Harvesting methods of Microalgae

By

Nurul Umi binti Sha'ari

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DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

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Student

Name: Nurul Umi binti Sha'ari

Date: 14 January 2018

I certify that the report of this final year project entitled “Harvesting Methods of Freshwater Microalgae” by Nurul Umi binti Sha'ari, matric number F14A032 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Agriculture Technology) with Honours, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan.

Approved by:

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Supervisor

Name: Associate Professor Dr Lee Seong Wei

Date: 14 January 2018

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HARVESTING METHODS OF MICROALGAE

ABSTRACT

The field studies of microalgae have increased in the last ten years due to the wide range of applications related to these aquatic microorganisms in the industry. Microalgae are an important source of oils and other biomolecules that can be used in the production of biofuels and high-valued products such as biodiesel, bioethanol, biogas and bio hydrogen, fish feed, animal feed, human food supplements and cosmetic products. However, the use of microalgae in these green processes is still not economically viable. One of the main costs associated to microalgal production is related to the harvesting process, as it usually accounts for about 20–30% of total cost. Harvesting microalgae basically is a process of removing the microscopic plants from the medium they grow and concentrate them into a paste. Therefore, this study highlighted on three harvesting method applied to microalgae which are bioflocculation, centrifugation and filtration, and identified the most effective method of harvesting by comparing the mean value of dry cell weight (DCW) determined from the separation of microalgal biomass from the culture medium. Among the three method of harvesting, filtration obtained the highest value of DCW which indicated as the most effective method for harvesting microalgae in this research. Even though, the weight of biomass obtained through the both processes of filtration and centrifugation were high, harvesting by filtration was considered as most effective in term of low energy consumption and cost.

Keywords: Bioflocculation, centrifugation, filtration, dry cell weight

PROSES PENUAIAN MIKROALGA

ABSTRAK

Kajian dalam bidang mikroalga telah meningkat dalam sepuluh tahun terakhir ini disebabkan oleh aplikasinya yang meluas dalam industri. Mikroalga adalah sumber terpenting dalam pemprosesan minyak dan biomolekul lain yang boleh digunakan dalam pengeluaran biofuel dan produk bernilai tinggi seperti biodiesel, bioethanol, biogas dan bio hidrogen, makanan ikan, makanan haiwan, makanan manusia dan produk kosmetik. Walau bagaimanapun, penggunaan mikroalga dalam proses hijau masih tidak berdaya maju dari segi ekonomi. Salah satu daripada kos utama yang berkaitan dengan pengeluaran mikroalgal adalah berkaitan dengan proses penuaian, kerana ia biasanya menyumbang kira-kira 20-30% dari jumlah kos. Pengambilan mikroalga pada dasarnya adalah proses menuai tumbuhan mikroskopik ini dari medium pembiakan lalu mengumpulkan menjadi pes. Oleh itu, kajian ini mengfokuskan kepada tiga kaedah penuaian yang digunakan untuk microalga iaitu bioflokulasi, sentrigugasi dan penapisan, dan mengenal pasti cara penuaian yang paling berkesan dengan membandingkan nilai purata berat sel kering yang diperolehi daripada pemisahan mikroalga biomas dari medium pembiakan. Antara tiga cara penuaian yang dinyatakan, kaedah penapisan memperolehi nilai purata berat sel kering yang tertinggi seterusnya meletakkan ia sebagai kaedah paling berkesan dalam kajian penuaian mikroalga ini. Walaupun, berat biomas yang diperolehi melalui kedua-dua proses penapisan dan sentrifugasi adalah tinggi, namun penuaian melalui kaedah penapisan di anggap paling berkesan daripada segi penggunaan tenaga dan belanja yang rendah.

Kata kunci: Bioflokulasi, sentrifugasi, penapisan, berat sel kering

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

DCW	Dry Cell Weight
TSB	Tryptic Soy Broth
TQD	Treated Quail Dung
FSP	Fermented Soy Protein
NPK	Nitrogen, Phosphorus, Potassium
MBF-UHF	Multi Band Repeater- Ultra High Frequency
B100	Bioflocculation :100 µL
B200	Bioflocculation :200 µL
B300	Bioflocculation :300 µL
C6000	Centrifugation : 6000 rpm
C7000	Centrifugation : 7000 rpm
C8000	Centrifugation : 8000 rpm

LIST OF SYMBOLS

min	minute
h	hour
mL	milliliter
µL	microliter
µm	micrometer
g	gram
mg	milligram
rpm	Revolution per minute

CHAPTER 1

INTRODUCTION

1.1 Research Background

There are several methods of algae harvesting applied in the industry such as biological method and physical method. From this study, biological method been applied was bioflocculation, while for physical methods were by filtration and centrifugation.

Okaiyeto in previous study stated that bioflocculation is a clumping together of fine organic particle by the action of certain microorganism such as bacteria and algae (Okaiyeto, Nwodo, Mabinya, & Okoh, 2013). In bioflocculation, enzyme extracted from bacteria *Bacillus licheniformis* is used as bioflocculant instead of using other common commercial flocculants such as chemical and organic flocculant which are more cost intensive and less feasible (Okaiyeto, Nwodo, Mabinya, & Okoh, 2013). This method is believed to supply significantly dewatering cost since less or no vitality utilization is required compared with other commercial methods generally applied in the industry (Ndikubwimana, Zeng, Murwanashyaka &Manirafasha,2016). Normally occurring microbial flocculants have been utilized to gather microalgae for aquaculture and biodiesel production as a result of their high harvesting efficiency, and biodegradability. The event of bioflocculation was observed in natural blossoms of microalgae happening in lakes, ponds or streams, and was most likely caused by extracellular polymeric substances (EPS) of phytoplankton in the water bodies (Zhou, Ruan, & Wang, 2016). Specialists in the previous years have exploited such bioflocculating property and studied its relevance in microalgae harvesting, and have extended the

scope of flocculating microorganisms from algae to bacteria and fungi through advantageous interaction (Zhou, Ruan,& Wang, 2016).Subsequently, bioflocculation is a dynamic procedure resulting from the synthesis of extracellular polymers by flocculant creating microorganisms. In biofloc framework, bioflocculation is the most broadly utilized process for treating the waste water from aquaculture industries as it is a viable and advantageous approach to take out suspended solids, colloids and cell debris. Bioflocculation has masive advantages, for example, biodegradability, less of optional contamination from sudden corruption and being safe to human (Hattab,Ghaly,&Hammouda, 2015). Bioflocculant is an optional metabolite discharged by microorganisms including bacteria, fungi and algae have been accounted for to comprise assortment chemical compositions, for example, polysaccharide, protein, glycoprotein, and nucleic acids (Okaiyeto, Nwodo, Okoli, Mabinya, & Okoh, 2016). During this study, three different dosage of the bioflocculant were added to the microalgae media with each of the medium were different type of fertilizers, and then the harvesting efficiency and weight of the DCW of each medium collected are observed and recorded.

Centrifugation is a conventional method which has been broadly utilized as a part of industrial and research for over a century as the earlier studies using centrifugation are from the first 1800s were recorded (Mäkeläinen & Heikkinen, n.d.). This strategy incorporates the utilization of the centrifugal force for the sedimentation of particles and partition of two distinctive insoluble particles by fast speed rotation. Mäkeläinen had mentioned, that centrifuges those are utilized in mechanical setting, can be sorted into two distinct classes which are sedimentation and filter centrifuges which depends on the general operation standards of the centrifuges. However the batch feeding and continuous type of centrifuge are additionally thought about in the classification. This method known as the best industrial method in getting high yielding

value by separating microalgae from it medium and were generally practiced in laboratory research in organic chemistry, science and in medicine. Recent research applications rely upon isolation of cells, subcellular organelles, and macromolecules, frequently in exceptional returns (Makeläinen and Heikkinen, n.d.). Recently, centrifuges are ordinarily utilized as a part of an assortment of disciplines ranging from vast scale business applications to lab scale scientific research including the utilization of the mineral, petrochemical, compound, therapeutic, pharmaceutical, civil/modern waste, dairy, nourishment, polymer, vitality and rural enterprises which appear to be nearly as various as the applications themselves .Besides, centrifuges are utilized all through many manufacturing industry e.g. agro-based and food industry, pharmaceutical/biotechnology, ecological industries and chemical industries (Makeläinen and Heikkinen, n.d.). In this study, the microalgae media were centrifuged with three different speed. The sediments of algal formed at the bottom were then observe and DCW weight were measured and recorded. Although the cost production of this method is higher, the efficiency of the flocculation and biomass collected is greater.

While filtration or otherwise called as membrane filtration is a common method for harvesting microalgae generally by using varieties of filters with various sort of materials and pore size, on which the green growth accumulate forming thick paste and the rest of the algae media are let to go through the filter and normally used to separate liquid solid mixture (Shah, Deokar, Patel, Panchal, & Mehta, 2014). Filtration process might be continuous or discontinuous and the systems can be categorized as microfiltration (pore size of 0.1– 10 μm), macro filtration (pore size of >10 μm), ultrafiltration (pore size of 0.02– 2 μm), and invert osmosis (pore size of <0.001 μm) (Huang, Chen, & Liu, 2012). Filtration aided by force or optimum force net energy for generally littler size algal cells can be abstemiously sluggish and tedious particularly if vast volumes of microalgae suspension are to be prepared (Shah, Deokar, Patel,

Panchal, & Mehta, 2014). Technology of membrane filtrations are being developed the world over for various applications. Some of these are city and industrial wastewater treatment, food processing wastewater, slaughterhouses wastewater and landfill leachates. The membrane can repel microorganisms and some of the dissolved organic matter found in drinking water. Membrane can supplant the post-treatment process that is currently being utilized as a part of conventional drinking water treatment. This developing innovation has a few advantages, for example, potential consistent separation and low vitality utilization. The developing applications and increment in new trends far and wide around the world regarding membrane technologies is an energizing and open-finished topic (Shah, Deokar, Patel, Panchal, & Mehta, 2014). Some of the primary featured part of membrane filtration is the material, fouling, synthesis and characterization. In this method, the characterization of the porous membrane is the most important. Three different pore size of filter cloths were used in this harvesting method. Algae paste formed after the media pass through the filters were collected and the weight were recorded.

Algae harvesting is a process of gathering the algae that had been cultured and cultivated in a medium. The harvesting of microalgae require some distinctive method to be done as the nature of algae and the various habitat that it grows in, for example, either from freshwater or marine. Thus, this research focused on the harvesting methods of freshwater microalgae which are by bioflocculation, centrifugation and filtration. The final result obtained by determining the DCW (dry cell weight) of the algal biomass, through each of these methods were then analyze to identify which are the best one to harvest the microalgae. This research is important as to identify the best method that may be one of the promising low cost with high efficiency harvesting technology that can be develop and apply in the industry which is comparable with other commercial technology. Among these three method of harvesting, filtration is the most effective method for harvesting microalgae

1.2 Problem Statement

Harvesting microalgae is a challenging procedure because of the extremely dilute culture (<1.0 g of solids) and normally tiny size of microalgae with a diameter around 3 – 30 μm which prompt to energy intensive process when huge volume of algal biomass should be dealt with (Lemos, Vargas, Mariano, Kava, & Ordonez, 2016). Consequently, it is hard to find the most ideal approach to harvest the microalgae with minimal cost, time consuming and energy intensive techniques. Most of the currently used harvesting techniques have several drawbacks, such as high cost, flocculant toxicity, or non-feasible of scale-up, which impact the cost and quality of products, As harvesting expense may itself contribute up to 33% of the biomass generation cost, considerable measures research and development are expected to build up a cost-and energy effective process for the harvesting of algae through the alternative method of bioflocculation, centrifugation and filtration which are the most widely recognized strategies utilized in the industry.

However, there is still lack of study on finding an effective method of harvesting this photosynthetic microorganisms, with intense cost of operation, man power and maintenance while maintaining a high achievement of biomass concentration which can be applied in both small and large scale harvesting industry.

1.3 Objectives of study

The objectives of this research study was to study the most effective method of harvesting fresh water microalgae which is able to obtain high value of dry cell weight (DCW).

1.4 Scope of Study

Harvesting of fresh water microalgae by using the method of bioflocculation, centrifugation and filtration.

1.5 Significance of Study

The findings of the research may improve the knowledge and facilitate understanding about the massive advantages of harvesting microalgae through microbial bioflocculation, centrifugation and filtration to the industry. For bioflocculation, an experiment was carried out by using different three dosage of bioflocculation extracted from bacteria *Bacillus licheniformis*. This bioflocculant acted as an accumulator between the algal cell and flocs were formed as a final result of the mechanism. While centrifugation is a common industrial application used to separate different substance in a mixture of solution aiding by different rotary speed. Three different speeds were conducted in the centrifugation process of microalgae during this study. Besides, filtration is method of harvesting microalgae by separating the dilute algal and it medium solution with the aided of membrane. This research were using three different pore size of macro filtration membrane and added with small pressure to facilitate the process of passing through of the algal medium. Final result for each of the method were recorded by determining the dry cell weight (DCW) of the biomass.

This study will be the reference to the future research and may lead to the improvement and development of harvesting method in Malaysia especially in aquaculture industry and others which are related to this field of study, in more affective and feasible ways.

CHAPTER 2

LITERATURE REVIEW

2.1 Harvesting Freshwater Microalgae

Novis in previous research stated that freshwater microalgae incorporate an extensive variety of living beings that buoy in the water or develop on submerged surfaces and can photosynthesize (utilizing sunlight, vitality, CO and water to manufacture organic matter + O_2). They incorporate individuals from various diverse kingdoms including the plant kingdom (the algae and red algae), the bacteria (blue green algae), protozoa (single-celled swimming groups) and numerous individuals from the chromista (for instance, diatoms) (Novis n.d.) One of the fundamental factors that impact the harvesting strategies is the strain and types of microalgae. Algae with various qualities require diverse harvesting methods.

Harvesting microalgae is a sequence of procedures of dewatering culture containing algal growth, and expanding the solids substance of the microalgae from <1.0 % to a consistency of up to 20 % solids, depending upon the downstream preparing necessities for transformation to fuel (Singh, Shukla, & Das, 2013). The term harvesting likewise refers to the concentration of dilute microalga culture suspension to sluggish or paste containing 5 % to, at least 25 %, add up to total suspended solids (TSS) (Vandamme, Foubert, & Muylaert, 2013). This slurry can be acquired in either a one stage or two stage harvesting process. Resulting handling of the algal paste relies upon the concentration of the algal paste. Increased product concentration diminishes the cost of extraction and purification, and also the effective unit cost of biomass. Concentration of algal paste fundamentally impacts downstream procedures, including

drying. Microalgae are particles characterized as colloidal particle in suspension. The action of electric repulsive between algal cells and cell interaction with the encompassing water give constancy to the algal suspension. Algal cells are typically described as adversely charged surfaces where the power of charge is an element of the species, ionic quality, and pH of the development media. These surface charges are useful in the microalgae culture since they facilitate the cells in the water section so they do not settle to the bottom of the lake, especially in parts of the lake where the water velocity is low. In any case, the charges represent a challenge to the dewatering procedure since they dispose of the choice of utilizing a basic settling tank or lake for harvesting. Harvesting and dewatering procedures can be isolated into two classifications which are those in which the dewatering is performed straightforwardly on the algae culture, and those including accumulation of the green growth into naturally visible masses to encourage the dewatering procedure (Singh et al., 2013).

2.1.1 Bioflocculation

Bioflocculation refers to the naturally induced flocculation due to the secreted biopolymers by the microbial cells which the stability of microalgae cell suspensions is consequent to the surface charge of the cells which being starts mostly from the existence of carboxylic (COOH), amine (NH^3) and phosphoryl (POH) groups on the cell surface (Ndikubwimana, Zeng, He, & Xiou, 2015). While Yuyan et al. in 2010 mentioned bioflocculant as a distinctive macromolecules secreted by microorganism such as bacteria, algae, bacteria and fungi those have been studied to produce bioflocculant, that will induce the bacteria, cells, solid and colloidal particles in a liquid medium to flocculate and form sedimentation (Xiong, Wang, Yu, Li,& Chen, 2010).Spontaneous flocculation assumed to be caused by extracellular polymer substances in the medium is called bioflocculatio. Bioflocculant is the most popular among other flocculants cause of several advantages such as biodegradability,

biocompatibility, harmless and environmentally friendly differs from either organic or inorganic flocculants such as polyacrylamide which can lead to toxicity and might be harmful to the environment cause of the intermediate degradation (Hattab, Ghaly & Hammouda, 2015).

Recently, bioflocculant has widely attracted and obtained attention from researchers around the world as this technology considered as cheap and easier in term of set up which can also act an agent that facilitates the accumulation of microorganism to form bioflocs. Poly-γ-glutamic acid (γ-PGA) broth was prove to be an excellent bioflocculant of microalgae (Ndikubwimana et al., 2015). The method of using specific bacteria to induce the biological flocculation of microalgae has been successfully applied especially for waste water treatment processes and one of another strategy to harvest microalgae with less cost and energy intensive. Currently, an innovative, economic and environment friendly microalgae harvesting from bacterial bioflocculant produced by *Bacillus licheniformis* CGMCC 2876 with active constituent of poly-γ-glutamic acid, γ- PGA was identified to be among the best bioflocculant for harvesting of microalgae (Ndikubwimana, Zeng, Murwanashyaka, Manirafsha et al.,2016). While the bioflocculant used is extracted from bacteria *Bacillus licheniformis* as mentioned by Carla Pinto in previous research in 2012, is a saprophytic bacterium which has an ability of producing and secreting sufficient amount of hydrolytic enzymes and make it proficient to grow on a great diversity of nutrient source, and has potential to degenerate several substrates. These abilities, make *Bacillus licheniformis* as remarkable economic importance in industry for a long time such as the production of antibiotic (Pinto, 2012).

The bioflocculants in the form of supernatant were obtained by storing in bottles capped, then centrifuged at 5000 rpm for 30 min and powder purified from the same volume supernatant, correspondingly. The flocculating activity of the supernatant and

the powder were measured at specific time to evaluate the stability and effectiveness of the bioflocculants. In order to determine the flocculating activity of the, 2mL sample was exchanged with the powder purified from the same volume of supernatant. The supernatant and powder were stored at 258C and initial pH (Ji, Zhang, Li , Z,Li et al., 2010).Under an optimal conditions, the maximum flocculating activity is usually attained at the optimal bioflocculant dosage . The flocculating activity of purified MBF-UFH was examined in a range of 0.01–0.5 mg/mL. The flocculating activity of 61.5% was achieved at 0.01 mg/mL, and a further increasing in MBF-UFH dosage resulted into a steady fall in flocculating activity. Nevertheless, the optimum bioflocculant dosage range for effective flocculation efficiency of over 90% was observed between 0.1 and 0.3 mg/mL, with the highest flocculating activity of 92.6% attained at 0.3 mg/mL (Hende, Vervaeren, Desmet & Boon, 2011). However, there was no significant rise in the flocculating activity of MBF-UFH when the dosage was increased from 0.1 up to 0.3 mg/mL. Even though, the flocculating activity of MBF-UFH was small at 0.01 mg/mL (61.5%) compared to the flocculating activity observed in the optimum range of 0.1–0.3 mg/mL which is above 90 % (Okaiyeto, Nwodo, Mabinya, Okoli & Okoh, 2015). At a smaller dosage, MBF-UFH was relatively small to destabilize the negative charge of the kaolin clay particles, and the excess kaolin particles re-stabilized and increased the turbidity of the suspension; lower flocculating activity was noted in comparison to the flocculating rate observed at 0.1 mg/mL. This showed that the bridging effect of MBF-UFH was lower at 0.01 mg/mL compared to when it was at a higher dosage. On the contrary, the flocculating activity slightly decreased to 87.7% on increasing MBF-UFH dosage to 0.5 mg/mL compared with the flocculating activity of over 90% observed at an optimum dosage range between 0.1 and 0.3 mg/mL. This observation was in agreement with those reported elsewhere. The decrease in flocculating activity of MBF-UFH observed at 0.5 mg/mL might be due to the excess addition of the negatively-charged MBF-UFH, generating strong repulsive forces between the kaolin clay particles and the bioflocculant. These processes restabilized the suspended particles,

increasing the viscosity of the suspension, blocking the adsorption sites and remarkably decrease the formation of floc. These findings are consistence with previous studies reported by Elkady et al. and Zheng et al. in 2010. It has been extensively documented that a lower concentration of bioflocculants with a high flocculating efficiency will lead to treatment cost reduction. Besides, the information of the dosage required for bioflocculation is essential for future research inwater treatment and other industrial application (Ji, X, Zhang, Z. Li et al., 2010).

Makapela in the previous study also found that the flocculating activity was above 90% within a dosage range of 0.1–0.5 mg/mL, and the highest percentage of flocculating activity (96.5%) was achieved at an optimum bioflocculant dosage of 0.1 mg/mL. While the flocculating activity was nearly linear between 0.2 and 0.5 mg/mL, presenting that there was no major difference in the flocculating activity as the dosage of the bioflocculant increased. The outcomes in this study revealed that the bioflocculant produced by bacterial strain exhibited high flocculating activity at very low dosage (0.1 mg/mL) which could lowering the cost for industrial scale applications. A lower dosage of bioflocculants with high flocculating activity will definitely decrease treatment cost (Makapela, Okaiyeto, Ntozonke, & Nwodo, 2016). From the study of Okaiyato also, stated that the flocs and the growth medium were detached after flocculation for recycle purpose. The pH of remaining flocculated medium, was accustomed back to the pH before flocculation by adding the necessary amount of 1M NaOH. Microalgae then were cultivated in the fresh medium and the flocculated medium. For the growth phase observation, the biomass concentration was studied (Makapela et al., 2016).

According to the study from Mallick et al in 2016, the samples of microalgae were diluted in a 10×10×45 mm polystyrene cuvette using filter sterilized tap water for the freshwater microalgae in order to achieve the value of optical density. Microalgae

suspension from the culture media were taken and diluted in a cuvette. After slow mixing, the suspension in the cuvette was left to settle at 27° C in the dark in a spectrophotometer. The temperature and pH of all samples were kept constant at 27°C and pH 7 by measuring in the beginning and at the end of the sedimentation period. The recovery percentage is measured in the top part of the cuvette, where individual cells and formed flocs independently sink. The effect of ratio between autoflocculating agent and target microalgae in bioflocculation was studied with prominence on the recovery, the rate of sedimentation and energy for harvesting the specific microalgae. Application of bioflocculation at a ratio of 0.25, followed by centrifugation reduces the energy demand for harvesting of the target microalgae (Mallick, Bagchi, Koley, & Singh, 2016).

Commonly, bioflocculation of microalgae suspensions can be classified to three main mechanisms which are the charge neutralization, bridging and patching. All of these mechanisms able to act individually or in combination. The positively charged polymers able bind partially or completely to the negatively charged microalgae cells. When the polymers bind partially, the unoccupied part of the polymers able to bind to other microalgae cells, thus bridging them and causing in a linkage of polymers and the microalgae cells. While if the polymers bind onto the microalgae cells totally, the charge of the microalgae cells surface will locally inverted, resulting in patches of opposite charge on the microalgae cells surface, and consequently the microalgae cells connect with each other through patches of opposite charge, then causing them to flocculate (Ndikubwimana, Zeng, He *et al.*, 2015)

2.1.2 Centrifugation

Centrifugation is widely utilized and important separation technique in many industrial fields such as food, pharmaceutical, biotechnical and chemical industries which followed the principal of centrifuges based on the force that facilitates the

separation of different components in the mixture based on their sedimentation features (Collection, 2017). The applied force on particles depends on the rotation speed and the radius of centrifuge rotor. Thus, heavier and bigger components were migrate outward from the axis of rotation caused from the centrifugal force. Centrifugation is a separation technique where different components of mixture or substance were separated based on their density or particle size. The separation of different substances is based on centrifugal force that is produced by high speed rotation. The heavier or denser components were directed away from the axis of rotation while the lighter components to be move and transfer towards it as the result of centrifugal force (Collection, 2017).

Centrifugation is the fastest harvesting method, but also the most expensive due to its high energy consumption, which restrict its application to only high valued products with abundance quantities, such as highly unsaturated fatty acids, pharmaceuticals and other products (Barros, Gonçalves, Simões, & Pires, 2015). Centrifuges are great to harvest most of microalgae. Some are even effective as one step separation procedure, while others require a pre concentrated algal slurry. However, there are proofs that the exposure of microalgaecells to high gravitational and shear forces results in cell structure damage. Normally, centrifuges are set to maximize capture efficiency. However, cost-effective microalgae harvesting may not correspond with the maximum capture efficiency (Beveridge & Canada, 2000). To achieve greater harvesting efficiencies, longer retention times in the bowl are needed to allow the sedimentation due to the small size of these microalgae cells. While high capture efficiency which is slower flow rates needed more energy per volume of culture, lower recoveries were counterbalance by the increase in the processed volume. This low energy conditions result in a decrease in whole cost per litter of produced oil. If coagulation/flocculation is applied prior to centrifugation, its high energy consumption might be lowered, as it reduces the volume to be processed in 65% which would

additionally resulted in a 3.8 fold increase in final algal concentration from this combination (Barros, Gonçalves, Simões, & Pires, 2015)

The separation of the microalgae from the suspension through the centrifugal force were depended on the retention time of the slurry, settling behaviors and depth of the microalgae in the centrifuge. The common types of centrifuges used include bowl centrifuge, disc stack and hydro cyclones. While Heasman et al. evaluated the cell recovery efficiency of nine different microalgae species using a disk stack centrifuge and noted a recovery efficiency greater than 95% at a force of 13,000g (Hattab, Ghaly & Hammoudae , 2015). They also had investigated that the recovery efficiency reduced with a decrease in the gravitational force to 60% and 40% at gravitational forces of 6,000g and 1,300g, respectively. Sim et al. noted a 90 % microalgae removal efficiency using a disc stake type of centrifuge. Hattab achieved an 18% microalgae concentration using a disc centrifuge (Hattab et al., 2015). Mackay used a disc centrifuge operating at a force of 4,000-15,000g for a biomass suspension containing 0.2-20% v/v algae cells. Chojnacka et al. harvested other species of microalgae which is the *Spirulina sp.* By using a disc type centrifuge operating at 6,000 rpm for 5 min. The microalgae cells were harvested using a Beckman Avanti J-251 high speed centrifuge at 8000 rpm for 10 min (Al-lwayzy, Yusaf, Al-juboori, & Road, 2014).

2.1.3 Filtration

Filtration or known as membrane filtration had been testified as an interesting low cost technique for microalgae harvesting, either in a separated process or in a coupled process as in a membrane photo bioreactor. However, the filtration performance can still be improved if the membrane fouling problem could be properly managed. There are two types of membranes that are classified according to characteristics: porous and nonporous membranes. Porous membranes contained

pores, whose measurements and distribution determine the separation ability of the membrane. Other factors that affect separation processes of microalgae biomass form this method are the concentration polarity, membrane fouling and pore geometry. Commonly found porous membranes applied in the harvesting industry are microfiltration and ultrafiltration membranes (Azizo,Wirzal, Billad et al., 2017). Ionic membranes are porous membranes whose characterize by the charged group present on the membrane that determine the separation process, in addition to pore size and distribution. These membranes are frequently used in electric driven process but can also be used in nano filtration, microfiltration and ultrafiltration processes. On the other side, nonporous membranes have the ability in performing molecular level separations. The factor that affect the separation process are the chemical which is mainly the permeability and physical properties of both the membrane and the permeability, and also the interaction between these two (Herrera et al*.,* 2013).This research were focusing on the filtration method characterized by the porous membrane of macro filtration membrane.

In all filtration a pressure of force must be applied through the medium in order to force the fluid to pass through while retaining the required microalgae cell (Wilson, 2012). Filtration is mainly a dewatering means and it is normally applied following coagulation/flocculation to improve harvesting efficiency. Its application requires the maintenance of a pressure drop across the system to force fluid flow through a membrane. In this process, microalgae deposits on the filtration membrane tend to grow thicker throughout the process, aggregate resistance and reducing filtration flux upon a continuous pressure drop (Shelef et al*.,* 1984). This phenomenon which is called as fouling or clogging indicates the main drawback associated to filtration methods, increasing their operational costs (Shah, Deokar, Patel, & Panchal et al*.,* 2014). Critical flux is defined as the lowest flux that creates irrevocable deposit on the membrane. However, limiting flux represents the highest consistency permeation flux that can be extended, for a given tangential velocity, by increasing transmembrane

pressure. Therefore, to maximize the performance and minimize cleaning steps, it is important to work in the subcritical zone even though the steady minor fouling and clogging can happen followed by a drastic increase that requires cleaning by using chemical. This phenomenon is further affected by the production of EPS, usually secreted by microalgae in stress conditions. These substances cause a gel-like layer in the filtration net, increasing the struggle to flow, also requiring chemical cleaning to be eliminated. Membranes must then be regularly cleaned to ensure purification, sanitization and reusability (Barros, Gonçalves, Simões, and et al., 2015).

Filtration is only sustainable for harvesting long length size microalgae or those forming large colonies. Despite microalgae cells of very low densities can be harvested by this method which is a main advantage, however membrane filtration is not commonly applied in large scale processes (Bejor, Mota, Ogarekpe, Emerson, & Ukpata, 2013). Both tangential flow and dead end filtration modes commonly applied in the industry cause of several benefits. Dead-end filtration is effective in the recovery of large microalgae cells (diameter over 70 mm). Tangential flow filtration (TFF) is deliberated more suitable for the harvesting of smaller suspended algae due to minor fouling problems. Furthermore, this method allows the separation of shear sensitive suspensions. As the medium flows tangentially across a membrane and the retentive is recirculated, the cells are kept in suspension, thus reducing membrane fouling. TFF has a well anti-fouling performance, given the deposit removal effect caused by the high fluid velocity tangential to the membrane. Additionally, higher filtration rates can be achieved, enabling complete removal of microalgae cells and debris. Micro or ultrafiltration membranes tend to be costly, energy intensive and need frequent membrane replacements when applied in large scale processing. Microfiltration (pore size from 0.1 to 10 mm) is suitable for harvesting fragile smaller cells. These membrane permit higher initial fluxes, but easily to clog. On the other hand, ultrafiltration (pore diameter: 1–100 nm) is appropriate for separation of solutes within 1–500 kDa of

molecular weight. The performances of both methods depend on hydrodynamic circumstances, concentration and culture properties of microalgae. Nevertheless, through ultrafiltration and for a long-term operation, low pressure and tangential velocity fluxes comparable to other industrial systems for biotechnological applications are accomplished. Ultrafiltration also seems to retain all cells and debris, which is suitable for these systems. High gradient magnetic filtration can also be proficiently applied. Bio-sorption of submicron-sized magnetic particles has great potential on microalgae harvesting. Efficiencies of over 95% have been reported. These particles must be low capital intensive, reusable, and chemically stable and have greater adsorption capacity. Filtration major costs are related with membrane replacement and pumping, thus it is cost-effective only for small volumes (Bejor, Mota, Ogarekpe, Emerson, & Ukpata, 2013). In fact, microfiltration can be more cost-effective than centrifugation when the volume to be processed is less than $2m^3$ as can meet the needs of cell harvesting for small sized microalgae species (2–40 μm). For volumes greater than $20m^3$ centrifugation may be more economic. The recommended design for force filtration, with detail to energy savings, dependability and concentration ability, are chamber filter press, cylindrical sieve and filter basket.

The operational factors in filtration include the pore size, cross-flow velocity, and transmembrane pressure. The permeation flux increases along with the increase in cross-flow velocity. The problem of membrane fouling can be decreased with the use of flow turbulence and shear force on the membrane surface (Ahmad, Mat Yasin, Derek, & Lim , 2017).

2.2 Dry Cell Weight of Biomass

The study of Ndikubwimana in 2015 had mentioned, the microalgae biomass concentration was first conducted by drying the dry cell weight (DCW) in an oven at 80◦C overnight(Ndikubwimana, Zeng, He & Xiou, 2015). While Axelsson in 2014 stated that the microalgae biomass was let to dried at 65°C overnight in air circulation oven (Axelsson et al., 2014). While in the study from Okaiyeto, algae biomass concentration identified were expressed by algal dry weight. A fixed volume of the algae suspension was fixed through a pre-weighed filter with a 0.45 μm porous membrane. The filter and algal cell were then dried at 105°C for 48 h. Finally, the algal dry weight was calculated by subtracting the mass of the filter from the total mass (Okaiyeto, Nwodo, Mabinya*et al.*, 2015).

CHAPTER 3

MATERIALS AND METHODS

3.1 Sampling of Fresh water Microalgae

The sampling of the microalgae was carried out in aquaculture laboratory of Universiti Malaysia Kelantan. Three different fertilizers which were the NPK (nitrogen:phosphorus:potassium) as control, fermented soy pulp and treated quail dung, with different percentage for each of the fertilizer were used to culture the growth of microalgae. The culture media with total volume size of 400 mL for each of the fertilizer and percentage used were harvested using the method of bioflocculation, centrifugation and filtration. Conventional method of centrifugation appeared as the control, while bioflocculation and filtration as biological and physical methods correspondingly.

3.2 Bioflocculation

3.2.1 Growth Medium for the Production of Biofloccullant

The medium composed of 15 g of Tryptic Soy Broth (TSB) was mixed with 500 mL distilled water, then was autoclaved for 15 min at 121 ˝C. 50 mL of each 6 falcon tube and 3/4 of 10 each visual bottle were filled up with the prepared medium and stored at 2-30 ˝C in the refrigerator until further use.

3.2.2 Isolation of Bioflocculant Producing Bacteria

1 mL of stock solution of bacteria *Bacillus licheniformis* was pippetted into each of the fresh water micoalgae media in the falcon tube, while 20 µLinto each of the visual bottle. All media were incubated at 30 ˝C for 24 h until the media turn into cloudy which indicated the bacteria growth. After the incubation period, 306 mL of cell-free supernatant of the broth was obtained by centrifuging at 4500 rpm for 5 min in order to separate the cells. The isolate *B.licheniformis* preserved in nutrient broth and in glycerol stock was then stored at -40 ◦C until further use.

3.2.3 Flocculation Analysis

The flocculation tests were performed with different dosage of bioflocculant which are 100, 200 and 300 µL. According to Salim in the research of harvesting of microalgae by bioflocculation, the microalgal samples were diluted in a 10×10×45 mm polystyrene cuvette. Each of the different dosage of bioflocculant was pipetted into each of 1.5 mL of 9 microalgae samples with different fertilizer type and it percentage. The flocs formed and the sedimentation of the flocs were observed.

3.3 Centrifugation

3.3.1 Centrifugation at Different Speed

Each of 9 microalgae sample (1.5 mL for each sample) with different fertilizers and it percentage were diluted in the 1.5 mL micro centrifuge tube. Each of the sample was centrifuge using a Beckman Avanti J-251 at speed 6000, 7000 and 8000 rpm each for 5 min. The sedimentation of microalgae at the bottom of the micro centrifuge tubes were observed

3.4 Filtration

3.4.1 Preparation of Macro filtration Membrane

Membranes with different pore size, 40, 70, 100 µm were prepared using stretch cotton fabric material type for 70 µm while both of the 40 and 100 µm size of pore were using the polyester fabric material. 1.5 mL of each of 9 dilute microalgae samples were let to pass through on each of the membranes with small pressure was applied.

3.5 Determining the Dry Cell Weight (DCW) of Microalgae Biomass

Microalgae biomass collected were let to dry in the oven at 115 C overnight. The DCW obtained were weighed by using the weighing scale, and the final weight were recorded.

3.6 Statistical Analysis of Microalgae Dry Cell Weight (DCW)

Statistical Analysis All measurements results are expressed as mean values ± 2S (standard deviation), Statistical differences between experimental groups were measured by one way ANOVA (post hoc Tukey analysis), at ($p \leq 0.05$).

CHAPTER 4

RESULT AND DISCUSSION

4.1 Comparison of DCW Biomass for Different Harvesting Methods

The differences of mean weight of DCW obtained through each of the method of bioflocculation, centrifugation and filtration were clearly identified from the Figure 4.1. Filtration shown the highest total mean value of DCW obtained which is 4.433 mg, followed by centrifugation which is 4.016 mg. While bioflocculation obtained the lowest total mean value which is 1.916 mg.

4.1.1 Bioflocculation

From this research of harvesting microalgae, bioflocculation showed the lowest DCW obtained as the final result (Figure 4.1). Three different dosage of bioflocculant produced by *B. licheniformis* were used, 100 µL (B100), 200 µL (B200) and 300 µL (B300) to harvest the algae cultures. Among the three dosages, 300 µL shown the highest DCW obtained which indicated the increasing of the dosage, might increasing the flocculant activity. However from the previous study from Okaiyeto in 2015, the increasing of bioflocculant dosage did not influenced in the increasing of flocculation efficiency(Okaiyeto et al., 2015). Among the drawback for the success of this bioflocculation method, might be came from the bacteria stain used and the species of the microalgae. The bacteria might be not be well cultured or contaminated thus affect the bioflocculant produced. Moreover, common species of freshwater microalgae which was used in this harvesting might not interact well with the type of flocculant, thus affect the formation of the flocs and the accumulation. Besides, one of the major

disadvantages of using bioflocculation in this research is this methods was relatively slower compared to the chemical flocculation which commonly applied in industrial sector.

4.1.2 Centrifugation

Generally, centrifuges are considered to be too cost and energy intensive to be suitable for microalgae harvesting for small production system. For most applications, centrifuges are adjusted primarily to maximize capture efficiency. However, cost-effective harvesting of algal cells may or may not correspond with the highest capture efficiency. At higher flow rates (>1 L/min), the lower capture efficiencies will be offset by the larger volumes of culture processed through the centrifuge.

This method showed a quite higher DCW collected (Figure 4.1) which indicated centrifugation at 7000 rpm (C7000) as the best speed, followed by 8000 rpm (C8000) and 6000 rpm (C6000), with time taken of each of the process were set to 5 minute and temperature at 4℃. Due to the microscopic size of the microalgae cells, longer retention times within the centrifuge bowl are needed for the sedimentation of the biomass (Taylor, Chen, Jang et al., 2014). As estimated, results indicated that longer retention times (slower flow rates) correlated with more energy being directed to a smaller volume of culture per min. The harvesting efficiency was determined by determining the DCW obtained at the final process. Even though this method was effective in obtaining high amount of biomass, it consumed too much energy and cost intensive which indicated, one of the major drawback when applying in large scale of harvesting microalgae. Thus, for this research, centrifugation was not effective method in term of energy and cost. Another main disadvantages are the cell of microalgae structure may be ruptured under the high gravitational and shear forces as fragility of the cell. Besides, the treatment time for a large amount of microalgae suspension takes

a longer period, and sometimes costly equipment, such as a continuous centrifuge, is required.

4.1.3 Filtration

Based on the test results, microalgae recovery rate for 70 micron (F70) pore size was significantly higher than the recovery rates for all other pore sizes (see Figure 4.1), followed by 40 micron (F40) and 100 micron (F100) of the pore size. Microalga biomass recovery rate was assumed to be independent of the concentration of the microalgae suspension and the culture volume(Barros, Gonçalves, Simões et al., 2015).

The microalgae size was not the main factor that affect the filtration efficiency as the smaller size of the freshwater microalgae ranging from 5 to 70 micron could pass through all the filtration mesh easily. However, the major factor indicated from this research was the type of the mesh filtration itself that affect the harvesting efficiency. 70 micron size of the filtration mesh was using stretch cotton fabric material while both of the 40 and 100 micron size of filtration mesh were using polyester linen fabric materials. This can be proved by the previous research which has shown that for most algae sizes commonly found in water and wastewater samples, efficient harvesting could be achieved using the stretch-cotton fabric material, whereas the polyester-linen would be best suited for pretreatment purposes as the microalgae accumulated better on the stretch cotton fabric material (Azizo, Wirzal, Bilad & Yusoff , 2017).The major advantages of this filter are easier to set up and design, and less maintenance costs. The main disadvantage is the difficulty of handling very fast-filtering materials on a large scale. Thus, the result obtained from this filtration method also was fundamentally influenced by the harvesting scale as small scale of harvesting was applied during harvesting process.

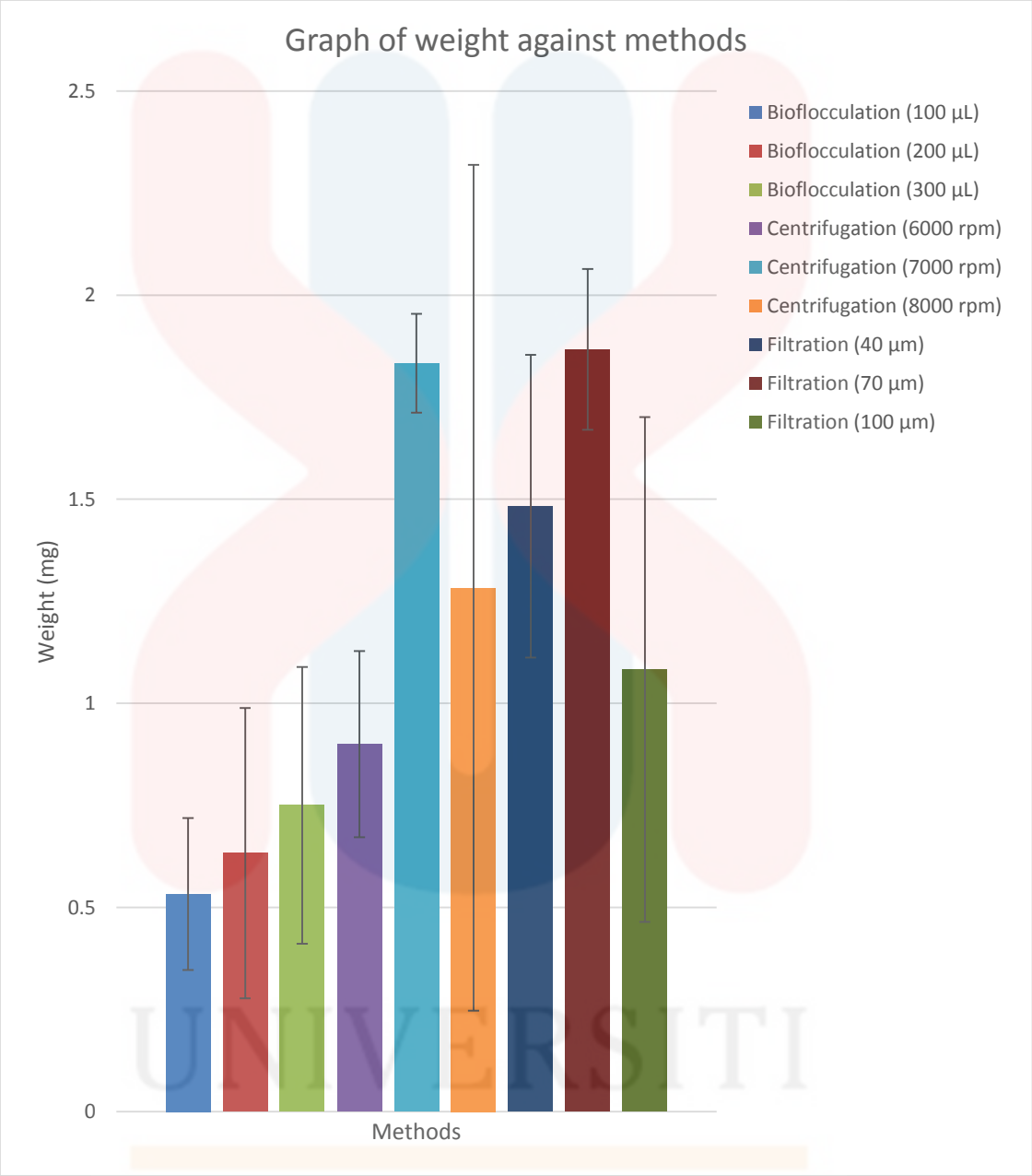

Figure 4.1 Dry Cell Weight (DCW) of biomass obtained from each methods of harvesting microalgae

Table 4.1 DCW Biomass in Microalgae from Different Harvesting Methods.

The value shown are the mean ±standard deviation of two replicates

Harvesting method		DCW biomass (mg)
Bioflocculation (µL)	100	0.5330 ± 0.1862 [a]
	200	0.6330 ± 0.3559 [a]
	300	0.7500 ± 0.3391[ab]
Centrifugation (rpm)	6000	0.9000 ± 0.2282[ab]
	7000	1.8330 ± 0.1211 [c]
	8000	1.2830 ± 1.0362[abc]
Filtration (µm)	40	1.4830 ± 0.3710[bc]
	70	1.8670 ± 0.1969 [c]
	100	1.0830 ± 0.6182[abc]

Mean ± standard deviation (number of samples). Different small letters (a,ab,c,abc) represent a significance difference between means

a : low significant difference

ab : moderate significant difference

c : higher significant difference

d : highest significance difference

Table 4.1 showed the mean performances of harvesting methods on the freshwater microalgae communities tested. Based on the result, for bioflocculation, there were no significance different of the result obtained from the dosage of 100 and 200 µL of bioflocculants, but exist between both of the dosage with 300 µL bioflocculant. However there was a difference between 300 µL bioflocculant and

centrifugation method at the speed of 6000 rpm. The difference also did not significance among the filtration at mesh size 100 micron and centrifugation at the speed 8000 rpm. But all of the methods mentioned, had bigger significance difference of DCW of biomass obtained by using the method of centrifugation at speed 7000 rpm and filtration at the pore size of 70 micron which indicated the highest harvesting efficiency. Thus, it was concluded that there was higher significant difference between each of the method, but lower difference between each of the sample within the method used. While the main result showed that filtration using 70 micron mesh size demonstrated the highest algae harvesting efficiency which (1.8670 ± 0.1969) for microalgae communities followed by centrifugation at 7000 rpm (1.8330 ± 0.1211) and bioflocculation with the dosage 300 µL (0.7500 ± 0.3391).

These outcomes demonstrated that for the scope of microalgae species regularly found in freshwater, the filtration technique could be effectively utilized as a harvesting instrument, while the centrifugation technique could best be utilized for pretreatment purposes where reducing in algae biomass concentration is required before next harvesting. Although the data presented appeared straightforward and it would be easy to compare the harvesting methods of microalgae by identifying the mean of DCW obtained, there are a variety of fundamental operational issues associated with each process. Therefore, it is important to carefully analyze several parameters, such as microalgae cell morphology, ionic strength of the media, pH, culture density and other factors, when selecting a suitable harvesting technique.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The freshwater microalgae was used to study harvesting methods because it is easy to grow and easy to get. High total mean value of DCW (dry cell weights) of 4.433 mg and 4.016 were obtained by filtration and centrifugation, respectively. For both of the methods, 70 micron size of filter pore and 7000 rpm of centrifuge speed at 5 min were demonstrated as the most effective to harvest higher amount of algae biomass during this research. For filtration, the pore size of the filtration membrane or mesh, the material type which is stretch- cotton also the bigger influencer of the high harvesting efficiency. This type of material is suitable for harvesting microalgae as it can retained even the cell smaller than the pore size and had capability to accumulate the cell better on the membrane. While, 7000 rpm was identified as the best rotary speed to capture the harvesting efficiency of the microalgae. Even though both filtration and centrifugation were comparably effective in harvesting the freshwater microalgae in term of the value of DCW obtained, meanwhile in term of effectiveness in other factor such as total energy consuming and cost supply, the filtration would be better choice especially in small scale application. While biofloculation method became the less effective of harvesting microalgae as the mean value of DCW obtained was the lowest.

From this finding, it can be concluded that filtration as the most efficient method for microalgae harvesting as the capability in decreasing capital costs, a higher efficiency of biomass obtained, and comparably low maintenance and energy requirements relative to other methods.

5.2 Recommendation

It is recommended to apply custom-designed multi stage harvesting method for microalgae harvesting in which by combining several harvesting method for example centrifugation with filtration method and also develop varieties harvesting technologies in order to attain the highest harvesting efficiency with lower capital and energy consumption. To lower the energy consumption from the method of harvesting microalgae, an integrated approach should be applied which is by either by combining, recycling and others.

Besides, specific method should be applied to specific microalgae and scale of harvesting system as to reduce the energy consumption and higher cost demand. For example, high-quality algae are to be produced for human consumption, continuous harvesting by solid ejecting or nozzle-type disc centrifuges is recommended. These centrifuges can easily be cleaned and suitable for all types of microalgae. While, small scale harvesting system should consider feasible methods.

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Appendices

APPENDIX A : FIGURES OF METHODOLOGY USED IN THE HARVESTING OF MICROALGAE

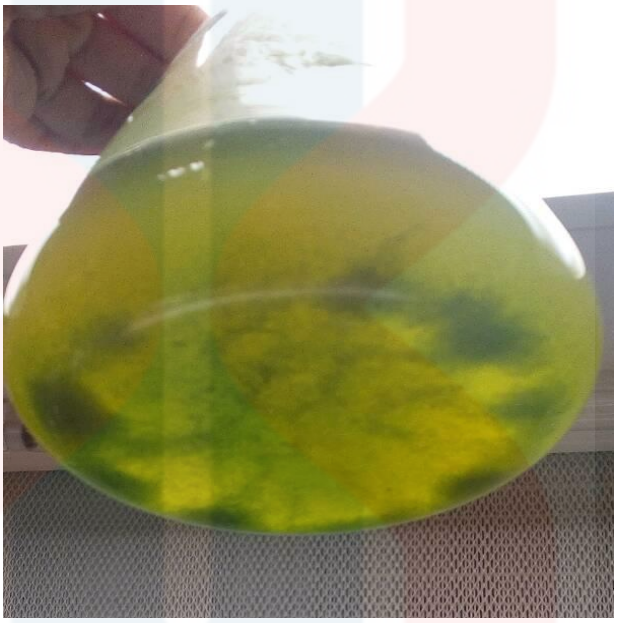

Figure 4.2 Bioflocculation interaction in fresh water microalgae (sample 1)

Figure 4.3 Bioflocculation interaction in fresh water microalgae (sample 2)

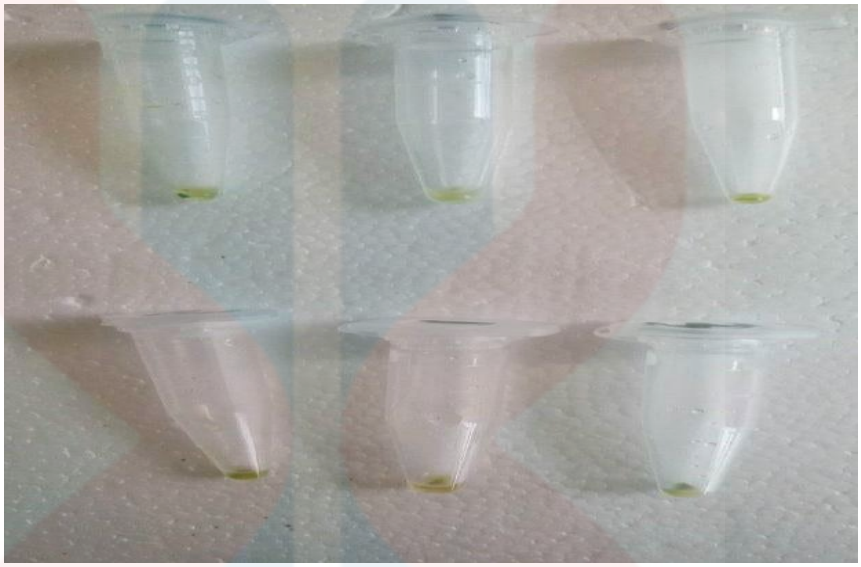

Figure 4.4 Biomass obtained after centrifugation

Figure 4.5 Biomass obtained from the filtration of microalgae

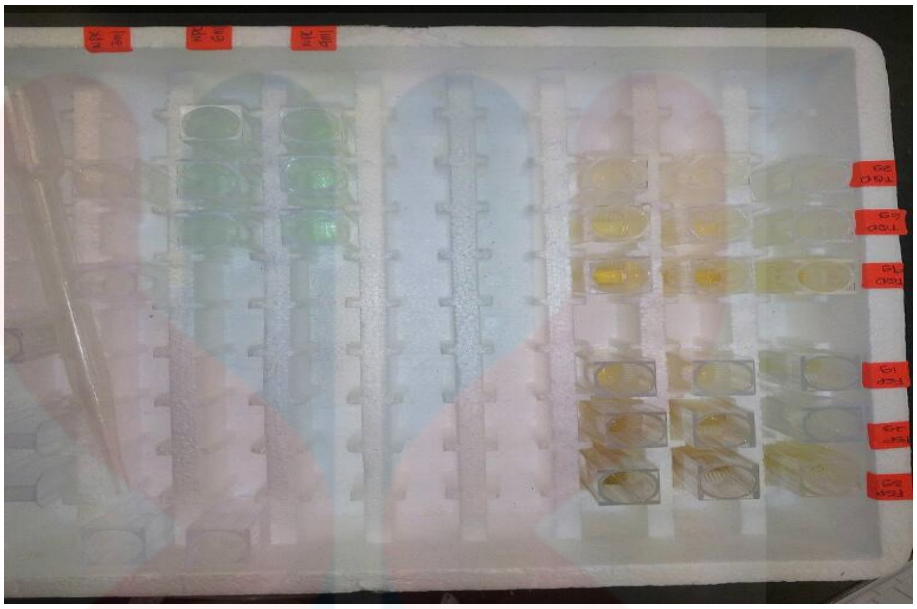

Figure 4.6 Samples of freshwater microalgae with three different fertilizers (TQD, FSP, NPK)

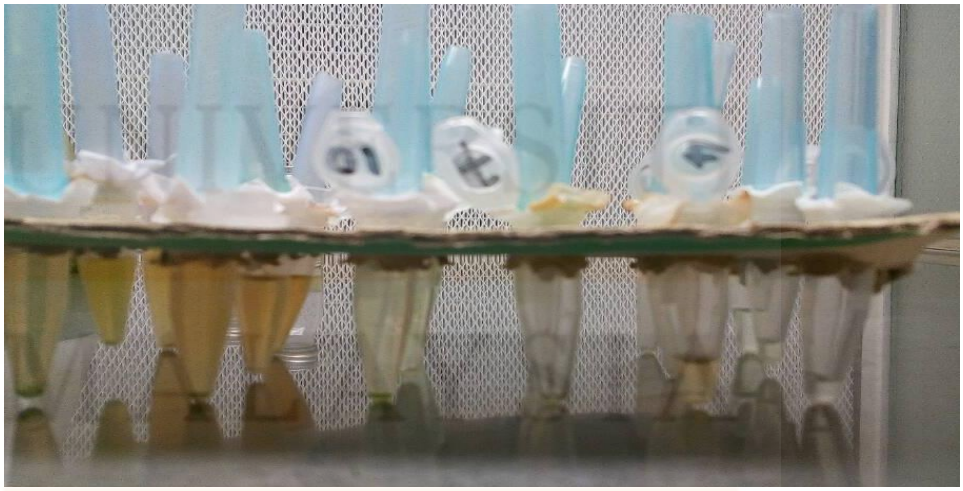

Figure 4.7 Filtration model of microalgae

APPENDIX B : STATISTICAL TEST FOR THE RESULT OF MICROALGAE HARVESTING

One-Way Descriptive of the Weight

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
B100	6	.000533	.0001862	.0000760	.000338	.000729	.0003	.0008
B200	6	.000633	.0003559	.0001453	.000260	.001007	.0001	.0011
B300	6	.000750	.0003391	.0001384	.000394	.001106	.0003	.0011
C6000	6	.000900	.0002280	.0000931	.000661	.001139	.0007	.0013
C7000	6	.001833	.0001211	.0000494	.001706	.001960	.0017	.0020
C8000	6	.001283	.0010362	.0004230	.000196	.002371	.0003	.0030
F40	6	.001483	.0003710	.0001515	.001094	.001873	.0010	.0020
F70	6	.001867	.0001966	.0000803	.001660	.002073	.0016	.0022
F100	6	.001083	.0002787	.0001138	.000791	.001376	.0007	.0014
Total	54	.001152	.0006182	.0000841	.000983	.001321	.0001	.0030

One-Way ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	8	.000	8.014	.000
Within Groups	.000	45	.000		
Total	.000	53			

Multiple Comparisons of The Microalgae Biomass Weight for each Method

Post Hoc Tests

Dependent Variable: Weight

Tukey HSD

(I) Method	(J) Method	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
B100	B200	-.0001000	.0002487	1.000	-.000910	.000710
	B300	-.0002167	.0002487	.993	-.001027	.000594
	C600	-.0003667	.0002487	.861	-.001177	.000444
	C700	-.0013000*	.0002487	.000	-.002110	-.000490
	C800	-.0007500	.0002487	.089	-.001560	.000060
	F40	-.0009500*	.0002487	.011	-.001760	-.000140
	F70	-.0013333*	.0002487	.000	-.002144	-.000523
	F1	-.0005500	.0002487	.416	-.001360	.000260

	00					
B200	B100	.0001000	.0002487	1.000	-.000710	.000910
	B300	-.0001167	.0002487	1.000	-.000927	.000694
	C6000	-.0002667	.0002487	.975	-.001077	.000544
	C7000	-.0012000*	.0002487	.001	-.002010	-.000390
	C8000	-.0006500	.0002487	.210	-.001460	.000160
	F40	-.0008500*	.0002487	.033	-.001660	-.000040
	F70	-.0012333*	.0002487	.000	-.002044	-.000423
	F100	-.0004500	.0002487	.676	-.001260	.000360
B300	B100	.0002167	.0002487	.993	-.000594	.001027
	B200	.0001167	.0002487	1.000	-.000694	.000927
	C6000	-.0001500	.0002487	1.000	-.000960	.000660

	C7000	-.0010833*	.0002487	.002	-.001894	-.000273
	C8000	-.0005333	.0002487	.458	-.001344	.000277
	F40	-.0007333	.0002487	.104	-.001544	.000077
	F70	-.0011167*	.0002487	.001	-.001927	-.000306
	F100	-.0003333	.0002487	.913	-.001144	.000477
C6000	B100	.0003667	.0002487	.861	-.000444	.001177
	B200	.0002667	.0002487	.975	-.000544	.001077
	B300	.0001500	.0002487	1.000	-.000660	.000960
	C7000	-.0009333*	.0002487	.013	-.001744	-.000123
	C8000	-.0003833	.0002487	.830	-.001194	.000427
	F40	-.0005833	.0002487	.338	-.001394	.000227
	F7	-.0009667*	.0002487	.009	-.001777	-.000156

	0 F1 00	-.0001833	.0002487	.998	-.000994	.000627
C7000	B1 00	.0013000*	.0002487	.000	.000490	.002110
	B2 00	.0012000*	.0002487	.001	.000390	.002010
	B3 00	.0010833*	.0002487	.002	.000273	.001894
	C6 000 0	.0009333*	.0002487	.013	.000123	.001744
	C8 000 0	.0005500	.0002487	.416	-.000260	.001360
	F4 0	.0003500	.0002487	.889	-.000460	.001160
	F7 0	-.0000333	.0002487	1.000	-.000844	.000777
	F1 00	.0007500	.0002487	.089	-.000060	.001560
C8000	B1 00	.0007500	.0002487	.089	-.000060	.001560
	B2 00	.0006500	.0002487	.210	-.000160	.001460
	B3 00	.0005333	.0002487	.458	-.000277	.001344

	C600	.0003833	.0002487	.830	-.000427	.001194
	C700	-.0005500	.0002487	.416	-.001360	.000260
	F40	-.0002000	.0002487	.996	-.001010	.000610
	F70	-.0005833	.0002487	.338	-.001394	.000227
	F100	.0002000	.0002487	.996	-.000610	.001010
F40	B100	.0009500*	.0002487	.011	.000140	.001760
	B200	.0008500*	.0002487	.033	.000040	.001660
	B300	.0007333	.0002487	.104	-.000077	.001544
	C600	.0005833	.0002487	.338	-.000227	.001394
	C700	-.0003500	.0002487	.889	-.001160	.000460
	C800	.0002000	.0002487	.996	-.000610	.001010

	F70	-.0003833	.0002487	.830	-.001194	.000427
	F100	.0004000	.0002487	.795	-.000410	.001210
F70	B100	.0013333*	.0002487	.000	.000523	.002144
	B200	.0012333*	.0002487	.000	.000423	.002044
	B300	.0011167*	.0002487	.001	.000306	.001927
	C6000	.0009667*	.0002487	.009	.000156	.001777
	C7000	.0000333	.0002487	1.000	-.000777	.000844
	C8000	.0005833	.0002487	.338	-.000227	.001394
	F40	.0003833	.0002487	.830	-.000427	.001194
	F100	.0007833	.0002487	.065	-.000027	.001594
F100	B100	.0005500	.0002487	.416	-.000260	.001360
	B200	.0004500	.0002487	.676	-.000360	.001260

	B300	.0003333	.0002487	.913	-.000477	.001144
	C6000	.0001833	.0002487	.998	-.000627	.000994
	C7000	-.0007500	.0002487	.089	-.001560	.000060
	C8000	-.0002000	.0002487	.996	-.001010	.000610
	F40	-.0004000	.0002487	.795	-.001210	.000410
	F70	-.0007833	.0002487	.065	-.001594	.000027

*. The mean difference is significant at the 0.05 level.

Tukey Test Analysis for the Homogeneous Subsets

Mean Weight				
Tukey HSD[a]				
Method	N	Subset for alpha = 0.05		
		1	2	3
B100	6	.000533		
B200	6	.000633		
B300	6	.000750	.000750	
C6000	6	.000900	.000900	
F100	6	.001083	.001083	.001083
C8000	6	.001283	.001283	.001283
F40	6		.001483	.001483
C7000	6			.001833
F70	6			.001867
Sig.		.089	.104	.065
Means for groups in homogeneous subsets are displayed.				
a. Uses Harmonic Mean Sample Size = 6.000.				