.74 ± 4.44 %. When the dosage of HMWC increased to 5 mg/L, the cell separation efficiency increased sharply and reached to the optimum that was at 62.71 ± 17.76 %. When the dosage of HMWC further increased to 60 mg/L, the cell separation efficiency decreased gradually and reached to only 11.45 ± 1.02 %. The cell separation efficiency when at dosage of 60 mg/L was the lowest among other concentrations in the range of 1 to 60 mg/L.

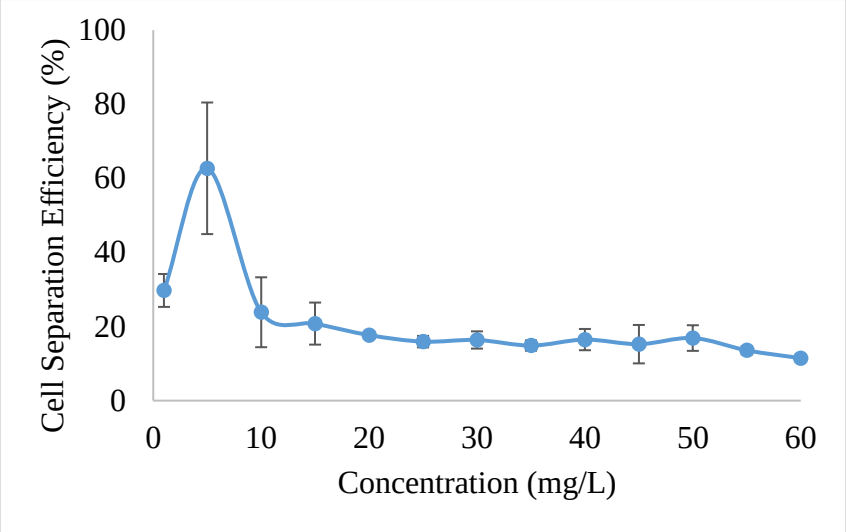

Figure 4.1: Cell Separation Efficiency of *Chlorella* sp. Microalgae in Function of the Dosage of HMWC.

When the dosage of LMWC was being investigated, Figure 4.2 shown that the cell separation efficiency when at 1 mg/L of LMWC was very low, which was at only 39.49 ± 0.34 %. However, the cell separation efficiency increased sharply when the dosage of LMWC increased to 5 mg/L. The cell separation efficiency reached to the optimum that is at 99.04 ± 1.00 %, when at 5 mg/L. When the dosage of LMWC further increased to 60 mg/L, the cell separation efficiency was decreased and reached to only 17.44 ± 9.54 %.

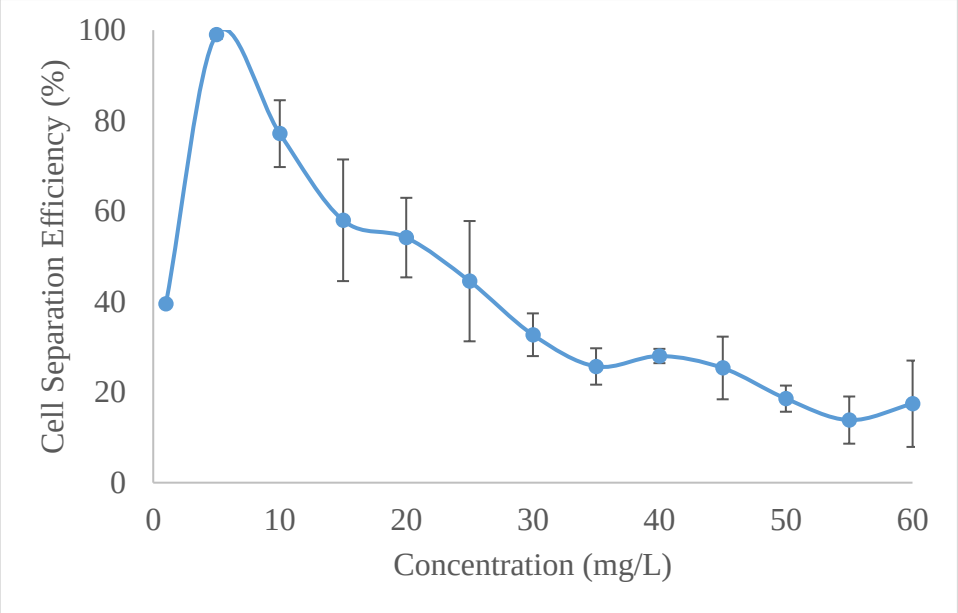

Figure 4.2: Cell Separation Efficiency of *Chlorella* sp. Microalgae in Function of the Dosage of LMWC.

From the result, the trend of the cell separation efficiency promoted by HMWC and LMWC is similar. The cell separation efficiency was low when at coagulant dosage more than or less than the optimum dosage. Moreover, the optimum dosage of HMWC and LMWC used to achieve optimum cell separation efficiency is same that is at 5 mg/L. Coagulation process takes place by charge neutralization, adsorption and electrostatic patch mechanism (Roussy et al, 2005; Ahmad et al, 2011). During the preparation of chitosan solution, 1% acetic acid is added to increase the number of protonated amine group in chitosan. Chitosan becomes soluble easily and the number of positively charged functional groups on chitosan increase too. The positively charged chitosan can adsorb easily onto the surface of negatively charged microalgae cells through electrostatic attraction force and achieve charge neutralization. The microalgae cells are destabilized due to the loss of surface charge and hence forming large floc which promoted by the bridging effect from the chitosan through continuous stirring (Roussy et al, 2005; Ahmad et al, 2011). Hence large and heavier cell flocs tend to achieve fast sedimentation. In this study, a very low dosage of chitosan, at only 5 mg/L of HMWC and LMWC, is sufficient to adsorb onto every cell surface to promote cell flocculation and sedimentation.

When the dosage of chitosan is lower than the optimum dosage, chitosan is not sufficient to attach onto the surface of microalgae cell. Charge neutralization between chitosan and microalgae cells cannot be achieved and maintained a net negative charge on cell surface (Ahmad et al, 2011; Tenney et al, 1969). The electrostatic repulsion between the microalgae cells tends to inhibit the flocculation of cells and so lead to ineffective electrostatic patch destabilization (Figure 4.3a) (Roussy et al, 2005; Ahmad et al, 2011). Therefore, the microalgae cells are hard to settle down and promote low cell separation efficiency due to the failure in produce large and denser flocs (Ahmad et al, 2011).

At optimum dosage of chitosan at 5 mg/L, negatively charged microalgae cells are attached strongly with the positively charged chitosan and tend to promote charge neutralization effect (Ahmad et al, 2011). Net surface charge on microalgae is reduced to neutral and thus the electrostatic repulsion between cells becomes negligible (Tenney et al, 1969). Hence promote the formation of cell agglomeration. Besides that, the coiling of chitosan molecules through bridging mechanism can improve the electrostatic patch destabilization of microalgae cells effectively and hence promote fast sedimentation of microalgae (Figure 4.3b) (Roussy et al, 2005; Ahmad et al, 2011). Therefore, the cell separation efficiency is the highest when at optimum dosage of chitosan (5 mg/L).

When at dosage of chitosan more than the optimum dosage, the supply of chitosan polymer is in excess. The chitosan molecules tend to attach on cell surface to form a layer of chitosan. When the cells are fully covered by the chitosan polymer, the cell surface will carry the charge of chitosan that is in positive charge. The microalgae cells that have been covered completely by a layer of chitosan will tend to repel each other through electrostatic repulsion force (Figure 4.3c). Hence, the cells are restabilized when the chitosan molecules are over-saturated and the collision frequency between the cells will increase. When the cells are restabilized, it is difficult to form cell agglomeration to promote sedimentation (Ahmad et al, 2011). Therefore, cell separation efficiency is low too when at excessive dosage of chitosan coagulant.

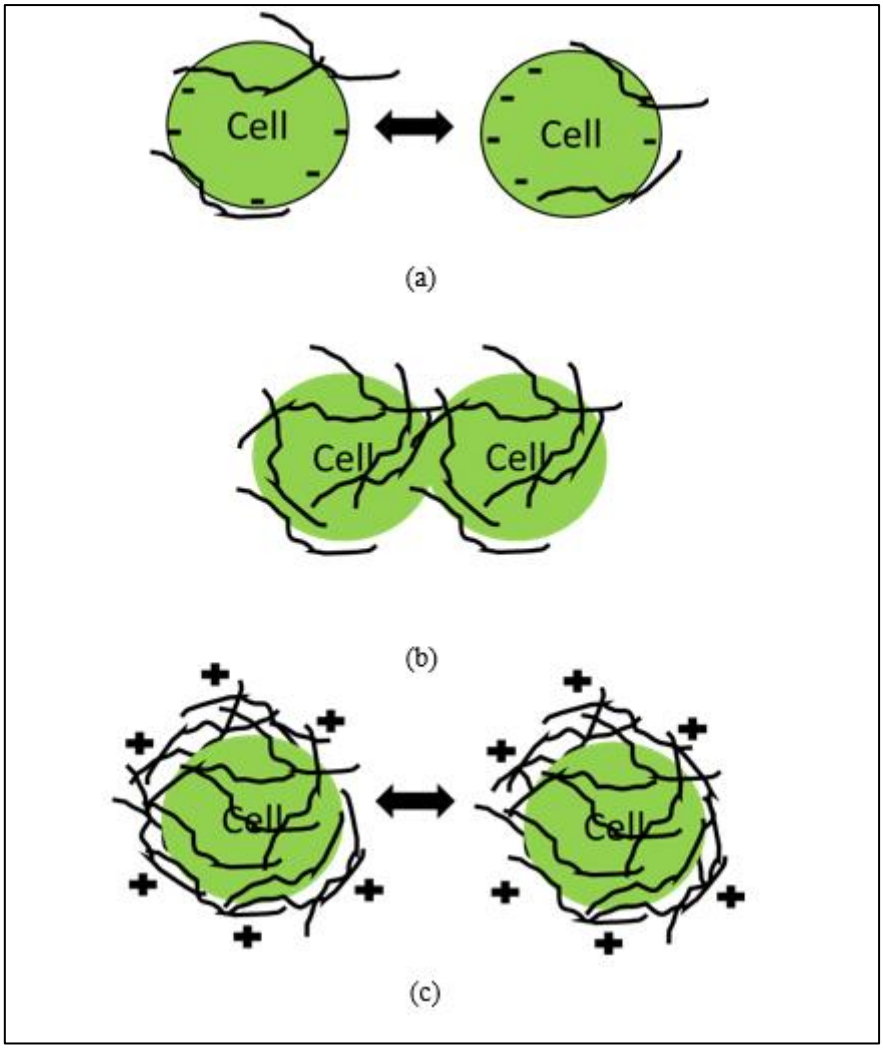

Figure 4.3: Microalgae Cells after Addition of Chitosan (a) When At Dosage of Chitosan Lower Than the Optimum Dosage, (b) At Optimum Dosage, and (c) When At Dosage of Chitosan More Than the Optimum Dosage.

4.2 Comparison between Optimum Dosage of HMWC and LMWC

4.2.1 Cell Separation Efficiency

From Figure 4.4, the average efficiency of HMWC is lower than LMWC. The average efficiency of HMWC is 62.71 ± 17.76 % while LMWC is 99.04 ± 1.00 %. Coagulation by HMWC, the performance was not stable by forming large standard deviation. The highest efficiency of HMWC can reach 82.60% but the lowest efficiency of HMWC

can only reach 57.09%. The difference between highest and lowest efficiency was very large, which was about 25.51%. On the other hand, the performance of LMWC was more consistent than HMWC. The highest efficiency of LMWC is 100.00% while the lowest efficiency of LMWC can reach 98.00%, the difference between highest and lowest efficiency was very small, which is about 2%.

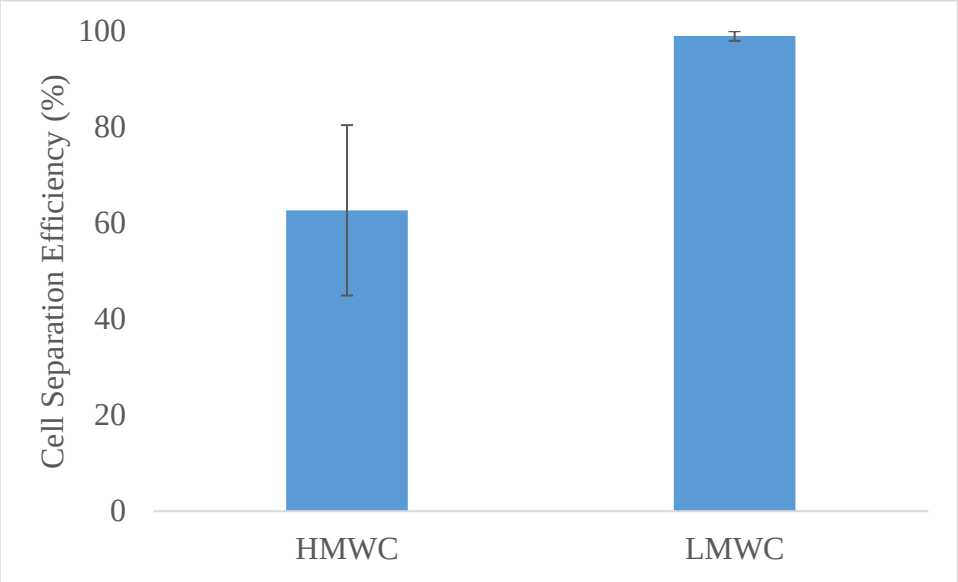

Figure 4.4: Efficiency of HMWC and LMWC When at Optimum Dosage of 5 mg/L.

From the comparison between the performance of HMWC and LMWC, HMWC is longer in chain. The HMWC tends to coat more completely onto the cell surface to form a thick layer of polymer than that of the LMWC (Santander-Ortega et al., 2011). Moreover, HMWC tends to form more tails and loops on cell surface with charged functional groups and extending out from the cell surface compare to that of LMWC as shown in Figure 4.5 (Zhou and Franks, 2006). Hence, the cells that have coated by a layer of HMWC will exhibit the positive charge of HMWC and form a colloidally stable cell suspension again through the electrostatic repulsion (Santander-Ortega et al., 2011). For the LMWC, it tends to attach onto cell surfaces and achieve charge neutralization on cell surfaces (Tenney et al, 1969). The neutralized cells become less likely to repel each other due to the weak electrostatic repulsion force (Ahmad et al, 2011). Hence, the cells tend to agglomerate by the aid of LMWC coagulant. Moreover, the agglomerated cells tend to form larger flocs through the

bridging between the LMWC by the aid of stirring. As a result, the efficiency of LMWC is higher than HMWC (Roussy et al, 2005; Ahmad et al, 2011).

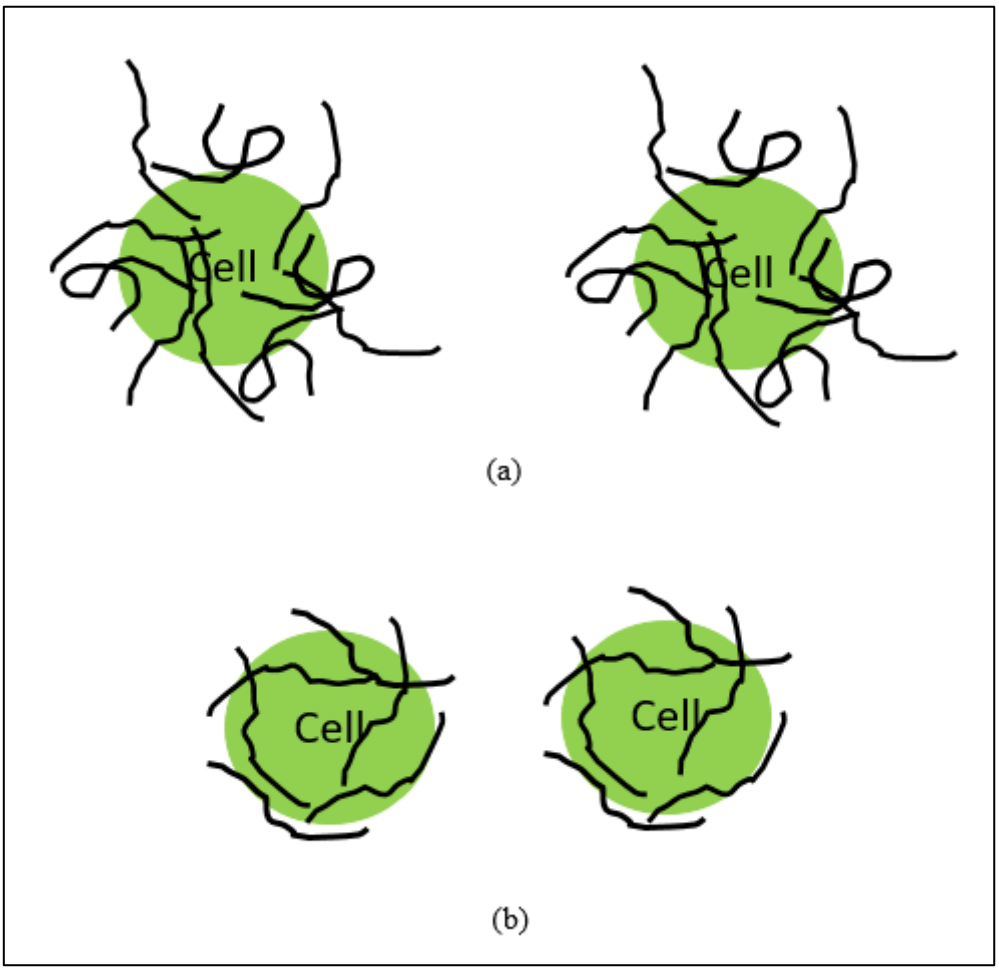

Figure 4.5: Polymer Layer on Microalgae Cells Promoted by (a) HMWC and (b) LMWC

4.2.2 Sedimentation Rate

From the comparison on the cell sedimentation rate between the case of HMWC and LMWC, Figure 4.6 and Figure 4.7 showed that the HMWC and LMWC tended to promote sedimentation rate at 0.30 $\pm$ 0.10 cm/h and 28.18 ± 4.71 cm/h respectively when at optimum dosage of 5 mg/L. The sedimentation rate promoted by the LMWC is 93.93 times faster than that of the HMWC. Moreover, Figure 4.8 showed that a total

5 mg/L of LMWC outperformed the HMWC and form a clear medium after all cell flocs have settle down.

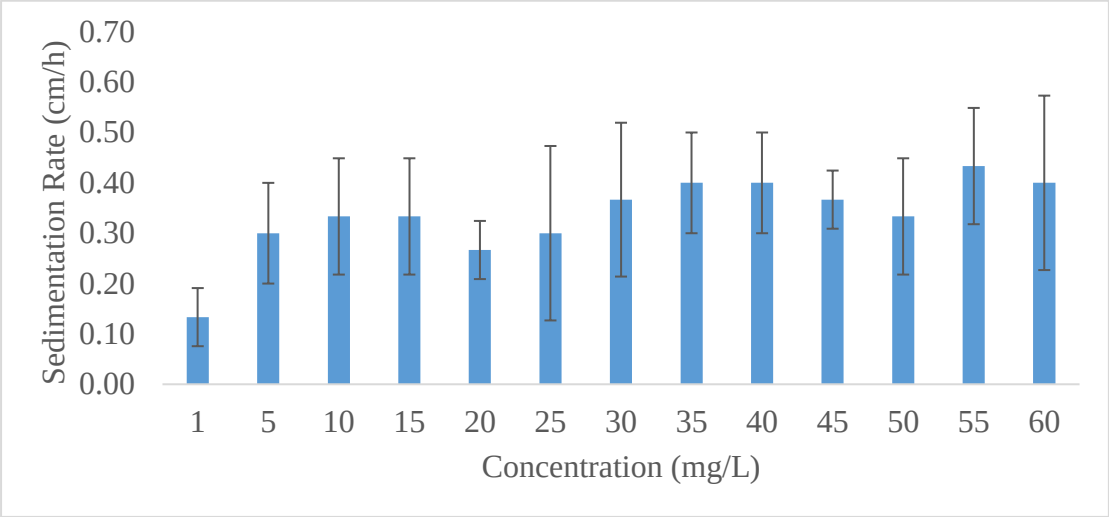

Figure 4.6: Sedimentation Rate of *Chlorella* sp. Microalgae in the Function of the Concentration of HMWC.

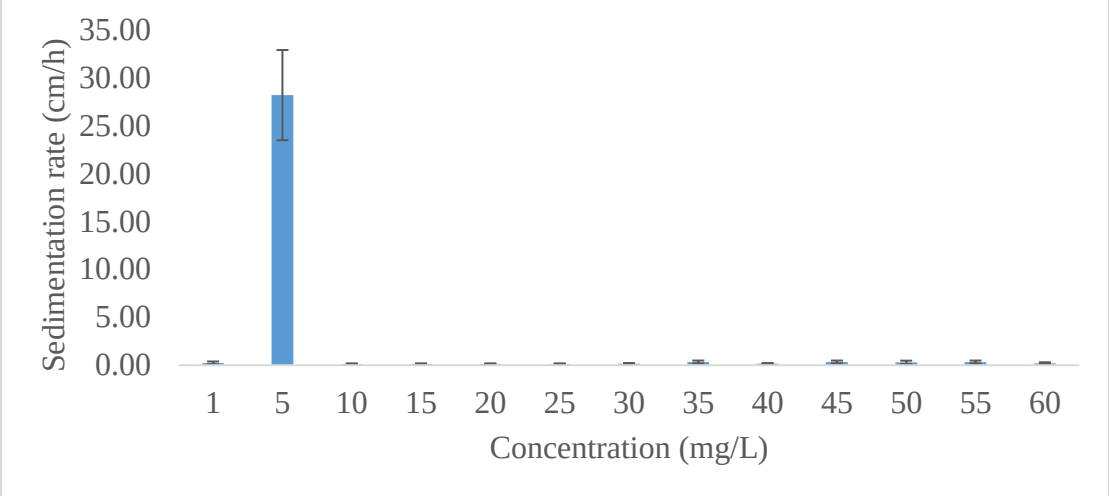

Figure 4.7: Sedimentation Rate of *Chlorella* sp. Microalgae in the Function of the Concentration of LMWC.

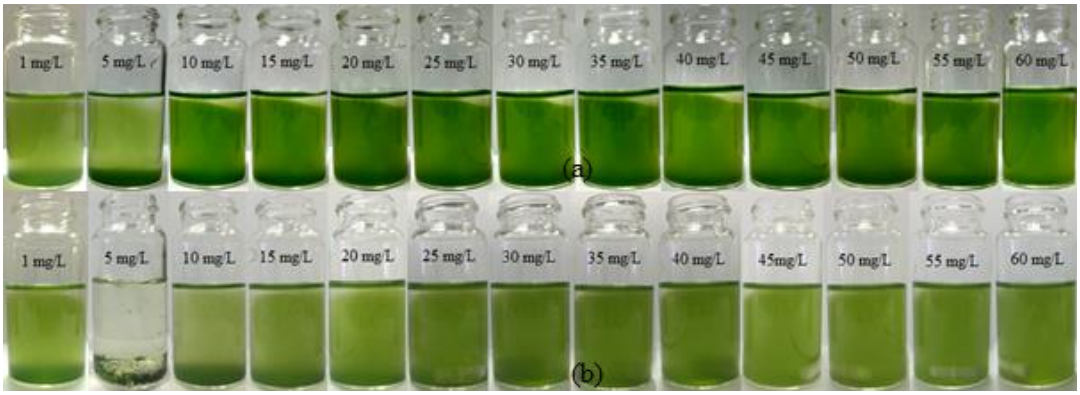

Figure 4.8: Images Show the Cell Sedimentation for 1 hour That Promoted by Different Concentration of (a) HMWC and (b) LMWC.

A total of 5 mg/L LMWC enable cells to settle down completely within an average sedimentation duration of 6.50 min because the neutralization effect between microalgae cell and LMWC (Roussy et al, 2005). It leads to less repulsion between cells and also cell flocs and the net surface charge of cells become more neutral, so the cells and flocs able to undergo gravimetric sedimentation (Tenney et al, 1969; Vedoy and Soares, 2015). In addition, more electrostatic patch destabilization and coiling of molecules formed, leading to better particle agglomeration by bridging between the dispersed cells. Therefore, it is sufficient to increase the cell settling rate (Roussy et al, 2005; Ahmad et al, 2011).

4.2.3 Floc Size

In addition, flocs size of LMWC and HMWC was studied. From Figure 4.9, it shown that the particle size of *Chlorella* sp. cell is about 3.67 µm. The size of the microalgae cell flocs formed by LMWC and HMWC can reach up to 29.46 µm and 24.80 µm. It means that coagulation by LMWC and HMWC formed quite large flocs after coagulation process and stirring. Result showed that the flocs size formed by LMWC was larger than that of HMWC, which was about 4.66 µm larger than HMWC.

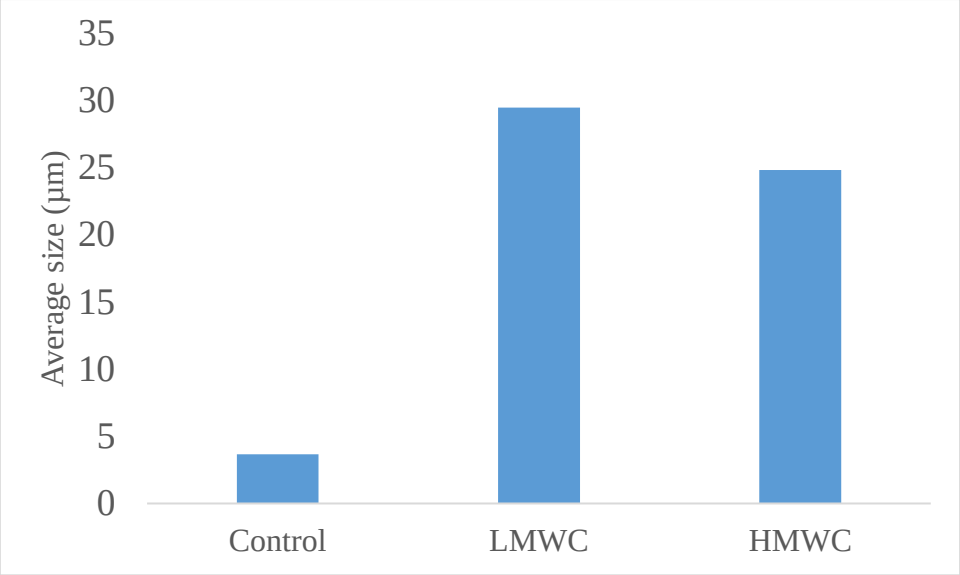

Figure 4.9: The Average Particle Size of *Chlorella* sp. cell, LMWC and HMWC.

From Figure 4.9, it was obviously shown that the particle size increased after coagulation by LMWC and HMWC since it formed flocs after coagulation process and stirring. From Figure 4.10, the particle size distribution of control sample showed only one peak, so its size is homogenous since there was no any coagulation process taking place and the cells are well dispersed in the medium. In contrast, the particle size distribution of HMWC and LMWC showed two peaks. In comparison, the particle size distribution of flocs formed by LMWC was more homogeneous compared to that of the HMWC. Therefore, the performance of coagulation by LMWC was better than HMWC because of the homogeneous size of flocs formed by LMWC tend to settle down simultaneously while for the non-homogeneous size of flocs formed by HMWC, the larger size of flocs will settle down faster and left behind the small flocs in the medium.

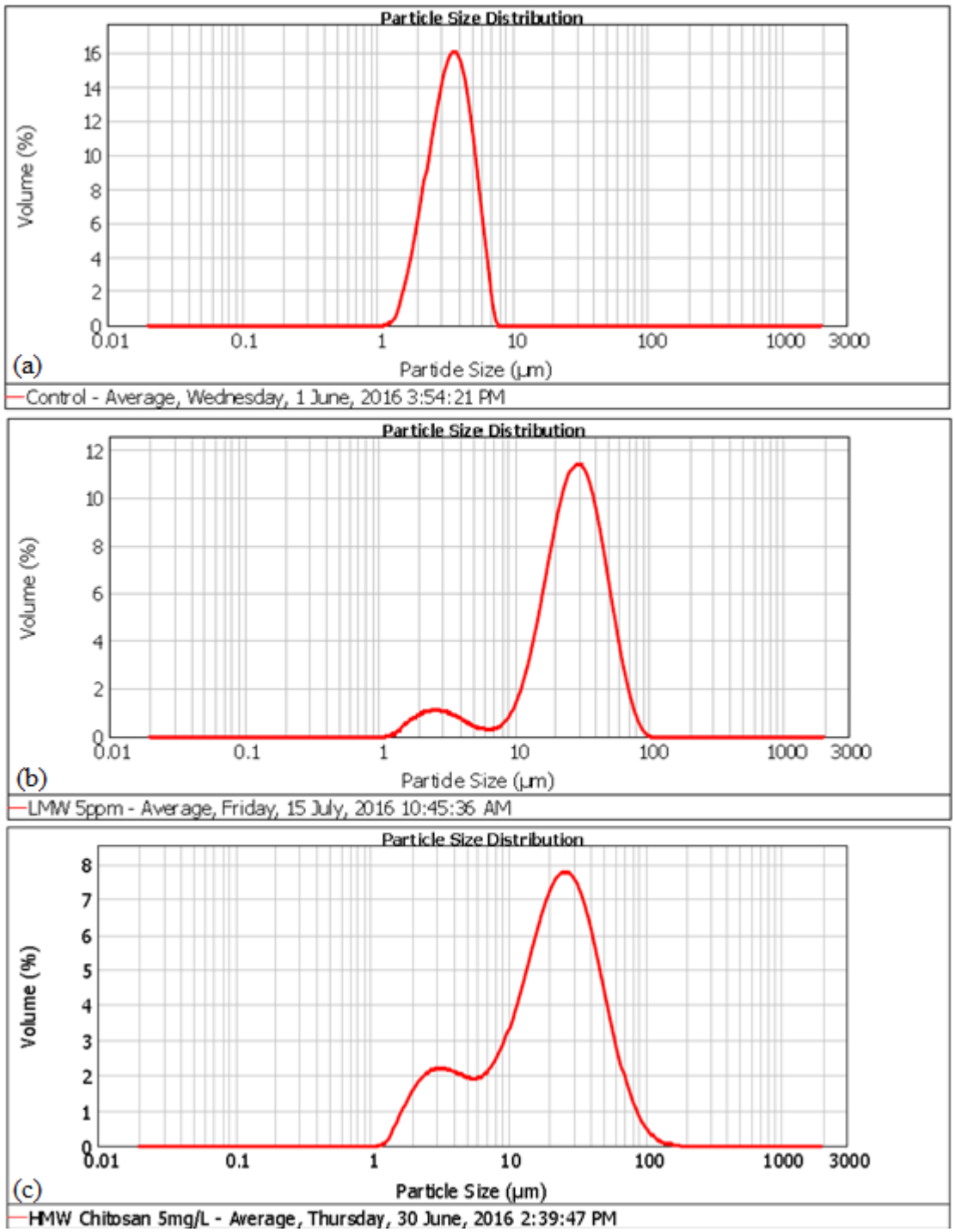

Figure 4.10: Particle Size Distribution of (a) Control, (b) LMWC, and (c) HMWC.

Mixing rate is the key to affect the floc size (Vedoy and Soares, 2015; Ahmad et al., 2011). Suitable mixing rate can promote the collision between coagulant and microalgae cell (Vedoy and Soares, 2015). However, high mixing rate can cause more intense mixing, leading to floc breakage (Vedoy and Soares, 2015; Ahmad et al., 2011;

Senaputra et al., 2014). The flocs cannot withstand the shear rate and become less stable, cause rupture, restabilization and redispersion of the coagulated cells (Ahmad et al., 2011; Senaputra et al., 2014; Blanco et al., 2002). Therefore, the efficiency of cell separation decreases. Microalgae medium mixed with 5 mg/L LMWC at stirring speed of 120 rpm withstands the shear and promotes the effective collision interactions between the cells and hence leading to successful polymer adsorption on cell surfaces and form larger and denser flocs so that the flocs can settle down fast and achieve high removal efficiency. However, higher concentration of coagulant may need higher agitation rate or higher mixing rate in order to maximize the collision between the cells and the coagulant. Moreover, low agitation rate will produces low mixing energy and hence causing local overdosing and achieve lower efficiency of coagulant (Vedoy and Soares, 2015).

4.3 Second Layer Flocculation Test by Using Polyethylene Glycol

4.3.1 Determination on the Optimum Dosage of for Second Layer Flocculation

After cell flocculation by using 5 mg/L HMWC, polyethylene glycol (PEG) in concentration range of 5 to 60 mg/L was added into the cell medium in order to study the optimum dosage of PEG for second layer flocculation test. From Figure 4.11, the cell separation efficiency of 5 mg/L PEG was low that is at 75.28 %. When the dosage of PEG increased to 45 mg/L, the cell separation efficiency increased gradually and reached to optimum, which is at 89.31 %. When the dosage of PEG further increased after optimum dosage, the cell separation efficiency decreased gradually. The cell separation efficiency of 60 mg/L can only reached 71.81% where it was second lowest cell separation efficiency among other concentration in a range of 5 to 60 mg/L PEG.

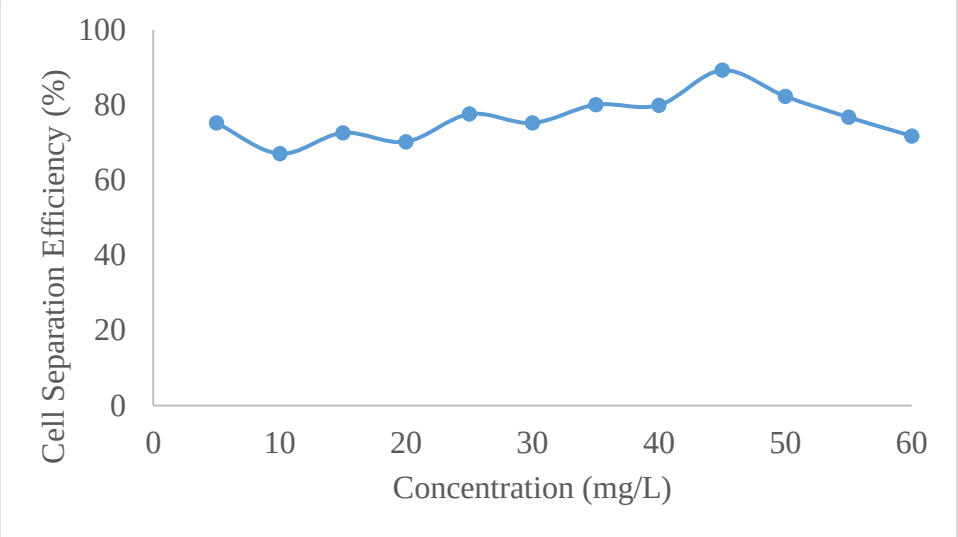

Figure 4.11: The Cell Separation Efficiency of *Chlorella* sp. Microalgae in Function of the Dosage of PEG.

When PEG concentration lower than the optimum dosage, PEG is not sufficient to attach on the surface of the HMWC-flocculated-cells (Abednejad et al., 2014). When at optimum dosage of PEG (45 mg/L), PEG can strongly attach on the surface of HMWC-flocculated-cells through hydrogen bonding interactions between PEG and HMWC to promote further flocculation o microalgae cells (Abednejad et al., 2014; Zhang et al., 2016). From Figure 4.12, it showed that ether group and hydroxyl group from PEG can form hydrogen bonding to the functional group of chitosan (Zhang et al., 2016). Hydrogen bonding is also formed between the polar groups, which is from hydrogen atom of PEG to nitrogen atom of chitosan (Fan et al., 2016) Hence, the cell flocs tend to become larger in size and heavier after the second layer flocculation. Therefore, the flocs can settle down faster and achieve high cell separation efficiency.

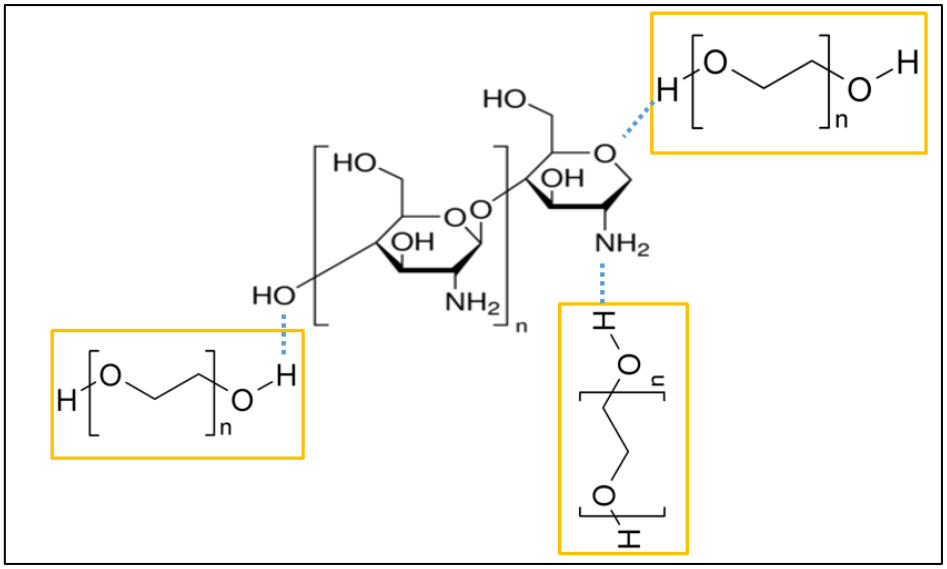

Figure 4.12: Hydrogen Bonding Formed From PEG To Chitosan.

4.3.2 Enhancement on Cell separation Efficiency by Second Layer Flocculation

In this study, the cell separation efficiency by the addition of PEG was studied and observed. The addition of 45 mg/L PEG onto the chitosan-flocculated-cells had improved the cell separation efficiency of *Chlorella* sp. microalgae. From Figure 4.13, the cell separation efficiency after the second layer flocculation, by the addition of 45 mg/L PEG, was at 99.15 ± 0.54%, which was slightly higher than the first layer cell flocculation by using 5 mg/L LMWC, which was about 0.11%.

From Figure 4.14, the cell separation efficiency of *Chlorella* sp. after second layer flocculation by the addition of 45 mg/L PEG into the cell medium after first layer flocculation by 5 mg/L HMWC was also improved. The cell separation efficiency reached to 93.61 ± 1.33 % from the first layer flocculation at only 62.71 ± 17.76 %. PEG had improved the performance of HMWC significantly compared to the case of LMWC in term of cell separation efficiency.

From the comparison between the case of HMWC and LMWC after the second layer flocculation as shown in Figure 4.12, the case of LMWC can achieve higher cell separation efficiency, at 99.15 ± 0.54 %, than the case of HMWC, at 93.61 ± 1.33 %.

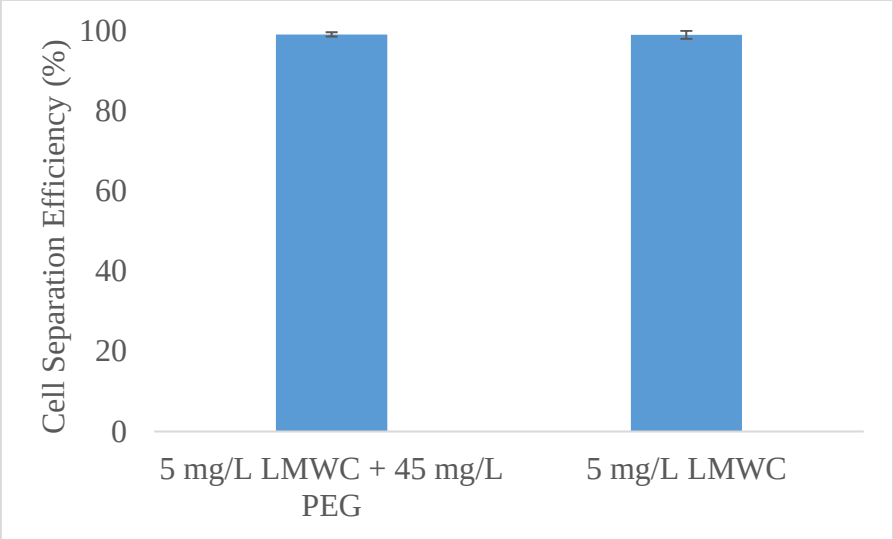

Figure 4.13: The Efficiency of *Chlorella* sp. Microalgae in the Function of the Concentration of LMWC with PEG and LMWC.

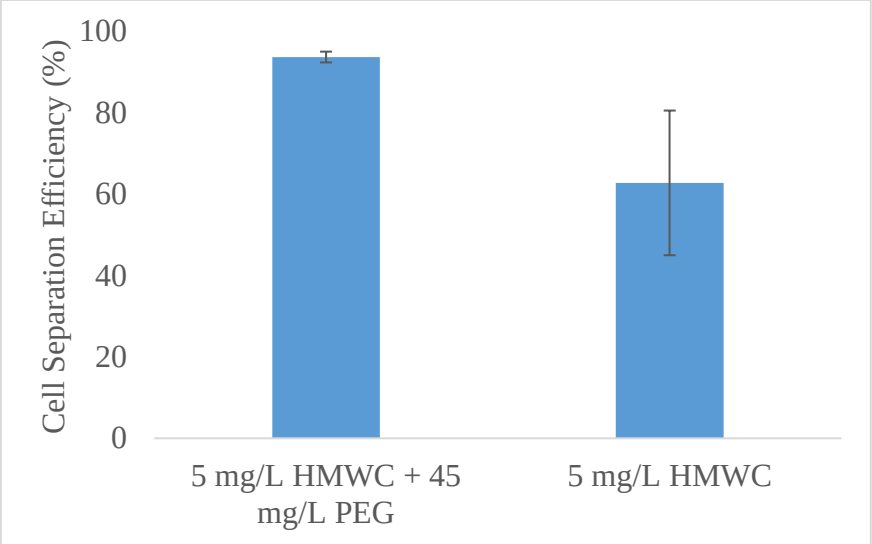

Figure 4.14: The Efficiency of *Chlorella* sp. Microalgae in the Function of the Concentration of HMWC with PEG and HMWC.

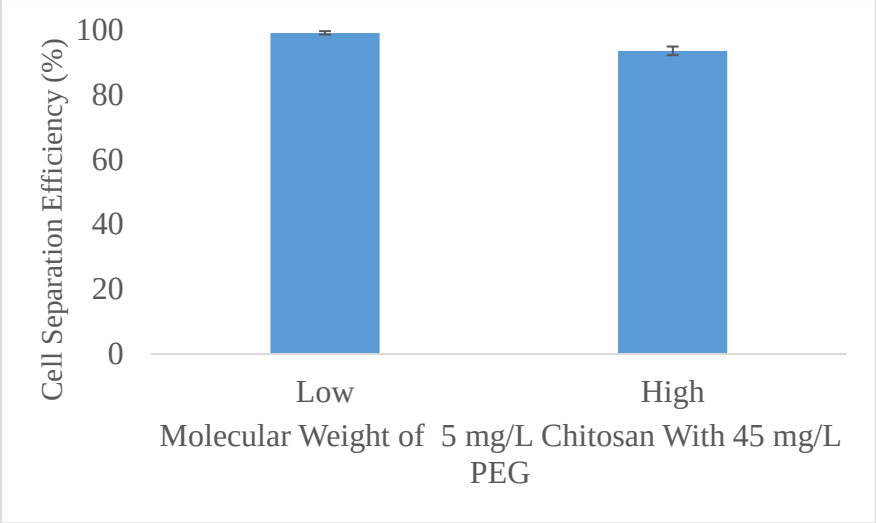

Figure 4.15: The Efficiency of *Chlorella* sp. Microalgae of the Case of LMWC and HMWC When at High Molecular Weight of 5 mg/L Chitosan with 45 mg/L PEG.

PEG is a non-ionic polymer where the hydrogen bonding is the key to promote effective PEG attachment onto the chitosan-flocculated-cells. (Abednejad et al., 2014; Şentürk et al., 2011; Fan et al., 2016). Then, PEG tends to promote the flocculation of cell flocs formed from the first layer flocculation to become larger in size by the aid of bridging mechanism to promote sedimentation and leading to higher cell separation efficiency (Abednejad et al., 2014; Ahmad et al, 2011).

4.3.3 Sedimentation Rate by Second Layer Flocculation

In this study, the sedimentation induced by second layer flocculation was studied and observed. The second layer flocculation using the optimal dosage of PEG (45 mg/L) tend to improve the sedimentation rate of first layer flocculation. After second layer flocculation, Figure 4.16 showed that the sedimentation rate for the case of LMWC was at 120.91 cm/h, which was about 4.29 times faster than the first layer flocculation.

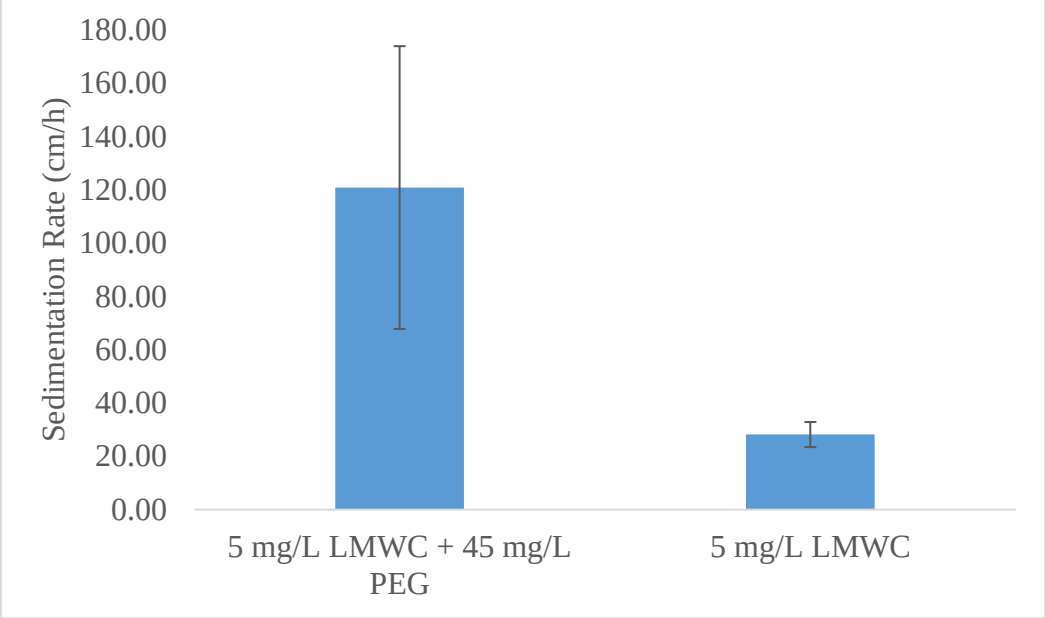

Figure 4.16: Sedimentation Rate of *Chlorella* sp. Microalgae in the Function of the Concentration of LMWC with PEG and LMWC.

For the case of HMWC, the sedimentation rate was not being compared because the cell flocs formed in first layer flocculation did not settle down completely even after 1 hour of sedimentation and left behind a greenish medium as shown in Figure 4.17 (Left). After second layer flocculation, most of the flocs tended to settle down within an hour as shown in Figure 4.14 (Right) but still left behind some cell flocs suspending in the medium (Figure 4.18).

In overall, second layer flocculation by using 45 mg/L PEG tended to improve the performance of cell separation in term of sedimentation rate for the case of LMWC while maintaining the high cell separation efficiency at about 99%.

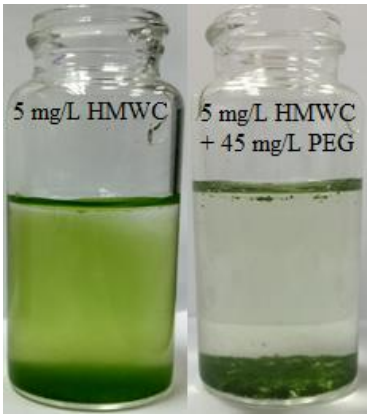

Figure 4.17: Imagine of *Chlorella* sp. Microalgae in the Function of the Concentration of HMWC with PEG and HMWC after Second Layer Flocculation Test.

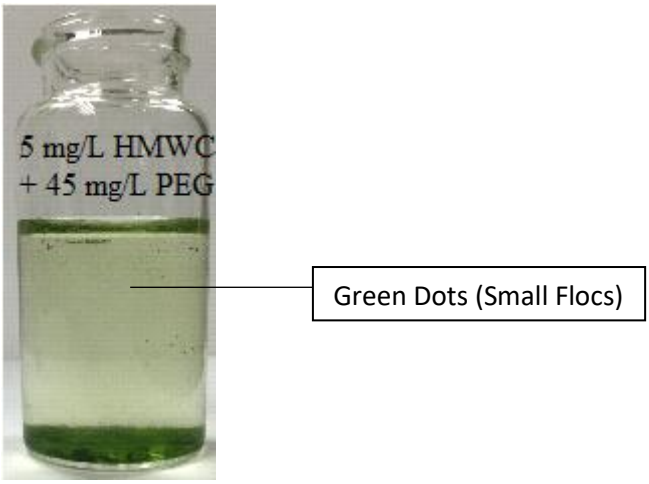

Figure 4.18: The Small Flocs Left behind In the Medium after Second Layer Flocculation of Case HMWC.

4.3.4 Floc Size Formed by Second Layer Flocculation

In this study, the flocs size after second layer flocculation by the addition of 45 mg/L PEG was studied and observed. From Figure 4.19, it showed that the flocs size was increased to 82.76 µm for the case of LMWC, which was about 2.80 times larger than that from first layer flocculation. While for the case of HMWC, the floc size was

increased to 57.75 µm, which was about 2.30 times larger than the first layer flocculation. In overall, the second layer flocculation by the addition of 45 mg/L PEG tended to increase the floc size of cells significantly.

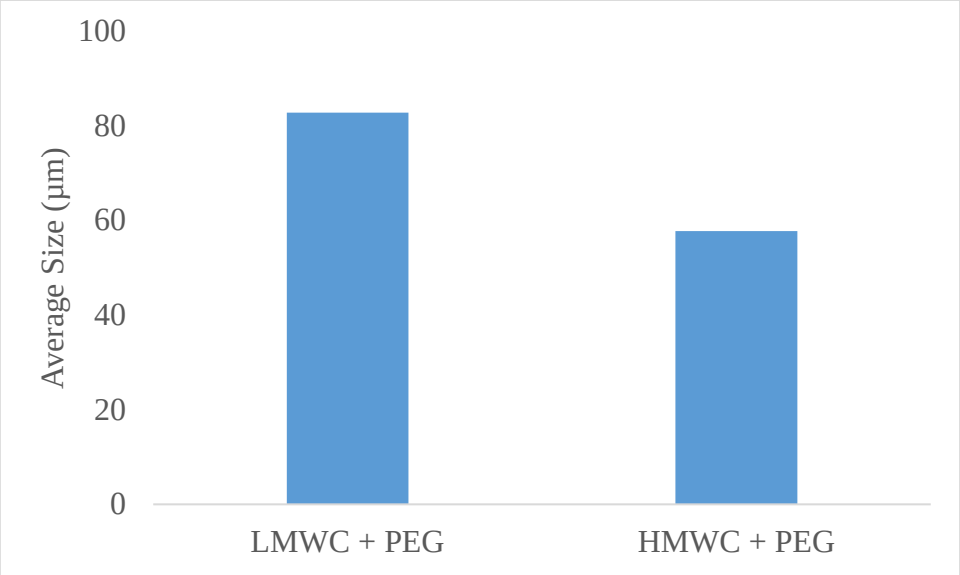

Figure 4.19: The Average Particle Size of Microalgae Cell with Different Molecular Weight of 5 mg/L Chitosan with 45 mg/L PEG.

Therefore, the second layer flocculation by the addition of 45 mg/L PEG was effective to increase the rate of cell sedimentation through the increasing of floc size especially for the case of LMWC.

CHAPTER 5

CONLUCSION AND RECOMMENDATIONS

5.1 CONCLUSION

The optimum dosage of LMWC and HMWC was 5 mg/L. The cell separation efficiency before and after optimum dosage of LMWC and HMWC was very poor, causing the cells difficult to settle down. When dosage less than optimum dosage, it failed to achieve charge neutralization, resulting in ineffective electrostatic patch destabilization. When the dosage more than optimum dosage, there is electrostatic repulsion force due to excess dosage of chitosan, leading to restabilization of the dispersed cells.

On the other hand, the performance of optimum dosage of LMWC was better than HMWC in term of cell separation efficiency, sedimentation rate and floc size formed. LMWC can achieve higher average cell separation efficiency than HMWC. The average cell separation efficiency promoted by HMWC was at 62.71 ± 17.76 % while for LMWC was at 99.04 ± 1.00 %. The performance of LMWC was also more stable than HMWC since the difference of highest and lowest cell separation efficiency of LMWC and HMWC can reach 2 % and 25.51 % respectively. There is electrostatic repulsion between the positive charged microalgae of HMWC while LMWC tends to perform more neutralized cells and form more cell agglomeration by bridging mechanism.

Furthermore, the cell mixed with optimum dosage of LMWC can settle down completely within an hour compared to HMWC and form a clear medium. Optimum dosage of HMWC and LMWC can reach the sedimentation rate at 0.30 ± 0.10 cm/h

and 28.18 ± 4.71 cm/h respectively. In comparison, optimum dosage of LMWC promoted cell settling rate at 94 times faster than that of HMWC because it tends to neutralize the surface charge of the microalgae cells, resulting in denser flocs. In addition, the microalgae cell flocs size formed by LMWC is larger than that of HMWC, which is up to 29.46 µm and 24.80 µm respectively.

In second layer flocculation test, a total of 45 mg/L PEG was the optimum dosage because it can achieve highest cell separation efficiency, which is about 89.31 %. It is because it can attach on chitosan-flocculated-cells strongly by forming hydrogen bonding. Therefore, the flocs become heavier and larger, resulting in high cell separation efficiency. Besides that, the addition of PEG can improve the performance of LMWC and HMWC. After addition of 45 mg/L PEG, the cell separation efficiency of the case of 5 mg/L LMWC and HMWC is 99.15 ± 0.54% and 93.61 ± 1.33 % respectively, improving about 0.11 % and 30.9% from first layer flocculation test respectively. Moreover, the sedimentation rate of cell flocs after mixed with 5 mg/L LMWC and the addition of 45 mg/L PEG can reach to 120.91 cm/h, which was 4.29 times faster than first layer flocculation test. For the case of 5 mg/L HMWC, the cell flocs cannot settle down completely within an hour and left behind a greenish medium. The floc size of LMWC and HMWC after addition of PEG were also improved. The floc size of LMWC and HMWC were improved to 82.76 µm and 57.75 µm, which are about 2.8 and 2.3 times higher than first layer flocculation test respectively.

In overall, double layer flocculation by using PEG can enhance the sedimentation of *Chlorella* sp. microalgae especially for the case of LMWC. This improvement can give contribution to high removal efficiency of microalgae for biofuel production and meet the time effectiveness.

5.2 RECOMMENDATIONS

There are some recommendations to improve this research, such as:-

- Surface charge should be measured so that the explanation of mechanism can be more in details and supportive.

- Temperature of room temperature should be fixed since temperature is also one of key to affect the performance of microalgae.

- pH should be further researched to increase the quality of the flocculation.

- Mixing rate is also needed to further research to decrease the dosage of chitosan and PEG.

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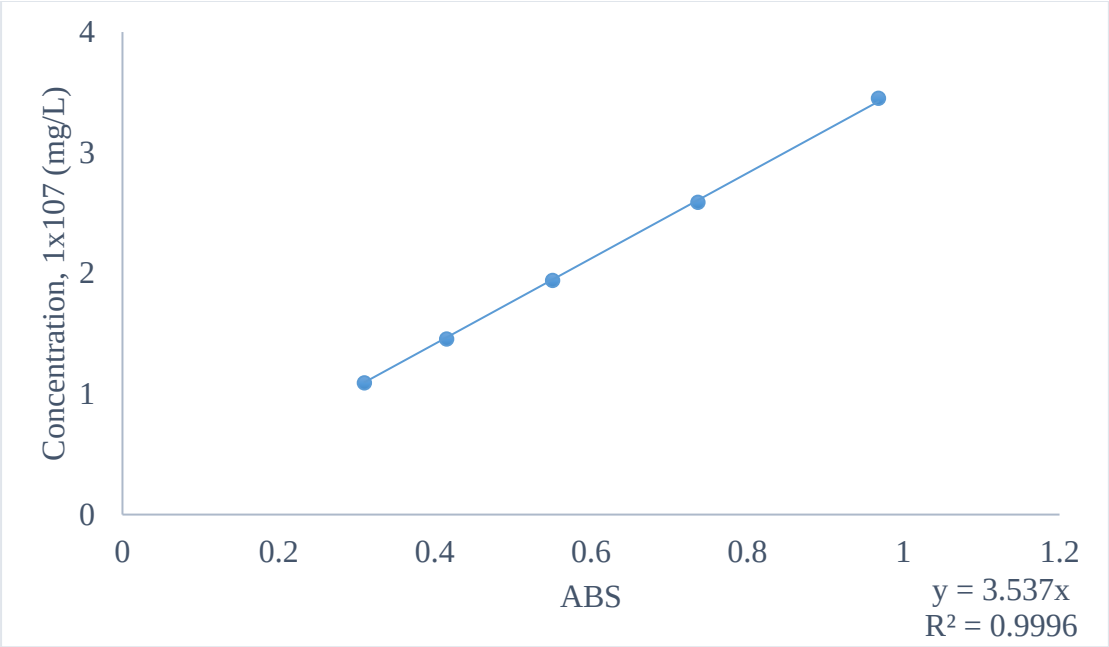
APPENDICES

APPENDIX A: Calibration Curve



APPENDIX B: Average Efficiency in the Function of in the Function of the Concentration of Coagulants

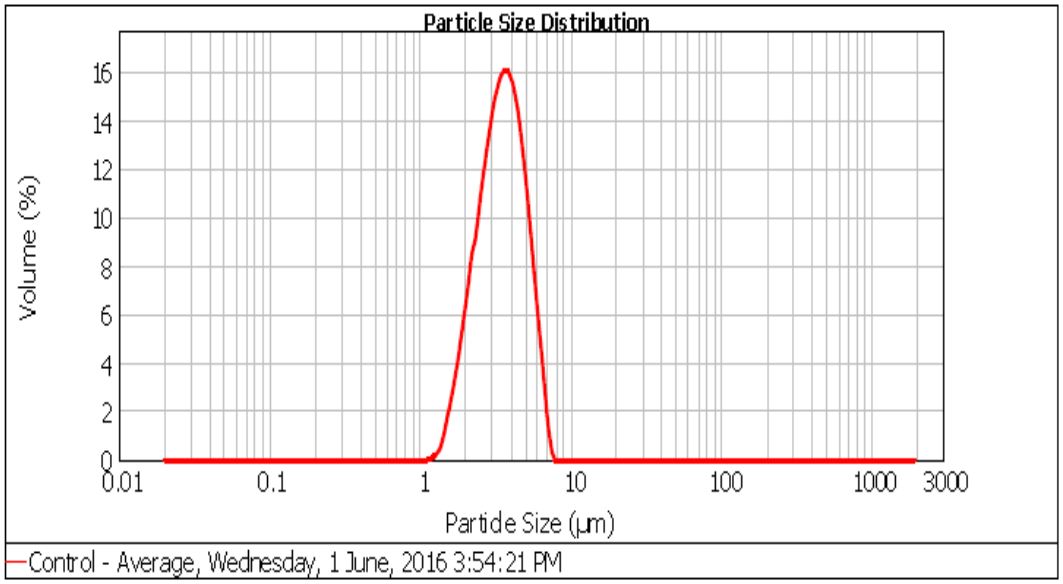
Coagulants	Concentration (mg/L)	Set 1		Set 2		Set 3		Average Efficiency (%)	SD
		ABS	Efficiency (%)	**ABS**	Efficiency (%)	**ABS**	Efficiency (%)		
HMWC	1	**0.522**	31.50	**0.555**	24.69	**0.531**	33.04	29.74	4.44
	5	**0.327**	57.09	**0.38**	48.44	**0.138**	82.60	62.71	17.76
	10	**0.603**	20.87	**0.617**	16.28	**0.52**	34.43	23.86	9.44
	15	**0.639**	16.14	**0.596**	19.13	**0.578**	27.11	20.80	5.67
	20	**0.633**	16.93	**0.608**	17.50	**0.645**	18.66	17.70	0.88
	25	**0.654**	14.17	**0.611**	17.10	**0.662**	16.52	15.93	1.55
	30	**0.631**	17.19	**0.603**	18.18	**0.684**	13.75	16.37	2.33
	35	**0.649**	14.83	**0.617**	16.28	**0.686**	13.49	14.87	1.40
	40	**0.613**	19.55	**0.619**	16.01	**0.683**	13.87	16.48	2.87
	45	**0.609**	20.08	**0.665**	9.77	**0.667**	15.89	15.25	5.19
	50	**0.607**	20.34	**0.638**	13.43	**0.659**	16.90	16.89	3.45
	55	**0.649**	14.83	**0.64**	13.16	**0.692**	12.74	13.58	1.11
	60	**0.678**	11.02	**0.644**	12.62	**0.708**	10.72	11.45	1.02
LMWC	1	**0.475**	39.80	**0.518**	39.13	**0.457**	39.55	39.49	0.34
	5	**0.007**	99.11	**0.017**	98.00	**0.000**	100.00	99.04	1.00
	10	**0.182**	76.93	**0.131**	84.61	**0.228**	69.84	77.13	7.38
	15	**0.376**	52.34	**0.227**	73.33	**0.391**	48.28	57.98	13.44
	20	**0.335**	57.54	**0.334**	60.75	**0.422**	44.18	54.16	8.79

	25	**0.326**	58.68	**0.576**	32.31	**0.434**	42.59	44.53	13.29
	30	**0.539**	31.69	**0.608**	28.55	**0.470**	37.83	32.69	4.72
	35	**0.603**	23.57	**0.593**	30.32	**0.581**	23.15	25.68	4.02
	40	**0.563**	28.64	**0.603**	29.14	**0.558**	26.19	27.99	1.58
	45	**0.618**	21.67	**0.672**	21.03	**0.504**	33.33	25.35	6.92
	50	**0.668**	15.34	**0.673**	20.92	**0.609**	19.44	18.57	2.89
	55	**0.669**	15.21	**0.696**	18.21	**0.695**	8.07	13.83	5.21
	60	**0.674**	14.58	**0.612**	28.08	**0.683**	9.66	17.44	9.54
PEG + 5 mg/L HMWC	5	**0.178**	75.28	**-**	-	**-**	-	75.28	-
	10	**0.237**	67.08	**-**	-	**-**	-	67.08	-
	15	**0.197**	72.64	**-**	-	**-**	-	72.64	-
	20	**0.214**	70.28	**-**	-	**-**	-	70.28	-
	25	**0.161**	77.64	**-**	-	**-**	-	77.64	-
	30	**0.178**	75.28	**-**	-	**-**	-	75.28	-
	35	**0.143**	80.14	**-**	-	**-**	-	80.14	-
	40	**0.144**	80.00	**-**	-	**-**	-	80.00	-
	45	**0.077**	89.31	**-**	-	**-**	-	89.31	-
	50	**0.127**	82.36	**-**	-	**-**	-	82.36	-
	55	**0.167**	76.81	**-**	-	**-**	-	76.81	-
	60	**0.203**	71.81	**-**	-	**-**	-	71.81	-
LMWC + 45 mg/L PEG	5	**0.002**	99.73	**0.010**	98.67	**0.007**	99.05	99.15	0.540
HMWC + 45 mg/L PEG	5	**0.059**	92.12	**0.04**	94.68	**0.044**	94.02	93.61	1.32

APPENDIX C: Sedimentation Rate in the Function of the Concentration of Coagulants

Coagulants	Concentration (mg/L)	Sedimentation Rate (cm/h)				SD
		R1	**R2**	**R3**	**Average**	
HMWC	**1**	0.10	0.20	0.10	0.13	0.06
	5	0.30	0.40	0.20	0.30	0.10
	10	0.40	0.40	0.20	0.33	0.12
	15	0.20	0.40	0.40	0.33	0.12
	20	0.20	0.30	0.30	0.27	0.06
	25	0.20	0.20	0.50	0.30	0.17
	30	0.40	0.20	0.50	0.37	0.15
	35	0.30	0.40	0.50	0.40	0.10
	40	0.30	0.40	0.50	0.40	0.10
	45	0.40	0.40	0.30	0.37	0.06
	50	0.40	0.40	0.20	0.33	0.12
	55	0.50	0.50	0.30	0.43	0.12
	60	0.50	0.50	0.20	0.40	0.17
LMWC	**1**	0.40	0.20	0.10	0.23	0.15
	5	26.28	33.54	24.72	28.18	4.71
	10	0.10	0.10	0.20	0.13	0.06
	15	0.10	0.10	0.20	0.13	0.06
	20	0.10	0.20	0.10	0.13	0.06
	25	0.10	0.10	0.20	0.13	0.06
	30	0.20	0.10	0.20	0.17	0.06
	35	0.50	0.30	0.20	0.33	0.15
	40	0.10	0.20	0.20	0.17	0.06
	45	0.50	0.20	0.30	0.33	0.15
	50	0.50	0.20	0.20	0.30	0.17
	55	0.50	0.30	0.20	0.33	0.15
	60	0.30	0.20	0.10	0.20	0.10
HMWC + 45 mg/L PEG	**5**	11.70	10.50	9.60	10.60	1.05
LMWC + 45 mg/L PEG	**5**	166.67	133.33	62.72	120.91	53.08

APPENDIX D: Particle Size Distribution and Average Particle Size of The Control



Size (µm)	Volume In %
0.010	0.00
0.011	0.00
0.013	0.00
0.015	0.00
0.017	0.00
0.020	0.00
0.023	0.00
0.026	0.00
0.030	0.00
0.035	0.00
0.040	0.00
0.046	0.00
0.052	0.00
0.060	0.00
0.069	0.00
0.079	0.00
0.091	0.00
0.105	0.00

Size (µm)	Volume In %
0.105	0.00
0.120	0.00
0.138	0.00
0.158	0.00
0.182	0.00
0.209	0.00
0.240	0.00
0.275	0.00
0.316	0.00
0.363	0.00
0.417	0.00
0.479	0.00
0.550	0.00
0.631	0.00
0.724	0.00
0.832	0.00
0.955	0.00
1.096	0.00

Size (µm)	Volume In %
1.096	0.03
1.259	0.31
1.445	1.64
1.660	3.40
1.905	5.75
2.188	8.42
2.512	11.07
2.884	13.25
3.311	14.44
3.802	14.13
4.365	12.15
5.012	8.84
5.754	5.10
6.607	1.47
7.586	0.00
8.710	0.00
10.000	0.00
11.482	

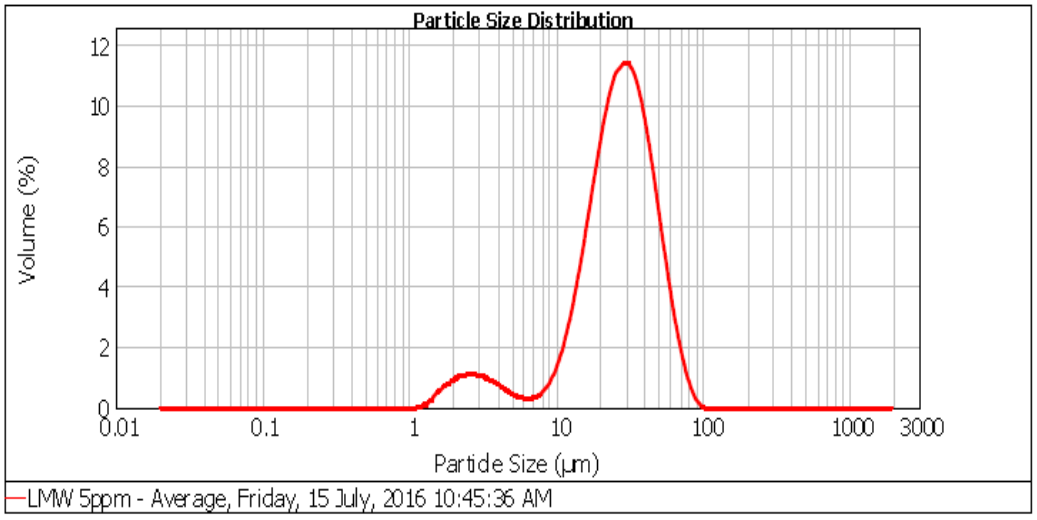
Size (µm)	Volume In %
11.482	0.00
13.183	0.00
15.136	0.00
17.378	0.00
19.953	0.00
22.909	0.00
26.303	0.00
30.200	0.00
34.674	0.00
39.811	0.00
45.709	0.00
52.481	0.00
60.256	0.00
69.183	0.00
79.433	0.00
91.201	0.00
104.713	0.00
120.226	

Size (µm)	Volume In %
120.226	0.00
138.038	0.00
158.489	0.00
181.970	0.00
208.930	0.00
239.883	0.00
275.423	0.00
316.228	0.00
363.078	0.00
416.869	0.00
478.630	0.00
549.541	0.00
630.957	0.00
724.436	0.00
831.764	0.00
954.993	0.00
1096.478	0.00
1258.925	

Size (µm)	Volume In %
1258.925	0.00
1445.440	0.00
1659.587	0.00
1905.461	0.00
2187.762	0.00
2511.886	0.00
2884.032	0.00
3311.311	0.00
3801.894	0.00
4365.158	0.00
5011.872	0.00
5754.399	0.00
6606.934	0.00
7585.776	0.00
8709.636	0.00
10000.000	

Average Size (µm)	Volume in %	Volume Size in %
1.178	0.03	0.035
1.352	0.31	0.419
1.553	1.64	2.546
1.783	3.40	6.061
2.047	5.75	11.767
2.350	8.42	19.787
2.698	11.07	29.867
3.098	13.25	41.042
3.557	14.44	51.356
4.084	14.13	57.700
4.689	12.15	56.965
5.383	8.84	47.586
6.181	5.10	31.521
7.097	1.47	10.432
SUM	100.00	367.083
Average Particle Size (µm)		3.671

APPENDIX E: Particle Size Distribution and Average Particle Size of Cell Flocs Promoted by 5 mg/L LMWC



Size (µm)	Volume In %
0.010	0.00
0.011	0.00
0.013	0.00
0.015	0.00
0.017	0.00
0.020	0.00
0.023	0.00
0.026	0.00
0.030	0.00
0.035	0.00
0.040	0.00
0.046	0.00
0.052	0.00
0.060	0.00
0.069	0.00
0.079	0.00
0.091	0.00
0.105	0.00

Size (µm)	Volume In %
0.105	0.00
0.120	0.00
0.138	0.00
0.158	0.00
0.182	0.00
0.209	0.00
0.240	0.00
0.275	0.00
0.316	0.00
0.363	0.00
0.417	0.00
0.479	0.00
0.550	0.00
0.631	0.00
0.724	0.00
0.832	0.00
0.955	0.00
1.096	0.00

Size (µm)	Volume In %
1.096	0.02
1.259	0.11
1.445	0.41
1.660	0.64
1.905	0.84
2.188	0.95
2.512	0.98
2.884	0.93
3.311	0.83
3.802	0.67
4.365	0.50
5.012	0.35
5.754	0.27
6.607	0.29
7.586	0.46
8.710	0.83
10.000	1.50
11.482	

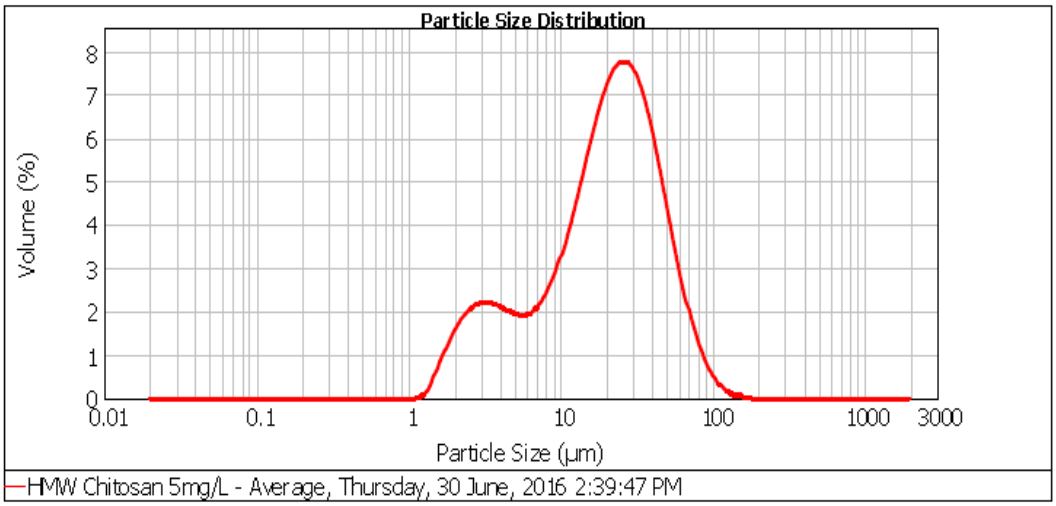
Size (µm)	Volume In %
11.482	2.47
13.183	3.78
15.136	5.32
17.378	6.96
19.953	8.48
22.909	9.65
26.303	10.25
30.200	10.15
34.674	9.34
39.811	7.94
45.709	6.17
52.481	4.33
60.256	2.66
69.183	1.36
79.433	0.50
91.201	0.05
104.713	0.00
120.226	

Size (µm)	Volume In %
120.226	0.00
138.038	0.00
158.489	0.00
181.970	0.00
208.930	0.00
239.883	0.00
275.423	0.00
316.228	0.00
363.078	0.00
416.869	0.00
478.630	0.00
549.541	0.00
630.957	0.00
724.436	0.00
831.764	0.00
954.993	0.00
1096.478	0.00
1258.925	

Size (µm)	Volume In %
1258.925	0.00
1445.440	0.00
1659.587	0.00
1905.461	0.00
2187.762	0.00
2511.886	0.00
2884.032	0.00
3311.311	0.00
3801.894	0.00
4365.158	0.00
5011.872	0.00
5754.399	0.00
6606.934	0.00
7585.776	0.00
8709.636	0.00
10000.000	

Average Size (µm)	Volume in %	Volume Size in %
1.178	0.02	0.024
1.352	0.11	0.149
1.553	0.41	0.637
1.783	0.64	1.141
2.047	0.84	1.719
2.350	0.95	2.233
2.698	0.98	2.644
3.098	0.93	2.881
3.557	0.83	2.952
4.084	0.67	2.736
4.689	0.50	2.344
5.383	0.35	1.884
6.181	0.27	1.669
7.097	0.29	2.058
8.148	0.46	3.748
9.355	0.83	7.765
10.741	1.50	16.112
12.333	2.47	30.461
14.160	3.78	53.523
16.257	5.32	86.487
18.666	6.96	129.912
21.431	8.48	181.735
24.606	9.65	237.448
28.252	10.25	289.578
32.437	10.15	329.236
37.243	9.34	347.845
42.760	7.94	339.514
49.095	6.17	302.916
56.369	4.33	244.076
64.720	2.66	172.154
74.308	1.36	101.059
85.317	0.50	42.659
97.957	0.05	4.898
SUM	99.99	2946.193
Average Particle Size (µm)		29.465

APPENDIX F: Particle Size Distribution and Average Particle Size of Cell Flocs Promoted by Concentration of 5 mg/L HMWC



Size (µm)	Volume In %
0.010	0.00
0.011	0.00
0.013	0.00
0.015	0.00
0.017	0.00
0.020	0.00
0.023	0.00
0.026	0.00
0.030	0.00
0.035	0.00
0.040	0.00
0.046	0.00
0.052	0.00
0.060	0.00
0.069	0.00
0.079	0.00
0.091	0.00
0.105	0.00

Size (µm)	Volume In %
0.105	0.00
0.120	0.00
0.138	0.00
0.158	0.00
0.182	0.00
0.209	0.00
0.240	0.00
0.275	0.00
0.316	0.00
0.363	0.00
0.417	0.00
0.479	0.00
0.550	0.00
0.631	0.00
0.724	0.00
0.832	0.00
0.955	0.00
1.096	0.00

Size (µm)	Volume In %
1.096	0.02
1.259	0.16
1.445	0.66
1.660	1.08
1.905	1.46
2.188	1.74
2.512	1.91
2.884	1.98
3.311	1.97
3.802	1.90
4.365	1.80
5.012	1.73
5.754	1.75
6.607	1.90
7.586	2.20
8.710	2.64
10.000	3.24
11.482	

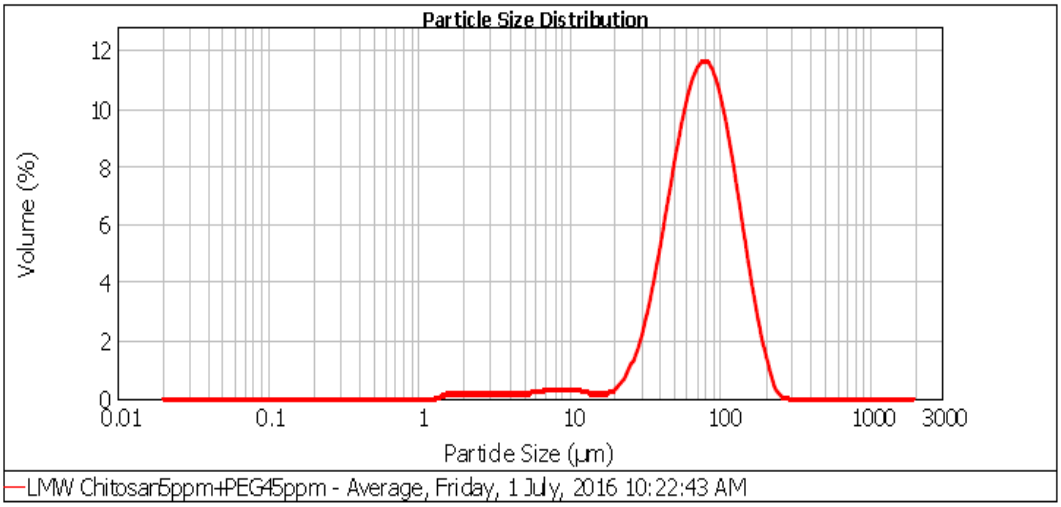
Size (µm)	Volume In %
11.482	3.95
13.183	4.73
15.136	5.49
17.378	6.18
19.953	6.70
22.909	6.98
26.303	6.96
30.200	6.63
34.674	6.01
39.811	5.16
45.709	4.18
52.481	3.19
60.256	2.27
69.183	1.52
79.433	0.94
91.201	0.53
104.713	0.28
120.226	

Size (µm)	Volume In %
120.226	0.11
138.038	0.05
158.489	0.02
181.970	0.00
208.930	0.00
239.883	0.00
275.423	0.00
316.228	0.00
363.078	0.00
416.869	0.00
478.630	0.00
549.541	0.00
630.957	0.00
724.436	0.00
831.764	0.00
954.993	0.00
1096.478	0.00
1258.925	

Size (µm)	Volume In %
1258.925	0.00
1445.440	0.00
1659.587	0.00
1905.461	0.00
2187.762	0.00
2511.886	0.00
2884.032	0.00
3311.311	0.00
3801.894	0.00
4365.158	0.00
5011.872	0.00
5754.399	0.00
6606.934	0.00
7585.776	0.00
8709.636	0.00
10000.000	

Average Size (µm)	Volume in %	Volume Size in %
1.178	0.02	0.024
1.352	0.16	0.216
1.553	0.66	1.025
1.783	1.08	1.925
2.047	1.46	2.988
2.350	1.74	4.089
2.698	1.91	5.153
3.098	1.98	6.133
3.557	1.97	7.006
4.084	1.90	7.759
4.689	1.80	8.439
5.383	1.73	9.313
6.181	1.75	10.816
7.097	1.90	13.483
8.148	2.20	17.926
9.355	2.64	24.697
10.741	3.24	34.801
12.333	3.95	48.713
14.160	4.73	66.974
16.257	5.49	89.251
18.666	6.18	115.353
21.431	6.70	143.588
24.606	6.98	171.750
28.252	6.96	196.630
32.437	6.63	215.057
37.243	6.01	223.827
42.760	5.16	220.642
49.095	4.18	205.217
56.369	3.19	179.816
64.720	2.27	146.913
74.308	1.52	112.948
85.317	0.94	80.198
97.957	0.53	51.917
112.470	0.28	31.491
129.132	0.11	14.205
148.264	0.05	7.413
170.230	0.02	3.405
SUM	100.02	2481.101
Average Particle Size (µm)		24.806

APPENDIX G: Particle Size Distribution and Average Particle Size of Cell Flocs Promoted by 5 mg/L LMWC and 45 mg/L PEG



Size (µm)	Volume In %
0.010	0.00
0.011	0.00
0.013	0.00
0.015	0.00
0.017	0.00
0.020	0.00
0.023	0.00
0.026	0.00
0.030	0.00
0.035	0.00
0.040	0.00
0.046	0.00
0.052	0.00
0.060	0.00
0.069	0.00
0.079	0.00
0.091	0.00
0.105	0.00

Size (µm)	Volume In %
0.105	0.00
0.120	0.00
0.138	0.00
0.158	0.00
0.182	0.00
0.209	0.00
0.240	0.00
0.275	0.00
0.316	0.00
0.363	0.00
0.417	0.00
0.479	0.00
0.550	0.00
0.631	0.00
0.724	0.00
0.832	0.00
0.955	0.00
1.096	0.00

Size (µm)	Volume In %
1.096	0.00
1.259	0.02
1.445	0.12
1.660	0.15
1.905	0.17
2.188	0.17
2.512	0.16
2.884	0.14
3.311	0.13
3.802	0.13
4.365	0.14
5.012	0.17
5.754	0.20
6.607	0.24
7.586	0.28
8.710	0.29
10.000	0.27
11.482	

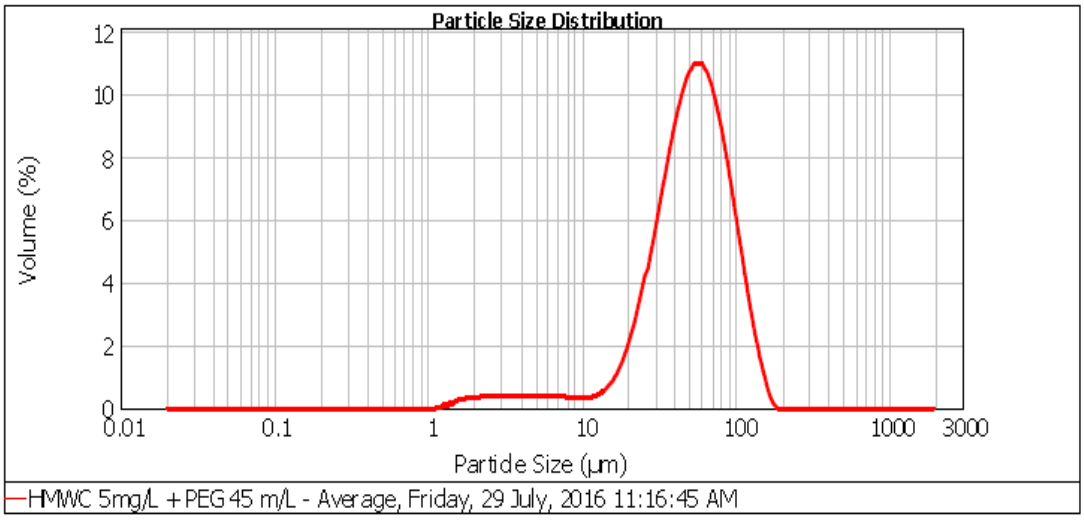
Size (µm)	Volume In %
11.482	0.22
13.183	0.16
15.136	0.12
17.378	0.17
19.953	0.36
22.909	0.78
26.303	1.50
30.200	2.54
34.674	3.89
39.811	5.45
45.709	7.09
52.481	8.60
60.256	9.78
69.183	10.41
79.433	10.40
91.201	9.73
104.713	8.46
120.226	

Size (µm)	Volume In %
120.226	6.81
138.038	4.97
158.489	3.24
181.970	1.77
208.930	0.69
239.883	0.08
275.423	0.00
316.228	0.00
363.078	0.00
416.869	0.00
478.630	0.00
549.541	0.00
630.957	0.00
724.436	0.00
831.764	0.00
954.993	0.00
1096.478	0.00
1258.925	

Size (µm)	Volume In %
1258.925	0.00
1445.440	0.00
1659.587	0.00
1905.461	0.00
2187.762	0.00
2511.886	0.00
2884.032	0.00
3311.311	0.00
3801.894	0.00
4365.158	0.00
5011.872	0.00
5754.399	0.00
6606.934	0.00
7585.776	0.00
8709.636	0.00
10000.000	

Average Size (µm)	Volume in %	Volume Size in %
1.178	0.00	0.000
1.352	0.02	0.027
1.553	0.12	0.186
1.783	0.15	0.267
2.047	0.17	0.348
2.350	0.17	0.400
2.698	0.16	0.432
3.098	0.14	0.434
3.557	0.13	0.462
4.084	0.13	0.531
4.689	0.14	0.656
5.383	0.17	0.915
6.181	0.20	1.236
7.097	0.24	1.703
8.148	0.28	2.281
9.355	0.29	2.713
10.741	0.27	2.900
12.333	0.22	2.713
14.160	0.16	2.266
16.257	0.12	1.951
18.666	0.17	3.173
21.431	0.36	7.715
24.606	0.78	19.193
28.252	1.50	42.377
32.437	2.54	82.390
37.243	3.89	144.873
42.760	5.45	233.042
49.095	7.09	348.084
56.369	8.60	484.769
64.720	9.78	632.957
74.308	10.41	773.546
85.317	10.40	887.297
97.957	9.73	953.122
112.470	8.46	951.492
129.132	6.81	879.389
148.264	4.97	736.870
170.230	3.24	551.544
195.450	1.77	345.947
224.407	0.69	154.840
257.653	0.08	20.612
SUM	100	8275.652
Average Particle Size (µm)		82.757

APPENDIX H: Particle Size Distribution and Average Particle Size of Cell Flocs Promoted by 5 mg/L HMWC and 45 mg/L PEG



Size (µm)	Volume In %
0.010	0.00
0.011	0.00
0.013	0.00
0.015	0.00
0.017	0.00
0.020	0.00
0.023	0.00
0.026	0.00
0.030	0.00
0.035	0.00
0.040	0.00
0.046	0.00
0.052	0.00
0.060	0.00
0.069	0.00
0.079	0.00
0.091	0.00
0.105	0.00

Size (µm)	Volume In %
0.105	0.00
0.120	0.00
0.138	0.00
0.158	0.00
0.182	0.00
0.209	0.00
0.240	0.00
0.275	0.00
0.316	0.00
0.363	0.00
0.417	0.00
0.479	0.00
0.550	0.00
0.631	0.00
0.724	0.00
0.832	0.00
0.955	0.00
1.096	0.00

Size (µm)	Volume In %
1.096	0.02
1.259	0.09
1.445	0.21
1.660	0.27
1.905	0.31
2.188	0.33
2.512	0.34
2.884	0.33
3.311	0.33
3.802	0.33
4.365	0.34
5.012	0.34
5.754	0.35
6.607	0.34
7.586	0.32
8.710	0.30
10.000	0.30
11.482	

Size (µm)	Volume In %
11.482	0.35
13.183	0.51
15.136	0.82
17.378	1.36
19.953	2.16
22.909	3.24
26.303	4.55
30.200	6.00
34.674	7.44
39.811	8.69
45.709	9.57
52.481	9.93
60.256	9.69
69.183	8.86
79.433	7.54
91.201	5.93
104.713	4.22
120.226	

Size (µm)	Volume In %
120.226	2.62
138.038	1.30
158.489	0.33
181.970	0.00
208.930	0.00
239.883	0.00
275.423	0.00
316.228	0.00
363.078	0.00
416.869	0.00
478.630	0.00
549.541	0.00
630.957	0.00
724.436	0.00
831.764	0.00
954.993	0.00
1096.478	0.00
1258.925	

Size (µm)	Volume In %
1258.925	0.00
1445.440	0.00
1659.587	0.00
1905.461	0.00
2187.762	0.00
2511.886	0.00
2884.032	0.00
3311.311	0.00
3801.894	0.00
4365.158	0.00
5011.872	0.00
5754.399	0.00
6606.934	0.00
7585.776	0.00
8709.636	0.00
10000.000	

Average Size (µm)	Volume in %	Volume Size in %
1.178	0.02	0.024
1.352	0.09	0.122
1.553	0.21	0.326
1.783	0.27	0.481
2.047	0.31	0.634
2.350	0.33	0.776
2.698	0.34	0.917
3.098	0.33	1.022
3.557	0.33	1.174
4.084	0.33	1.348
4.689	0.34	1.594
5.383	0.34	1.830
6.181	0.35	2.163
7.097	0.34	2.413
8.148	0.32	2.607
9.355	0.30	2.807
10.741	0.30	3.222
12.333	0.35	4.316
14.160	0.51	7.221
16.257	0.82	13.331
18.666	1.36	25.385
21.431	2.16	46.291
24.606	3.24	79.723
28.252	4.55	128.544
32.437	6.00	194.622
37.243	7.44	277.084
42.760	8.69	371.584
49.095	9.57	469.839
56.369	9.93	559.739
64.720	9.69	627.132
74.308	8.86	658.369
85.317	7.54	643.290
97.957	5.93	580.885
112.470	4.22	474.621
129.132	2.62	338.326
148.264	1.30	192.743
170.230	0.33	56.176
SUM	99.96	5772.682
Average Particle Size (µm)		57.750