A STUDY ON FOULING DURING HARVESTING OF *CHLORELLA VULGARIS* USING MICROFILTRATION

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FACULTY OF ENGINEERING
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2018

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DISSERTATION SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF ENGINEERING SCIENCE

FACULTY OF ENGINEERING
UNIVERSITY OF MALAYA
KUALA LUMPUR

2018

UNIVERSITY OF MALAYA

ORIGINAL LITERARY WORK DECLARATION

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Matric No: KGA110081

Name of Degree: MASTER OF ENGINEERING SCIENCE

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A STUDY ON FOULING DURING HARVESTING OF *CHLORELLA VULGARIS* USING MICROFILTRATION

ABSTRACT

Microalgae harvesting using membrane filtration is economically attractive and does not require any addition of chemicals. Membrane filtration has a major drawback related to membrane fouling that can be minimized by controlling filtration hydrodynamic parameters. The effect of hydrodynamic control is only secondary compared to the fouling materials existed in microalgae culture. A few recent studies have found that one of the microalgae components, transparent exopolymer particle (TEP) is a key factor causing fouling. Understanding TEP production in microalgae culture is important to reduce membrane fouling phenomena. In this study, *Chlorella vulgaris* was cultivated in a batch pure culture using 5L closed bubbled column PBR to investigate its physiological state during its growth phase cycle. Fouling caused by TEP was studied during microfiltration (MF) of *C. vulgaris* at their physiological ages of 2, 4, 6, 8, and 10 days. MF was conducted at constant operation condition consists of 0.5 bar transmembrane pressure (TMP) and 2.13 m/s crossflow velocity (CFV) using titanium oxide tubular membrane with pore size of 0.45 µm. During the growth cycle, TEP production from *C. vulgaris* was low during exponential growth phase then suddenly rose towards stationary phase and highly accumulated until its senescence phase. The highest amount of TEP concentration accumulated was 0.31 g/L during microalgae cultivation of 14d. The fouling phenomena was determined using modified fouling index (MFI) and resistance factor. The highest MFI value is 11.49 x 10^5 sL^{-2} during filtration on 6d microalgae physiological age. Pore blocking resistance (R_b) has the highest value at 5.43 x 10^{12} m^{-1} during MF of 6d microalgae physiological age. TEP concentration gave significant influence to R_b but MFI values showed that high total biomass concentration influence cake resistance (R_c) that caused severe fouling.

Keywords: fouling; microfiltration; *Chlorella vulgaris*; membrane filtration; microalgae.

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ABSTRAK

Penuaian mikroalga menggunakan teknologi membran didapati menarik secara ekonomi dan tidak memerlukan penambahan bahan kimia. Penapisan membran mempunyai satu kelemahan ketara iaitu kotoran pada membrane yang boleh dikurangkan dengan pengawalan parameter hidrodinamik penapisan. Kesan pengawalan hidrodinamik hanyalah sekunder jika dibandingkan dengan bahan kotoran yang wujud dalam kultur mikroalga. Beberapa kajian terbaru menjumpai satu daripada komponen mikroalga, *transparent exopolymer particle* (TEP) adalah faktor utama yang menyebabkan kotoran. Pemahaman mengenai pengeluaran TEP di dalam kultur mikroalga adalah penting bagi mengurangkan fenomena kotoran membrane. Dalam kajian ini, *Chlorella vulgaris* ditanam di dalam kultur kumpulan menggunakan 5L photobioreaktor lajur gelembung tertutup untuk mengkaji keadaan fisilogi semasa kitaran fasa pertumbuhan. Kotoran disebabkan oleh TEP dikaji semasa penapisan mikro (MF) *C. vulgaris* pada umur fisiologi mereka di 2, 4, 6, 8, dan 10 hari. MF dilakukan pada keadaan operasi kekal terdiri daripada 0.5 bar tekanan transmembran (TMP) dan 2.13 m/s kelajuan silang-aliran (CFV) menggunakan membran titanium oksida berbentuk tiub dengan saiz liang pada 0.45 μm. Semasa kitaran fasa pertumbuhan, pengeluaran TEP daripada *C. vulgaris* didapati rendah semasa fasa eksponen kemudian meningkat kepada fasa pegun dan terkumpul secara mendadak pada fasa penuaan. Jumlah tertinggi kepekatan TEP terkumpul adalah 0.31 g/L pada umur penanaman 14 hari. Fenomena kotoran ditentukan menggunakan Indeks Kotoran Diubahsuai (MFI) dan faktor rintangan. Nilai tertinggi MFI adalah 11.49×10^5 sL^{-2} semasa penapisan mikroalga pada umur fisiologi 6 hari. Rintangan penyekatan liang (R_b) adalah tertinggi sebanyak 5.43×10^{12} m^{-1} semasa penapisan mikro mikroalga pada umur fisiologi 6 hari. Kepekatan TEP memberi pengaruh besar kepada R_b tetapi nilai MFI

menunjukkan jumlah kepekatan biomas yang tinggi mempengaruhi rintangan kek (R_c) yang menyebabkan kotoran yang teruk.

Kata kunci: kotoran; penapisan mikro; *Chlorella vulgaris*; penapisan membran; mikroalga.

ACKNOWLEDGEMENTS

Alhamdulillah. Thanks to Allah for bringing me the strength to finish my dissertation. There are many people that contribute to this work and I would like to take this opportunity to thank them

First and foremost, I would like to express my sincere gratitude to my supervisor, Prof. Dr. Nik Meriam Nik Sulaiman for the guidance, advice, encouragement, and support throughout this project. Thanks for your help that you give during my up-and-down in this Master project.

I also would like to thank Prof. Dr. Phang Siew Moi for the wonderful opportunity to collaborate on this project. I also would like to express my gratitude to Tan Cheng Yau for his kindness and guidance during my stay in Algae Lab.

A lot of thanks to my fellow friends in Makmal Zarah for their useful references and information as well as emotional support and priceless friendship that brings me joy and wonderful time spent in the lab.

I would like to acknowledge Skim Biasiswa Universiti Malaya (SBUM) and Peruntukan Penyelidikan Pascasiswazah (PPP) (PG094-2013A) for scholarship and financial support of the project.

Last but not least, I would like to give my thanks to my siblings and parents for their emotional support.

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LIST OF SYMBOLS AND ABBREVIATIONS

μ	:	True Mean
AOM	:	Algagonic Organic Matter
ASTM	:	American Society for Testing and Materials
BBM	:	Bold's Basal Medium
CA	:	Cellulose Acetate
CFV	:	Crossflow Velocity
CO_2	:	Carbon Dioxide
DOM	:	Dissolved Organic Matter
EOM	:	Extracellular Organic Matter
EPS	:	Extracellular Polymeric Substance
FO	:	Forward Osmosis
GX	:	Gum Xanthan
HGRP	:	High Growth Rate Phase
IFM	:	Improved Flux Step Method
LGRP	:	Low Growth Rate Phase
M	:	Mean
MF	:	Microfiltration
MFI	:	Modified Fouling Index
NaOH	:	Sodium Hydroxide
NaOCl		Sodium Hypochlorite
NOM		Natural Organic Matter
OM	:	Organic Matter
PAN	:	Polyacrylonitrile
PBR	:	Photobioreactor

PES	:	Polyethersulfone
PET	:	Polyester
PS	:	Polysulfone
PVA	:	Polyvinyl Alcohol
PVC	:	Polyvinyl Chloride
PVDF	:	Polyvinylidene Fluoride
R_a	:	Resistance of Adsorption
R_b	:	Resistance of Pore Blocking
R_c	:	Resistance of Cake Formation
R_{cp}	:	Resistance of Concentration Polarisation
R_m	:	Resistance of Membrane
SDI	:	Silt Density Index
SE	:	Standard Error
RO	:	Reverse Osmosis
TEP	:	Transparent Exopolymer Particle
Ti-Zr	:	Titanium - Zirconia
TMP	:	Transmembrane Pressure
UF	:	Ultrafiltration
UMACC	:	University of Malaya Algae Culture Collection

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CHAPTER 1: INTRODUCTION

1.1 Background

For the last two decades, microalgae have been receiving a lot of attention since the discovery of its potential motivated by depleting fuel resource, instability of oil fuel price, environmental pollution and global warming concern (Ahmad et al., 2011; Benemann, 2008; Chisti, 2007; Safi et al., 2014). Microalgae are photosynthetic microorganisms that are found in both marine and freshwater environments. They are unicellular species that have sizes ranging from a few micrometers to hundreds of micrometers, depending on their species. Microalgae require sunlight, nutrients and carbon dioxide to perform photosynthesis; thus contributing approximately half of the atmospheric oxygen on earth (Safi et al., 2014) and carbon sequestration during their production. Microalgae are regarded as 'crop' for the third generation of biodiesel by reason of its sustainable production and possess similar properties in term of energy content, chemical and physical properties of fossil fuel. Microalgae seem promising as a renewable energy resource due to its requirement of a small area of cultivation, high growth rate compared to other energy crops (Demirbas, 2011), low water consumption (Yang et al., 2011), and high biomass conversion to lipids (Widjaja et al., 2009). Microalgae also have a nutritional value and been sources for wide range of proteins, carbohydrates, vitamins, pigments, fatty acids, and minerals that beneficial to human health.

Chlorella vulgaris has a good growth performance and biomass production. *C. vulgaris* has been used for multiple purposes like food supplements, animal feed (Eguchi et al., 2004), cosmetic, food coloring and preservation (Fradique et al., 2010; Gouveia et al., 2007). Natural antioxidant properties in *C. vulgaris* is studied to be used

as medical drugs to prevent degenerative diseases such as cancer, diabetes, heart disease, osteoporosis, Parkinson's and Alzheimer's disease (Goiris et al., 2015; Rodriguez-Garcia & Guil-Guerrero, 2008). Additionally to its nutritional benefits, *C. vulgaris* has been observed to be a good candidate for renewable biodiesel and aviation fuel resources (Huang et al., 2010; Singh et al., 2014; Slade & Bauen, 2013). *C. vulgaris* has been found to accumulate high amount of lipid productivity as much as 79.08 $mgL^{-1}day^{-1}$ and fatty acid profiles suitable for biodiesel production (Talebi et al., 2013).

C. vulgaris is a readily available microalgae species in the market due to its robust characteristic and easy to cultivate. *C. vulgaris* could be grown either through photosynthesis process with sufficient supply of light energy and CO_2 or non-photosynthesis process that only needs ample amount of nutrients in the dark conditions. Recovery of microalgae biomass from the culture is called harvesting. Microalgae biofuel at industrial-scale production is not cost-effective in harvesting and extraction methods when compete with petroleum-based fuel (Danquah et al., 2009; Kim et al., 2013).

Microalgae harvesting could potentially contribute 20-30% of total microalgae biodiesel production cost (Grima et al., 2003). Harvesting of microalgae suffers from a major bottleneck due to microscopic cell sizes (2–200 μm), similar density to water, and dilute biomass concentration in its culture media (Pittman et al., 2011; Rashid et al., 2014; Ríos et al., 2013). Microalgae also have high surface charges which caused microalgae suspension to be stable and difficult to settle in water. Harvesting of microalgae in large volumes requires several steps of solid-liquid separation methods for bulk harvesting and dewatering before proceeding to extraction of microalgal oil.

There is no ideal stand alone or combination of harvesting methods introduced yet which is commercially viable; an efficient, versatile and sustainable dewatering method. Harvesting is the most challenging of upstream processes that hindering microalgae biofuel into commercialization (Kim et al., 2014; Kiran et al., 2014; Tan et al., 2016). Various solid-liquid separation methods can be used to harvest microalgae like flocculation, gravity sedimentation, floatation, centrifugation, ultrasound aggregation and filtration.

Membrane filtration has been used in a wide range of industries including water and wastewater treatment. Membrane filtration is seen as a promising dewatering application as it only uses physical separation and enhanced the quality of end products due to non-chemical addition to product stream. In addition, the membrane separation processes are easy to scale-up from laboratory scale into industrial scale. Other advantages are steadily decreasing cost of membrane and the ability of membrane to remove protozoan and viruses while retaining the residual nutrient (Zhang et al., 2010). Harvesting of microalgae typically using low pressure driven membrane such as microfiltration (MF) and ultrafiltration (UF) according to microalgae particle size.

1.2 Problem Statement

Membrane fouling is the major constraint in membrane processes implementation. Fouling is demonstrated through flux decline and increase in membrane resistances which inadvertently increases operational costs and transmembrane pressure (TMP) requirements (Her et al., 2007). Microalgae harvesting using membrane filtration involves reversible and irreversible fouling where reversible fouling can be curbed through mechanical cleaning but irreversible fouling can only be removed by chemical

cleaning (Dvorák et al., 2011). Frequent chemical cleaning to remove foulants could deteriorate the membrane performance and shorten the membrane life. Besides, the chemical cleaning of microalgae could give serious effect to microalgae composition as valuable resources.

Understanding membrane fouling is crucial to obtain the sustainable biomass concentration using membrane technology. Fouling frequently happen to the pressure driven membrane processes such as MF and UF due to handling of high feed concentration factor (Pavez et al., 2015). Particles foul the membrane using the mechanisms: pore blocking, adsorption, cake formation, and concentration polarization. Hydrodynamic parameters such as transmembrane pressure (TMP) and crossflow velocity (CFV) influences fouling of membrane but their effect is secondary to the presence of fouling material available in microalgae culture (Amy, 2008). The most problematic foulants in microalgae culture are identified as polysaccharides and protein in colloidal form that commonly found in microbial origin (Amy, 2008).

Extracellular polymeric substance (EPS) is a bound polymer coating outside of microalgae cells that consists of polysaccharides, proteins, nucleic acids, and humic substances and can be dissolved in water during cell lysis (Drews et al., 2006; Sutherland, 2001). Transparent exopolymer particles (TEP) are one type of EPS that show sticky gel-like rigid organic polymer that mainly consists of acidic polysaccharides and exists as individual particles rather than dissolved substances (Passow, 2002b). TEP was found to be predominantly produced by diatom microalgae like *C. vulgaris*. The abundance of TEP and TEP precursors is dependent on the species, physiological status, and environment condition of the microorganisms involved (Passow, 2002b).

The interaction between EPS particle like TEP and membrane fouling is still not well understood (Kennedy et al., 2009; Villacorte et al., 2009a). The primary focus of the study is on the physiological status of *C. vulgaris* in terms of organic foulants production by TEP and its role in membrane fouling of MF observed through permeate flux, resistances and modified fouling index (MFI).

1.3 Objectives of Study

The aim of this research is to study the fouling phenomena during MF harvesting of microalgae due to the production of TEP during its growth.

The objectives of study are:

i. To determine TEP production during the growth of *Chlorella vulgaris*.
ii. To evaluate the microfiltration performance of *Chlorella vulgaris* at different physiological age.
iii. To determine the effect of TEP formation on membrane fouling.

1.4 Scope of Study

C. vulgaris is obtained from University of Malaya Algae Culture Collection (UMACC) at Algae Research Laboratory, University of Malaya. A laboratory scale of artificial illuminated closed bubbled photobioreactor was used with 5 L of Bold's Basal Medium (BBM) as culture medium. The biomass of *C. vulgaris* and organic foulant, i.e. transparent exopolymer particle (TEP) production was studied for each growth phase.

Harvesting of *C. vulgaris* was conducted on different cultivation ages using ceramic membrane MF with pore size of 0.45 µm. The performance and fouling of membrane was assessed on different physiological state of *C. vulgaris* in terms of biomass and TEP production at different physiological ages.

Fouling parameters that studied were modified fouling index (MFI) and membrane resistances which consist of resistance of pore blocking (R_b) and resistance of cake formation (R_c) to indicate fouling severity and its interaction with physiological state of *C. vulgaris*.

1.5 Significance of Study

The contribution of this study is to provide a suitable harvesting protocol according to microalgae physiological age that can reduce cost in harvesting using membrane technology. The contribution can be achieved from understanding of the membrane fouling mechanisms by TEP produced in microalgae cultivation system and its correlation on fouling parameters in terms of resistances and MFI. Other significant aspect is to develop a proper membrane filtration configuration and clean-in-place methods appropriate to TEP fouling mechanisms to curb severe fouling, prolong membrane shelf life, and produce high biomass recovery.

1.6 Thesis Outline

This thesis is divided into five chapters described briefly as follows:

i. Chapter 1 introduces the background of this study, problems addressed with appropriate scope and objectives of study

ii. Chapter 2 encompasses relevant review on microalgae involves, previous works on techniques used to harvest microalgae, and also selected organic foulant associated with microalgae.

iii. Chapter 3 provides experimental technique and equipments used in the study from cultivation to harvesting of microalgae. The analytical procedure for determination of organic foulant also included in this chapter.

iv. Chapter 4 presents the experimental outcomes with detailed discussion on the issue.

v. Chapter 5 concludes the findings and contributions of the study. Recommendations for future work is included in this chapter.

CHAPTER 2: LITERATURE REVIEW

2.1 Microalgae

Microalgae are photosynthetic microorganisms. They convert light energy, CO_2 and nutrients into lipids, proteins and carbohydrates within the cellular cells while generating high level concentration of oxygen. Microalgae can be found in both marine and freshwater environments in any geographical location from hot to cold climate. Microalgae could be classified into green algae, red algae, and diatoms. Microalgae covers every unicellular species and multi-cellular microorganisms that have sizes ranged from a few micrometers to hundreds of micrometers depends on their species.

Microalgae can be cultivated into commercial scale by using open system, i.e. raceway pond, and closed system, i.e. photobioreactor. The microalgae that cultivated using this method must be cultured in the growth medium that contains essential nutrients, e.g. nitrogen, iron, and phosphorus. Microalgae development is only toward the cellular level and they have high adaptability to the surrounding resulting them to last longer (Brennan & Owende, 2010). Microalgae can be either autotrophic, heterotrophic, or mixotrophic. Autotrophic microalgae require carbon dioxide, salts and light energy for growth; photosynthesis process is vital for their existence. Heterotrophic microalgae do not need the light energy but only require the nutrients in their growth medium as an energy source. Mixotrophic microalgae could survive through autotrophic and heterotrophic behaviour; it is able to perform photosynthesis process and also could acquire energy from nutrients as well.

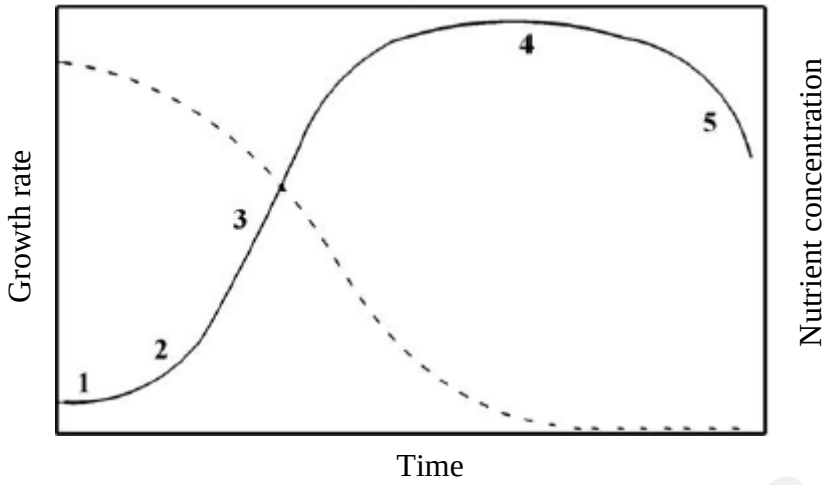

Figure 2.1: Different phases of microbial growth curve in batch culture (solid line) and nutrient concentration (dashed line) (Mata et al., 2010)

Microalgae growth phase in batch culture can be described as shown in Figure 2.1. The microalgae stock culture is typically grown in agar medium before being introduced to the fresh liquid medium. The start of inoculation of microalgae in liquid culture medium in phase (1) (refer to Figure 2.1) is called lag phase. Lag phase is a period of time where cells adjust to their new environment which can either be brief or extended. Microalgae cells do not immediately multiply their cells instead they mature; increase cells size and cellular metabolism for preparation of cell division.

Phase (2) is the log or exponential phase where the matured cells rapidly grow and start to multiply by binary fission at constant rate. The microalgae cell numbers doubles during unit time period called as generation time in which they start slow initially and increase in exponential leading to the next growth phase. Phase (3) is the linear phase where the exponential growth curve reached the maximum growth rate and continues growing in the exact rate developed a linear slope. Higher growth rate produces steep slope. Microalgae cell number continuously grows until the conditions become less favorable.

Phase (4) is a stationary phase where the cells generation slows down under unfavorable condition of limiting nutrients and waste products accumulation in the environment. The new cells generated equals to cells that dies resulting a constant microalgae cell numbers, producing an elevated steady state line in growth curve. Lastly, phase (5) is senescence or death phase where the cells death rate overcomes the cells generation rate to form a uniform negative slope. Due to continuous exhaustion of nutrients and wastes accumulation, some living cells go through an involution process as a survival mechanism. Microalgae excreted extracellular polymeric substance (EPS) as a survival mechanism typically promoted in stationary and senescence phase that will be explained in more detail in Subtopic 2.4.

Microalgae are biomass that capable to turn 'waste to energy' and the production of microalgae is a carbon free process. Massive consumption of CO_2 in photosynthesis process favors microalgae as a way to sequestrate global carbon emissions. The sequestration of carbon from microalgae cultivation could help to mitigate the global climate change in addition with the benefits of microalgal biofuels, food, feed and highly added value products (Chen et al., 2011). Direct feed of CO_2 from industrial plant flue gases does not affect the growth phase of microalgae. The ideal commercialize site for microalgae cultivation would be nearby the industrial plant and the sewage treatment plant for ultimate utilization of wastewater nutrients and carbon dioxide emissions. This is called the 'symbiosis' industrial complex.

2.1.1 *Chlorella vulgaris*

Chlorella vulgaris is a spherical single-celled green algae with diameter ranging in between 3-12 µm. Figure 2.2 shows the microscopic image of *C. vulgaris*. *Chlorella* sp. was used in the experiment due to their availability in the market, easy handling and cultivation in the laboratory scale (Ahmad et al., 2011). *C. vulgaris* is produced and available in the market due to its extraordinarily flexibility and adaptation against unfavorable growth conditions and invaders. Approximately 2000 tons dry weight of *C. vulgaris* was produced in 2009 and the main producers are Japan and Taiwan (Eguchi et al., 2004). *C. vulgaris* is a mixotrophic microalgae species that can be grown in mixotrophic condition where it uses CO_2 as inorganic carbon source through photosynthesis proses or utilizing only organic carbon source depending on the concentration of carbon compound or light intensity available (Mata et al., 2010). *C. vulgaris* cultivated in 50% growth media nutrients produced higher biomass compared to that of 100% nutrients composition which can reduce chemical cost for large scale production (Blair et al., 2014).

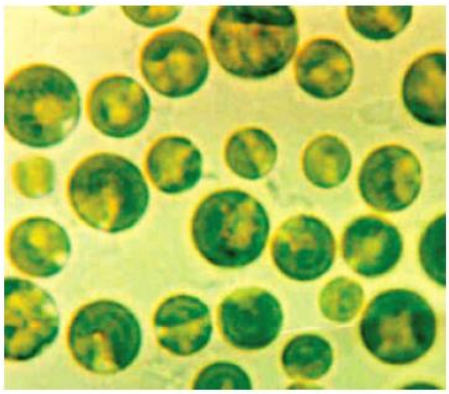

Figure 2.2: *Chlorella vulgaris* (Benemann, 2008)

Microalgae can be cultivated in the wastewater through heterotrophic metabolism (Blair et al., 2014). Cultivation of microalgae in wastewater seems to be promising due to the possible minimization of fresh water, abundance available nutrients in wastewater to support microalgae survival, and elimination of pesticide and herbicide uses. Different species of microalgae could reduce 50-90% the amount of metal ions along with reduction of biochemical oxygen demand and chemical oxygen demand in various type of wastewater (Suali & Sarbatly, 2012). *C. vulgaris* is considered one of the best microalgae to be used for wastewater bioremediation. *C. vulgaris* was demonstrated to be able to remove 100% ammonium, 45-90% nitrogen, 28-96% phosphorus and 61–86% reduction in chemical oxygen demand for various type of wastewater (Mata et al., 2010; Pittman et al., 2011; Safi et al., 2014).

C. vulgaris generally has great benefits and been used in various application such as food supplements, food preservation, food coloring, animal feeds, and also in wastewater treatment. *C. vulgaris* extracts contain the highest antioxidant activity among other microalgae tested (Rodriguez-Garcia & Guil-Guerrero, 2008). *C. vulgaris* is a natural antioxidants source used as additives to replace chemical additives in food processing to prevent lipid peroxidation which is the main cause of food deterioration (Goiris et al., 2015). Addition of microalgae biomass increases the quality and nutrient in human food and animal feed resulting in the enhancement of health and life expectancy of human and animals (Safi et al., 2014).

Microalgae also received interest in literature for prevention of wide range of degenerative diseases (Rodriguez-Garcia & Guil-Guerrero, 2008). *C. vulgaris* is also suitable to be used as food coloring. Addition of *C. vulgaris* on pasta (Fradique et al., 2010) and traditional butter cookies (Gouveia et al., 2007) significantly increases appeal

and firmness of the food. It gives food nutritional advantages without affecting or deteriorating the cooking and texture properties of food. The green color from *C. vulgaris* remains stable for 3 months of storage periods for the tested butter cookies.

2.1.2 Potential of microalgae as renewable energy

Reliance on energy sources offers dramatic consequence on energy security and environment. Resource depletion, instable fossil fuel prices, and emission of greenhouse gases contributed to increase of global climate which has induced the research institutes to find alternative renewable fuel resources. Renewable energy contributed 4% of global energy demand with biofuels production increased by 2.6% in 2016 (BP, June 2017).

Biofuels are found to be potentially attractive as an alternative to replace fossil fuel. Even though the biofuels innovation is very creative, the earlier generation of biofuel has caused a few controversial debates among the researchers and industrial people. Microalgae was discovered to be more beneficial to convert to biofuel than the previous two generations of biofuel crops. Table 2.1 shows the generation of biofuel crops and their issues.

Microalgae are receiving attention and have been considered as one of the alternative renewable and environmental friendly feedstocks for biofuels production. Majority of microalgae has 10-50 times higher cell growth rate compared to terrestrial plants (Yen et al., 2013). Microalgae are a good candidate for biofuel production attributing to fast growth rate, high oil content, high photosynthesis efficiency, low water consumption, high oil yield per unit area of land.

Table 2.1: Generation of biofuel crops and their issues

Biofuels	Crops	Issues
First Generation	Food crops, e.g., corn, sugar beet, sugarcane, palm oil, and edible oil.	• Negative impact on food security mainly in food constraint regions causing imbalance global food market. • Huge water consumption and deforestation for plantation of crops. • Increase in food prices. • Pressure on production of crops.
Second Generation	Non-food feedstock: • Lignocellulosic of forest and plantation residues, e.g. palm oil empty fruit bunch, and saw dust. • Energy crops, e.g. jojoba oil, jatropha, and tabacco seeds. • Waste, e.g. waste cooking oil, restaurant grease and animal fats.	• Difficult in collection of raw materials. • Challenging in conversion of raw materials to biofuels
Third Generation	Microalgae	• Promising due to fast biomass production, easy to cultivate, low water consumption and low land occupation. • Eliminate competition of 'energy versus food'. • High lipid content up to 80% of biomass dry weight and able to produce algal oil 25 times greater than any other crops. • Non-toxic and highly biodegradable microalgae-derived biofuels. • Not competitive with petroleum due to high cost from harvesting and extraction processes.

Table 2.2 shows the comparison between biofuel crops. Microalgae eliminate the competition of energy feedstock with food. Microalgal lipids can produce different biofuels including biodiesel, bio-oil, biosyngas, bioelectricity and bio-hydrogen. Microalgae biofuels are definitely environmental-friendly energy sources since the microalgae biofuels do not produce any toxic effluent and emission from the system. Bio Fuel Systems (BFS) is a company located in Spain that is able to produce microalgae biofuel at an industrial scale and introduced them as Blue Petroleum. The process system developed produces 35,000 tons of fuels and 3,000 tons of nutritious byproducts. In one barrel production of BFS Blue Petroleum, the system managed to absorb 2,168.76 kg of industrial CO_2, neutralized 937.95 kg CO_2 emission and released 452 kg of oxygen into atmosphere from photosynthesis (BFS, 2013).

Table 2.2: Comparison of biofuel crops (Chisti, 2007; Mata et al., 2010)

Crops	**Oil content (%)**	**Oil Yield (L/ha.year)**
Corn	44	172
Hemp	33	363
Soybean	48	446 - 636
Jatropha	28	741 – 1,892
Camelina	42	915
Canola	18	974 – 1,190
Sunflower	41	1,070
Castor	40	1,307
Palm Oil	36	5,366 – 5,950
Microalgae (low oil content)	30	58,700
Microalgae (medium oil content)	50	97,800
Microalgae (high oil content)	70	136,900

2.2 Mass production of microalgae

Microalgae could produce various types of biofuels comparable to the former generation of biofuels. Microalgae go through many kind of processes such as cultivation, harvesting, extraction and conversion before producing the algal biofuels. Figure 2.3 shows the flowchart of microalgae from cultivation process until conversion to various type of biofuels.

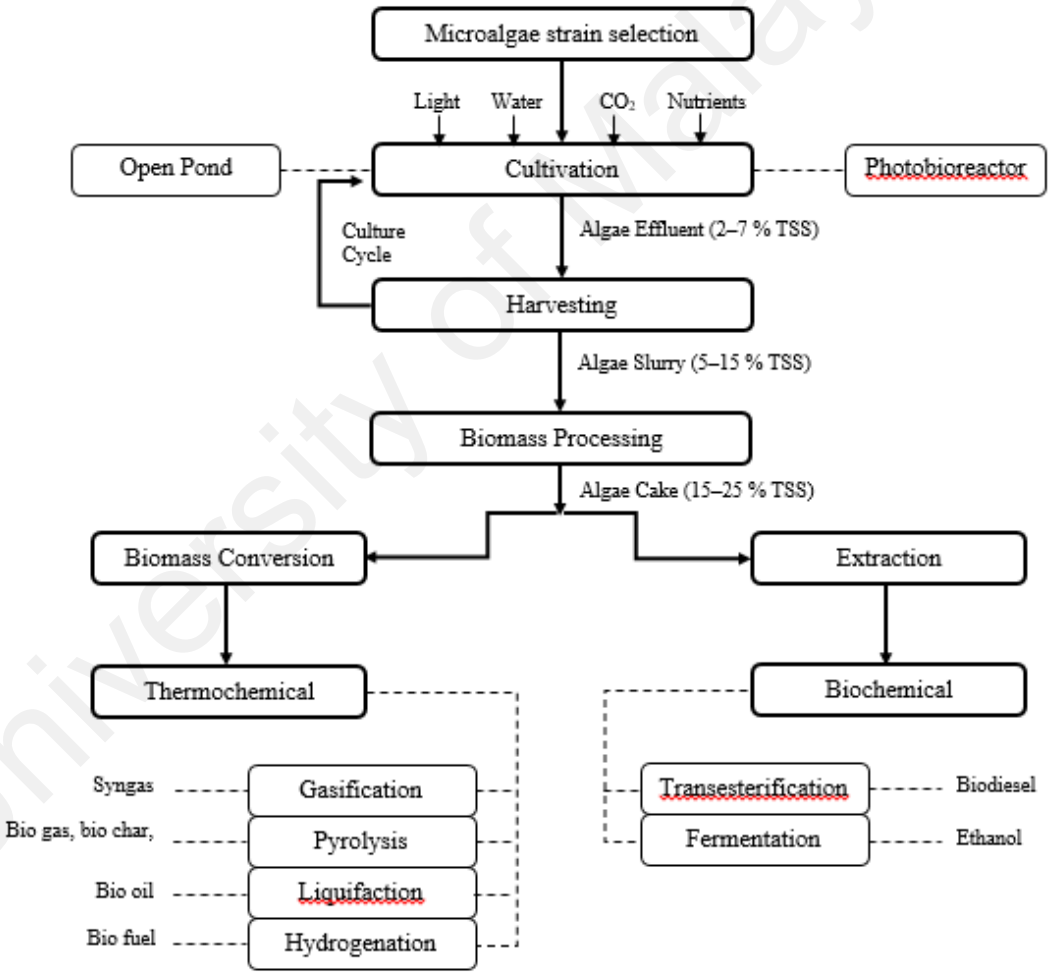

Figure 2.3: Microalgal biofuel processes flowchart. Adapted from (Mata et al., 2010; Suali & Sarbatly, 2012)

2.2.1 Selection of microalgae strain

Before the cultivation of microalgae, selection of microalgae species is an essential step that must be taken into consideration. Different species of microalgae produce different amount of lipids and hydrocarbons. The most important aspects of microalgae selection for biofuels production are biomass productivity and percentage of lipid content. Hundreds of microalgae strains are capable of producing high content of lipid. Depending on the species, strains and growth conditions, lipids can constitute up to 80% of the algal dry mass.

Chlorella sp. has frequently been reported to be the best strain for biofuel production. The species could produce 63% of lipid content per dry weight biomass. *C. vulgaris* has high growth rates; it could generate 5.7×10^7 cells per millimeter volume for 34 days of cultivation. *C. vulgaris* has a low water footprint compare to other microalgae species which are 1-6 times higher (Yang et al., 2011). Biodiesel derived from *C. vulgaris* has the exact petroleum diesel characteristics that comply with various international standards (Safi et al., 2014). *C. vulgaris* cultivated in steel plant wastewater could sequestrate 0.624 g CO_2 per liter per day and reduced 10-50% flue gases concentration (Brennan & Owende, 2010).

2.2.2 Cultivation of microalgae biomass

High yield of microalgae biomass indicates the efficiency of the cultivation system. The designation of cultivation system requires understanding of photosynthetic metabolism and physiology of microalgae used (Yen et al., 2013). Different species of microalgae respond differently to environmental parameters that can affect the growth

rate and lipid productivity. The cultivation process of microalgae can be done in open system, i.e. open pond or in close system, i.e. photobioreactor (PBR). The method of cultivation is very much dependent on the type of microalgae species and their adaptability to the environment.

Open pond is the most common cultivation method for large scale biomass production that can either be a natural water catchment area like brackish water for marine microalgae (Widjaja et al., 2009) or artificial pond using containers. Open pond is suitable to cultivate robust autotrophic microalgae species due to its possibility of risks in contamination, invading bacteria, and difficulty to control temperature difference, sunlight and CO_2 concentration. Thus, open pond is suitable to be built in tropical region next to power plants or industries that can supply massive CO_2 emission.

PBR was designed to overcome the limitations of open pond. There are various types of PBR: flat-plate PBR, tubular PBR, and column PBR. PBR can generate higher biomass productivity due to its ability to control the medium environment such as pH, light intensity, temperature and CO_2 concentration; thus making it suitable to cultivate fragile and heterotrophic microalgae species. Benefits hold by PBR are less land footprint and culture medium recycle. However, PBR's construction and operation cost is relatively higher than that of an open pond (Suali & Sarbatly, 2012).

There are several types of harvesting methods to recover microalgae biomass which will be explained in detail in the next. Drying method would be required after dewatering of microalgae biomass to ease some conversion methods that converting oil into biofuel.

2.2.3 Harvesting techniques for microalgae biomass

Recovery of microalgae from culture medium is done after the cultivation process to concentrate the microalgae biomass to 5–25% solid content (Mata et al., 2010). Harvesting microalgae is tricky and presents some difficulties due to small size microalgae cells (less than 10 μm) in dilute culture (less than 2 g/L) with density similar to water (Kim et al., 2013). Negative surface charge from extracellular polymeric substance (EPS) carried by microalgae make them disperse from each other and hard to settle. Microalgae harvesting usually needs one or more solid-liquid separation operation units. The selection of harvesting method depends mainly on microalgae characteristics, desired product and the subsequent processes (Kiran et al., 2014; Pragya et al., 2013).

Microalgae harvesting employs different combination water purification technologies that are divided into two-stage processes involving bulk harvesting and thickening. Bulk harvesting separates biomass from bulk culture medium and concentrates to 2-7% solid content (Brennan & Owende, 2010). Bulk harvesting techniques are such as flocculation, flotation, and gravity sedimentation. Thickening is a technique to concentrate the biomass slurry and is more energy demanding compared to bulk harvesting. Thickening techniques include filtration, centrifugation, and ultrasonic aggregation. Combination of harvesting methods could overcome the weakness of individual technique. Table 2.3 shows the summary of pros and cons of microalgae harvesting techniques.

Table 2.3: Summary of microalgae harvesting techniques (Rashid et al., 2014; Rawat et al., 2013; Tan et al., 2016)

HARVESTING METHOD	ADVANTAGES	DISADVANTAGES
Flocculation	Increase size of microalgae; low cost; low energy consumption; able to handle large volume; high removal of microalgae.	Plentiful chemical usage; biomass contamination; sensitive to changes of pH; low harvesting efficiency for small cell size.
Flotation	Does not require addition of chemicals; small microalgae naturally float; easy application on large scale; efficient to harvest microalgae from wastewater.	Species-specific; high capital and operational cost.
Gravity Sedimentation	Low cost; depends on settling characteristics; commonly used in wastewater treatment; recycle of culture medium.	Slow process; dependent on microalgae cell density; product deterioration.
Filtration	Wide range of filter and membrane types; recycle of culture medium.	Periodically replacement of membrane; requirement of pump; fouling; species-specific.
Centrifugation	Rapid; efficient; for high value product; able to handle large volume.	Energy intensive; high maintenance cost; cell damages due to high shear force.
Ultrasonic Aggregation	Efficient separation; non-fouling technique.	High cost; limited evidence on technique

2.2.3.1 Flocculation

Flocculation bridges the particles physically to each other to form larger particle size with addition of flocculants that neutralize the negative charges of microalgae surface thus disrupt the stability of dispersed state of microalgae cells. Flocculation helps microalgae cells to coagulate and settle before going through other separation process such as flotation, filtration, centrifugation or sedimentation.

There are two types of flocculants, i.e. organic and inorganic. Inorganic flocculants are multivalent metal salts, i.e. ferric chloride, aluminium sulphate, and ferric sulphate that are very frequently used in the industrialize sedimentation tank. Residual of inorganic flocculant gives negative impact for nutrients and water recycling, production large quantity of sludge and reducing the quality of end products due to contamination of aluminium and iron salts (Kiran et al., 2014). Organic flocculants are biodegradable such as chitosan and starch can overcome the drawback of inorganic flocculant.

Flocculation is not suitable to harvest marine microalgae due to the ionic strength of seawater (Kim et al., 2013). Microalgae can have auto-flocculation when their negative surface charge decrease in stationary and declining growth phase as pH increases due to accumulation concentration of CO_2, nitrate, and phosphate (Safi et al., 2014). However, the most suitable period of microalgae harvesting is during the end of exponential stage where the microalgae biomass is the most abundant.

2.2.3.2 Gravity sedimentation

Gravity sedimentation is a process that utilizes the suspended solid density and the settling characteristics of particle to let the particle settled by gravitational force in the settling tank. Gravity sedimentation process always had flocculation process as a preparatory step to enhance the settling characteristics of particle. Wastewater treatment always applies flocculation-sedimentation techniques to recover biomass since the biomass has a low value and is treated in large volume. Gravity sedimentation is only suitable for large algae size more than 70 mm like *Spirulina sp*. Gravity sedimentation is a slow process that could deteriorate microalgae biomass in hot climate (Rawat et al., 2013). Moisture in microalgae biomass could influence the end product of biofuels. Dewatering process either thermally or mechanically is required after harvesting from sedimentation process which would be more costly and energy intensive.

2.2.3.3 Flotation

Flotation is a method that uses air bubbles buoyancy to attach and trap microalgae cells on top of water surface and later collected by skimming. Flotation can naturally occur when the lipid content in microalgae increases (Safi et al., 2014). Flotation using the fine bubbles to float the microalgae cells and managed to collect up to 7-10% of total suspended solid of algae suspension (Rawat et al., 2013). The only constraint of flotation process is that it can only float the microalgae species that has small particle size (10-30 μm) and small molecular weight. Flotation process can be divided into dissolved air flotation, dispersed flotation and electrolytic flotation based on the air bubble size used. The smaller the bubble size the longer of flotation to carry the microalgae cells.

Flotation efficiency is affected by hydraulic air flow rate, time retention, and microalgae surface characteristic such as hydrophobicity and surface charge (Rashid et al., 2014). Addition of ozone could change the hydrophobicity of microalgae cell wall by releasing protein and ease the flotation process (Henderson et al., 2010). Flotation is the most chosen technique for large scale production if the construction and operating cost is high (Tan et al., 2016).

2.2.3.4 Centrifugation

Centrifugation process are used to harvest biomass to produce high value product especially food and aquaculture products and to extent the concentrates' shelf-life for succeeding processes. Centrifugation is highly efficient where 80-90% of microalgae biomass could be recovered within 2 to 5 minutes (Chen et al., 2011). Centrifugation is the preferable harvesting method to help prolong algae shelf-life as it using centrifugal force without contamination of chemicals and bacteria in raw product (Grima et al., 2003). The concentration of slurry after centrifugation is increased by 150 times (Brennan & Owende, 2010).

There are different type of centrifuges: nozzle type centrifuge, solid-ejecting centrifuge, solid-bowl-decanter centrifuge, and multi-chamber centrifuge. Multi-chamber centrifuge gained attention due to its ability to separate biomass based on their particle sizes (Rashid et al., 2014). Although centrifugation technique is very effective for thickening suspension in large volume, it is very costly in related to maintenance of its moving equipment and energy demanding that makes it negative for commercialization of biofuels. In addition, microalgae cell could be damaged due to high gravitational and shear force (Chen et al., 2011).

2.2.3.5 Ultrasound aggregation

Ultrasound aggregation is a new technique and limited literature available reporting about its application in harvesting microalgae. Ultrasound can be used for microalgae harvesting and lipid extraction by manipulating the frequency and amplitude of wavelength (Kim et al., 2013). Ultrasound wavelength agitates and aggregates microalgae cells and then the cells settle by gravity force when the ultrasonic field is turned off. Ultrasonic aggregation has many advantages: very efficient, no introduction of shear force to the cells, and it is a non-fouling method. Despite its advantages, ultrasound cannot be used in a large volume harvesting due to high energy input and low separation efficiency.

2.2.3.6 Filtration

Filtration is a solid-liquid separation process that separates solid through filter media with particular pore size. Conventional filtration such as filter presses and rotary drum are capable of harvesting large microalgae species with size more than 70 mm like *Spirulina* sp. in large volume of culture medium but the process is relatively slow. Chamber filter press could produce 245 times more concentrated biomass, 27% cake slurry for large microalgae species such as *Coelastrum proboscideum* (Grima et al., 2003). Membrane filtration is more suitable for microalgae size less than 30 mm such as *Dunaliella* sp., *Scenedesmus* sp., and *Chlorella* sp. Membrane filtration is alternative to conventional filtration that suitable for harvesting of fragile microalgae when operates in low transmembrane pressure (TMP) and low crossflow velocity (CFV) condition (Brennan & Owende, 2010).

The cost of membrane filtration are mainly due to replacement of membrane and pump equipment but this condition can be controlled by operating parameters and backwashing to prolong the membrane's shelf-life. Based on Tan (2016) fuzzy analytical hierarchy process study, filtration is the preferred alternative when the environmental criteria, carbon footprint, and land footprint are considered.

2.2.4 Downstream processes for microalgae biofuel products

Microalgae would have to go through extraction or directly conversion method to produce biofuels. Extraction processes can be done chemically or mechanically. Chemical extraction is the most common method for extraction that involves solvent such as hexane, chloroform, and alcohol mixtures to extract lipid. Physical extraction uses microwave and ultrasonic.

Biochemical processes convert the extracted lipid into biofuel products through transesterification and fermentation. Transesterification process produces biodiesel as a main product and glycerol as byproduct. Microalgae biodiesel can be used directly or as a blend with diesel fuel that is very suitable to be used as transportation fuels especially for aircrafts due to its less sulfur content; and the emission of sulfur dioxide to the atmosphere through vehicle exhaust can be avoided (Suali & Sarbatly, 2012). Fermentation uses microorganism such as yeast and bacteria to convert microalgae carbohydrates into bioethanol. *C. vulgaris* is a good source for bioethanol production and achieved 65% conversion in yeast fermentation (Safi et al., 2014).

Thermochemical processes use high temperature to convert microalgae biomass that rich in carbohydrate and protein after lipid extraction into various biofuels. Pyrolysis occurred at temperature condition of 200-750°C and it does not require oxygen in its heating process. Fast pyrolysis process produces bio-oil and bio-char while slow pyrolysis process produces pyrolysis gases, i.e. methane, carbon dioxide and bio-char as byproduct. Liquefaction is a catalytic process of microalgae biomass in water at high temperature in between 200-500°C and high pressure of more than 20 bar. Liquefaction products are bio-oil, gas mixture and ashes. Gasification is an aerobic heating process with presence of oxygen at temperature in between 200-700°C to produce gases such as hydrogen, carbon monoxide and methane. Hydrogenation is a process of hydrogen gas addition into the hydrocarbon double-bond to add the molecular weight of feedstock for further conversion into biofuel.

The production of microalgae biofuels in large scale has a lot of drawbacks even though the procedure is feasible and clearly organized. The main problem would be the huge cost to build large-scale production systems and infrastructure including wastewater treatment, water supply and any other support system. Cultivation system using PBR is costly due to addition of light energy and nutrient supplies. Also, extra attention should be given towards delicate microalgae to avoid contamination as this will highly affect the productivity of biofuels.

Harvesting and drying are the most challenging of upstream processes that hindering microalgae biofuel into commercialization due to their effect on operation cost and energy intensive as several processing units are needed for large production (Tan et al., 2016). Harvesting and dewatering processes contribute 90% of equipment cost for microalgae biomass production in open systems (Tan et al., 2016).

There is no cost-efficient technologies available for harvesting and extraction of microalgae biomass (Benemann, 2008; Chen et al., 2011). A trade-off in economy can be done if excess nutrients and water were recycled back into cultivation system during harvesting stage to recover microalgae biomass (Yang et al., 2011). Harvesting of microalgae must be done quickly to avoid microalgae concentrates (5-15% solid content) spoiled in a few hours in hot climate (Mata et al., 2010). Development of efficient harvesting method is critical to ensure economic and environmental sustainability of microalgae-based fuel.

2.3 Membrane technology

Membrane separation was discovered since the late 19th century. However, the research on application of membrane did not receive great attention until a few decades ago. Membrane technology is used by a wide range of industries such as food and beverages, pharmaceutical, oil and gas, chemicals, water and wastewater treatment (Mulder, 1996).

Membrane separation system has many advantages: 1) separation could be done physically without alteration to the feed, 2) low energy consumption, 3) continuous operation, 4) easy scale-up, and 5) capability to combine with other separation operation or system. Despite its great advantages, membrane separation system also has a few drawbacks that can be controlled through its operation parameters and membrane material. The disadvantages are: 1) fouling, 2) low flux, 3) low membrane lifetime, and 4) linear up-scaling.

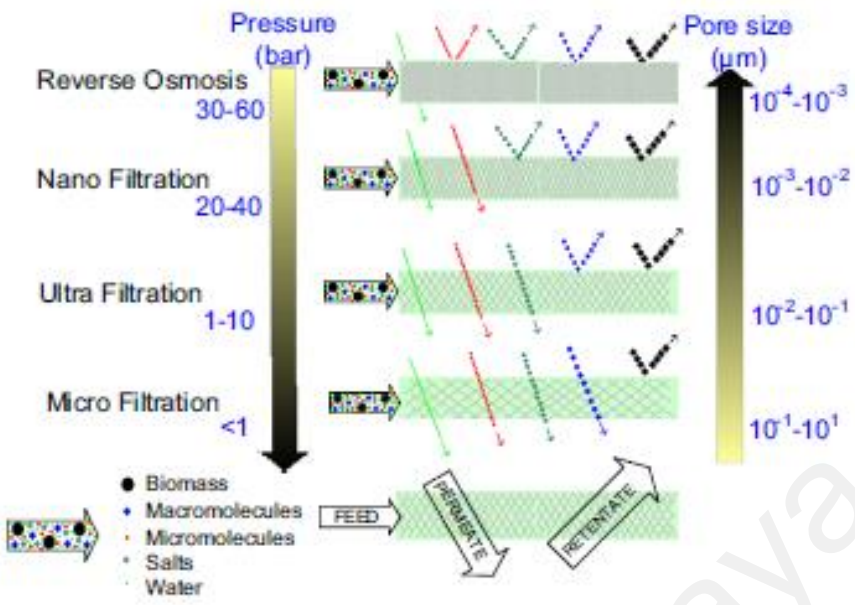

Figure 2.4: Solute rejection in pressure driven membrane processes (Bilad et al., 2014)

There are four primary pressure-driven membranes that can be divided into different range of pore size known as microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO). The application of membrane is very much dependent on the particle size of solute. Figure 2.4 shows the schematic illustration of solute rejection on pressure driven membrane processes.

There are two types of conventional membrane configuration, i.e. dead-end and cross-flow. Dead-end filtration mode operated when the feed flow is perpendicular to the membrane surface. The particles are directly retained on the membrane surface and accumulated as the filtration time increase while decreasing the permeation flow rate. Cross-flow filtration mode operates when feed flow is along the membrane surface, thus only a part of the particles from the feed channel is accumulated on the membrane surface. Many new membrane configurations have been developed to improve the conventional configuration such as submerged membrane, dynamic or shear-enhanced module and forward osmosis (Barros et al., 2015; Mo et al., 2015).

2.3.1 Application of membrane technology in microalgae harvesting

Membrane filtration has been studied for harvesting of microalgae using various membrane technologies. Several reviews have been conducted on the potential of multiple membrane technologies for dewatering microalgae (Bilad et al., 2014; Gerardo et al., 2014; Mo et al., 2015). A summary of studies on microalgae harvesting using membrane technologies is shown in Table 2.4. The table shows the membrane applications for single-step microalgae harvesting according to microalgae species, membrane configurations, targeted filtration parameters and fouling management used.

Most of membrane technologies used for microalgae harvesting are MF and UF due to microalgae particle size that lies within micrometers. Most of membrane material used is polymer-type flat sheet membrane with crossflow filtration configuration system. Crossflow filtration helps to reduce fouling due to tangential flow compared to dead-end (Kim et al., 2015). Membrane filtration is applicable to both freshwater and marine microalgae as summarized in Table 2.4.

Unfortunately, most of the studies observed that membrane fouling and flux decline as the main concern during the microalgae harvesting as this will increase the operational cost. Ahmad (2012) found that high TMP and CFV increased high permeate flux but this condition also caused fouling dominated by cake layer formation. High biomass concentration factor caused high energy consumption and low biomass recovery which lead to rapid flux decline (Danquah et al., 2009; Huang et al., 2012; Pavez et al., 2015).

Table 2.4: Summary of studies of microalgae harvesting using membrane technology

Membrane Filtration (mode, type, operating parameters)	Microalgae species	Findings	Reference
CA, circular flat sheet, batch, crossflow, MF, 1.2 µm, hydrophilic, negative surface charge. TMP: 0.5-1.5 bar; CFV: 0.13-4.0 ms^{-1}	*Chlorella* sp.	- The permeate flux increased with increasing TMPs and CFVs. - R_c becomes the dominant resistance to the filtration rate.	(Ahmad et al., 2012)
PVDF, flat sheet, batch, crossflow, MF, 0.22 µm. TMP: 30 psi; CFV: 3-5 L/min. Cleaning: 0.1 M NaOH.	*Tetraselmis suecica*	- 100% retention of microalgae. - Less energy consumption and better dewatering performance for harvesting of LGRP culture compared to HGRP culture.	(Danquah et al., 2009)
PET, crossflow, MF, 4.0 µm. PVDF, crossflow, MF, 0.45 and 0.20 µm Coating: PVA, hydrophilic TMP: 200 kPa, CFV: 1.0 ms^{-1}	*Chlorella* sp.	- Surface-coated membrane with PVA shows better performance compared to unmodified membrane. - Hydrophilic property of coating limits foulant attachment on membrane surface.	(Hwang et al., 2013)
PVDF, flat sheet, submerged, MF, 9%, 12%, 15% w/w IFM and batch filtration comparison	*Chlorella vulgaris, Phaeodactylum tricornutum*	- Lower fouling degree using IFM submerged MF compared to batch submerged MBR.	(Bilad et al., 2012)
PVDF, flat sheet, continuous, crossflow, MF, 0.1-1.5 µm. PAN, flat sheet, continuous, crossflow, UF, 40 kDa. TMP: 105 Pa, CFV: 2.5 ms^{-1}	*Haslea ostrearia, Skeletonema costatum*	- 100% retention of microalgae. - UF performance better than MF. - Hydrophilic, negative charge UF membrane recommended for long term, high concentration operation	(Rossignol et al., 1999)

Table 2.4: Summary of studies of microalgae harvesting using membrane technology, continued

Membrane Filtration (mode, type, operating parameters)	Microalgae species	Findings	Reference
PS, flat sheet, crossflow, UF, 60 kDa CFV: 0.14 ms^{-1} Cleaning: NaOH, NaOCl	*Microcystis aeruginosa*	- *Microcystis* acted as secondary membrane to NOM. - Strong repulsion existed due to negative charges of membrane and *Microcystis* cells.	(Kwon et al., 2005)
PVC, hollow fiber, UF, 50 kDa TMP: 34.5 kPa, CFV: 0.17 ms^{-1} Air-scouring Cleaning: NaOCl	*Scenedesmus quadricauda*	- Fouling dominated by cake layer caused by adsorption of AOM on membrane surface. - Air scour, optimized backwash interval and CFV maintained membrane high flux. - Fouling and flux decline can be predicted by resistance-in-series model.	(Zhang et al., 2010)
PAN, UF, 40 kDa, coupling with PBR TMP: 1 bar Crossflow and rotating disk module comparison	*Cylindrotheca fusiformis, Skeletonema costatum*	- Rotating disk module permeate flux twice higher than crossflow module - Dynamic module reducing microalgae feed flow and lowering EPS synthesis	(Frappart et al., 2011)
PVDF, hollow fiber, UF, 0.2 µm TMP: 50 kPa Cleaning: 1% NaOH	*Chlorella* sp.	- Periodical backwash with permeate was more effective than air–water flushing. - A high volume concentration factor or a high initial biomass resulted in a low biomass recovery.	(Huang et al., 2012)
PES, tubular, UF, 30 nm CFV: 1.5 ms^{-1} Cleaning: NaOCl	*Nannochloropsis gaditana, Chlorella sorokiniana*	- Low recovery for high concentration of biomass - Membrane filtration not recommend as sole concentration method for non-axenic culture. - Recommended for post concentration alternative that normally provide low volume concentration factor.	(Pavez et al., 2015)

Recent studies have focused on fouling with the aim to find the optimum condition with the best possible configuration to prolong membrane life, reduce chemical and financial demand in microalgae harvesting. A few studies came up with anti-fouling measures for better efficiency such as membrane modification using hydrophilic negative-charge membrane (Hwang et al., 2013; Rossignol et al., 1999), and clean-in-place procedures like backwashing (Huang et al., 2012; Zhang et al., 2010) and chemical cleaning (Kwon et al., 2005). Futhermore, dynamic membrane modules were developed to reduce fouling such as improved flux step method in submerged MF (Bilad et al., 2012), rotating disk membrane (Frappart et al., 2011; Kim et al., 2015), magnetic vibration membrane (Bilad et al., 2013; Nurra et al., 2014), and electro-membrane (Kim et al., 2014). Pavez (2015) suggested to use the membrane filtration as post-concentration dewatering technique as the condition provides less biomass concentration factor.

It is critical to understand how a membrane is fouled to be enable choosing proper membrane properties and design a suitable membrane configuration that helps to mitigate fouling and enhance the membrane performance to cater for unique characteristic of each microalgae species (Hwang & Liao, 2012; Kim et al., 2013).

2.4 Membrane fouling

In membrane operation, the feed solution is split into retentate and permeate. The performance of membrane filtration usually is observed by flux-time behavior changes throughout the operating time and solute rejection (Bilad et al., 2014). Permeate flux gives quantitative value of how much permeate passes through membrane and rejection is how much targeted solute retained in the retentate.

Fouling is a phenomenon that occurred to pressure driven membrane processes as flux decline continuous after some elevated operation time. MF and UF are often associated with severe fouling during which flux declined to less than 5% of pure water flux due to complex interaction between feed solution characteristic or elements and porous membrane surface (Mulder, 1996). Membranes that retain low molecular particles i.e. NF and RO are subjected to low fouling tendency.

Application of MF and UF for microalgae harvesting is very effective but they are prone to severe fouling attributed to interaction of factors that involves microalgae complex characteristics and membrane operating conditions. Microalgae harvesting using membrane filtration also involves in reversible and irreversible fouling. The reversible fouling can be reduced with physical clean-in-place procedure such as backwashing. However, irreversible fouling can only be removed by chemical cleaning (Dvorák et al., 2011). Knowledge about causes and mechanisms of fouling helps to mitigate the fouling symptoms and find the appropriate solution to avoid severe fouling from happening.

2.4.1 Fouling mechanisms

Fouling mechanisms include adsorption, pore blocking, cake formation, and concentration polarization. Adsorption is a situation where the particle cells retained at the pore wall of membrane. This happens when the particle size is smaller than the membrane pore size. The particle started to deposit on the membrane surface due to the application of pressure causes some particle restricting the pore opening to certain degree allowing development of initial fouling layer. Initial pore blocking can be completely plugging of a pore, limiting the pore opening through particle deposition around the pore or combination of the two (Pearce, 2007).

Continuous deposition of particles sufficiently on initial layer will form a cake layer on the membrane surface and control the permeation of membrane. Cake layer has some beneficial effects such as improving removal efficiency and protection from adsorptive fouling. Cake formation phenomenon needs to be controlled as a dense cake will be formed after a period of filtration time resulted in low permeability or poor membrane performance. TMP, particle size and particle deformability could be factors affecting the cake permeability.

Low performance of membrane from fouling can be resulted from polarization phenomena. Concentration polarization happens when concentration of feed channel builds up as the particulates retained on membrane surface; the filtration and permeation becoming more limited until a steady-state condition established. Concentration polarization strongly influences the selectivity especially macromolecules or high molecular weight particulates as the retention gets higher when concentration of feed increased. Thus, concentration polarization can be quite severe in MF and UF operating system which mostly used for filtration of micro and macromolecules. Concentration polarization is lower with filtration of low molecular weight solutes in NF and RO and hardly occurs in gas separation (Mulder, 1996).

The phenomenon of fouling is very complex that involves physical and chemical interactions i.e., concentration, pH, temperature, ionic strength, chemical bonding etc. (Pearce, 2007). Thus, fouling mechanisms that happened in the process are difficult to explain theoretically through graphical flux-time behavior. A reliable method to describe flux decline is by using resistance-in-series model, in which total fouling resistance could be a complete series of membrane, adsorption, pore blocking, cake layer, and concentration polarization resistances (Mulder, 1996).

The degree of fouling mechanisms phenomena will determine the degree of attachment to membrane or fouling severity. Every process has different fouling state depending on membrane process, operating parameters, and condition of feed solution involved. Resistance-in-series mathematical model used in this study is described in Subtopic 3.4.

The most ideal situation is when only membrane resistance R_m involved where the barrier to the permeability of flux is just the existence of membrane itself but this situation is almost impossible to maintain in most cases of macromolecules membrane processes (Mulder, 1996). Flux decline indicates that some resistances other than R_m may involve in the process. A resistance from adsorption phenomena R_a arises upon deposition on surface or within pore of membrane. The pore blocking resistance R_b could arise from adequate blockage of membrane pore.

Continuous deposition of particulates formed a cake layer obstructs transport of solvent to membrane surface which can be evaluated as cake resistance R_c. Accumulation of particulates on membrane surface increases concentration of solution near membrane that create concentration polarization resistance R_{cp}. Knowing the resistance values can give views on situation happened on membrane and strategies effective to control it.

2.5 Membrane foulant

Membrane fouling can be categorized into 4 general groups: organic fouling, inorganic fouling, colloidal fouling, and biofouling. Colloidal fouling is caused by particulates or colloids foulant that block the membrane surface which can either be organic or inorganic in nature.

Inorganic foulant typically comes from chemical residual presents in coagulation processes such as calcium carbonate and silica that used in pretreatment to destabilize the colloids and reduce fouling on membrane. Organic foulant consists of organic matter (OM) that precipitated on the membrane surface due to pH change or oxidation (Hung & Liu, 2006). Organic fouling category can be overlapped with colloidal fouling and biofouling. Biofouling is a biotic form of organic fouling caused by OM derived from microorganisms such as microalgae and bacteria that form biofilms. Biofilms are a complex network associations of microbial cells, extracellular products and debris that bind together by polysaccharides (Sutherland, 2001).

Fouling causes by algae-related membrane processes can be categorized into organic fouling as the microalgae and its OM deposited on membrane surface during filtration and can formed biofilms that similar to activated sludge, biofilm, and anaerobic sludge in membrane bioreactor wastewater treatment, just the differences between them are by the foulant's composition and content (Liao et al., 2018).

Almost all microalgae species can produce OM during normal growth, in response to unfavorable environment conditions, and as well as during cell-rupture in the death growth phase (Zhang & Fu, 2018). There are several different terms to define organic

foulant caused by microalgae such as algoganic organic matter (AOM), extracellular organic matter (Her et al.), extracellular polymeric substance (EPS), and transparent exopolymer particles (TEP).

A study claimed biofouling during MF and UF of marine bloom-algae was caused by AOM which consists mostly of protein and carbohydrate (Ladner et al., 2010). The same foulant characteristics has been observed from EOM (Her et al., 2007) that has polysaccharide and protein substances on the surface of *Chlorella* sp. during MF (Hung & Liu, 2006). The most problematic foulants are identified as polysaccharides and protein in macromolecular or colloidal form that commonly found in microbial origin (Amy, 2008). EPS and TEP which mainly derived from polysachharides are said to be the main culprit of biofouling and will be discussed in the next section.

2.5.1 Extracellular Polymeric Substance

The main fraction of extracellular polymeric substance (EPS) are polysaccharides, proteins, nucleic acids, and humic substances from cell lysis (Drews et al., 2006; Sutherland, 2001). Microorganism can synthesize wide range of polysaccharides including intracellular polysaccharides, structural polysaccharides and extracellular polysaccharides. Polysaccharides are polymeric carbohydrate structure that can differ in chemical and physical properties in different microorganism species depending on their monosaccharide building blocks.

EPS produced by *C. vulgaris* are mainly dominated by glucose and fucose (Angelis, 2009). The composition and structure of some EPS resembles the cellulosic backbone of xanthan from *Xanthomonas campestris* and the molecular mass of the polysaccharides is

ranged 0.5-2.0 x 10^6 Da (Sutherland, 2001). The EPS has wide range of structure from soluble bound cell coating to weak gel-like polymeric colloidal that shed off from the exposed surface of biofilm which called TEP. EPS is insoluble gel polymer when interacted with ions. Polysaccharides from microalgae are rigid and non-deformable gels due to interaction with Ca^{2+} or Sr^{2+} (Sutherland, 2001).

EPS concentration can be influenced by cultivation conditions such as temperature, mixing hydrodynamic, initial pH and microbial growth rate. The amount of polysaccharides synthesis inside or outside the cells are greatly depending on the carbon substrate and nutrients. EPS synthesized in all growth phase but predominantly promoted during stationary growth phase of microorganism as seen in planktonic cells, microalgae, and enterobacterial species like *Pseudomonas aeruginosa* and *Escherichia coli* (Sutherland, 2001; Vanysacker et al., 2014). EPS acted as antibodies and metabolic strategy for stress response in unfavorable condition due to limiting nutrients and excess of carbon substrate (Angelis, 2009). EPS significantly influences biogeochemical cycling of aquatic environment such as mobility, bioavailability, and ecotoxicity (Shammi et al., 2017).

Diatom phytoplankton like *C. vulgaris* is known to generate a lot of EPS especially when growth condition deteriorate. A study from Angelis (2009) found 0.95 g/L of EPS production was observed after 24 days *C. vulgaris* culture cultivation. The main component of EPS can be varied based on the origin of microbial community (Vanysacker et al., 2014).

Humic substances is a major component EPS in the wastewater activated sludge (Dvorák et al., 2011) while protein and carbohydrate can be found in microbial biofilms. Colloidal EPS from polysaccharides exhibit higher fouling potential while humic substance will pass through the membrane (Drews et al., 2006; Dvorák et al., 2011). A study from Hung & Liu (2006) found that polysaccharides-like organic matter cause severe fouling especially for hydrophobic membrane due to hydrophobic bonding between the non-polar segment of polysaccharides and the membrane.

2.5.2 Transparent Exopolymer Particles

Transparent exopolymer particles (TEP) are one type of EPS that show sticky gel-like organic polymer that mainly consist of acidic polysaccharides and exist as individual particles rather than dissolved substances (Passow, 2002b). Eventhough TEP is EPS but not all EPS can occur as TEP. Formation of TEP by EPS could occur by complex mechanism between dissolved organic matter (DOM) and metal which causes ion neutralization due to exposure to sunlight (Shammi et al., 2017).

TEP are available in natural surface water (i.e., lake, river, pond, seawater) and wastewater. Colloidal TEP particle size can range from 0.001-0.4 μm (Kennedy et al., 2009). TEP are transparent and cannot be detected through microscopic thus they are visualized by staining with cationic Alcian Blue dye that absorbed by acidic polysaccharides. The amount of Alcian Blue dye absorbed representing the TEP need to be standardized using Gum Xanthan (Passow & Alldredge, 1995) as the real molecular weight of TEP are unidentified due to difficulty to isolate TEP individually.

2.5.2.1 Formation of Transparent Exoploymer Particles

Transparent Exopolymer Particles (TEP) are defined to be larger than 0.4 μm whereas the particles smaller than that are considered as TEP precursors (Shammi et al., 2017). Formation of TEP can be divided into two pathways. The first pathway of TEP formation is from TEP precursors consist of dissolved organic matter (DOM) that released by aquatic organisms during lysis or excretion products and formed continuously until it become colloidal DOM. The colloidal DOM later coagulated further to form large aggregate until eventually the TEP.

The second pathway of TEP formation is directly released as particulate by microorganisms especially for phytoplankton cells such as microalgae. TEP easily slough off from microalgae surface coatings due to shear force introduced from bubbling. The abundant of TEP and TEP precursors depend on the species releasing them, environment condition and also the physiological status of the microorganisms involved as the complex function of their growth condition (Passow, 2002b). The production of TEP by microalgae is very species-specific and achieved peak concentration during decline of algae-bloom dominated by diatom phytoplanktons such as *C. vulgaris* (Passow, 2002a).

Villacorte (2009a) found that TEP concentration in seawater is 2-6 times higher than freshwater source. TEP behaves like a gel existing in reversible interface transition between condensed particulate and hydrated dissolved material which is influenced by environmental factors such as pH, temperature, and ion density (Passow, 2002a). TEP have stickiness 2-4 magnitude higher than other phytoplankton and mineral particles (Berman et al., 2011).

TEP can be used as bioflocculant that helps aggregation of non-sticky particles like heavy metals and trace elements and promotes sedimentation. TEP most of the time acted as polymer particle than dissolved materials which can aggregate and possible to be collected through filtration (Passow, 2002a).

2.5.2.2 Role of Transparent Exopolymer Particles in membrane fouling

Transparent Exopolymer Particles (TEP) play important role in membrane fouling during low and high TMP operation. Many studies of membrane fouling from TEP were conducted using sources from wastewater or drinking water treatment plant.

A study by de la Torre (2008) monitored the TEP concentration in three units of submerged MF-MBR wastewater treatment plant and found that the TEP can be retained with total retention varying 40-96%. A linear correlation was found in TEP and polysaccharides to the critical flux measured (de la Torre et al., 2008).

The gel-like behavior of TEP gives it flexibility and thus can pass through pore size smaller than their particle size especially in raw water source treatment. Several studies found that soluble TEP still existed in RO system even after pretreatment processes (Bar-Zeev et al., 2009; Villacorte et al., 2009a; Wu et al., 2013). Clusters of colloidal and particulate TEP were found on support membrane of forward osmosis (FO) system (Linares et al., 2012). All the studies using high TMP operating membrane systems; RO and FO claimed that fouling caused by TEP does not come from the feedwater but instead by the development of biofilm on the membrane that begins since the first exposure to feedwater.

UF membrane system is efficient in removal of colloidal TEP compared to other pretreatment method (Villacorte et al., 2009a). Kennedy (2009) modified the calibration method for TEP quantification and monitored the TEP removal from integrated membrane system. 100% removal of TEP from secondary wastewater UF treatment plant causing 7% and 11% increase in irreversible and reversible fouling respectively over 30 hours operation period (Kennedy et al., 2009).

Li (2016) studied the impact of coagulation on the transformation of TEP in seawater and the effectiveness of combination of pretreatment of coagulation and UF membrane system. Ferum ion from ferric chloride increased the TEP size and contribute to fouling reduction thus enabling some degree of fouling control since UF system without coagulants could cause severe fouling (Li et al., 2016). However, the usage of coagulant is disadvantageous to microalgae harvesting as addition of chemical can change the composition of product in further processes for biofuel production.

Discart (2014) studied the origin and behavior of TEP in UF raw water treatment system for over 8-month period. The author also found that Fe-organic complex from addition of flocculant caused irreversible fouling even after clean-in-place procedure was done (Discart et al., 2014). An earlier study by the same author has studied the role of TEP on membrane fouling by different *C. vulgaris* broth solution and fraction. The results showed that all TEP variables caused fouling in membrane performance (Discart et al., 2013). However, the TEP production during all growth stages including death growth phase correlated with membrane fouling has not been studied by the author as the nutrients for microalgae cultivation was continuously refreshed.

2.6 Summary

Microalgae is a valuable material rich in proteins, carbohydrates, lipids, fatty acids, and pigments that can be used in various type of industries. Scientific discoveries from the past few decades also found that microalgae can be converted into biofuels due to its advantageous characteristics compared to other crop candidates. This opens up a new possibility of commercialization of microalgae production in order to fulfill the global demand. *C. vulgaris* has been a favorable choice among the many microalgae species due to its robust characteristics and high biomass production.

According to many literature sources, harvesting of microalgae using membrane filtration has proven to be the most environmental-friendly method compared to other methods that have been discussed. However, fouling in membrane filtration continues to be a challenging issue for large scale production of microalgae. Continuous operation of membrane filtration can lead to fouling that can increase operational and maintenance costs mainly due to the need for replacement of membranes and pumps. Thus, reducing the fouling conditions should be the priority together with maintaining the quality of microalgae in the process.

Most of the membrane fouling studies were performed on the hydrodynamic effect and operating parameters (shear rate, temperature, pH, transmembrane pressure, feed concentration) and membrane properties (materials, porosity, hydrophobicity or hydrophilicity affinity). Membrane fouling and its control has yet to be solved satisfactorily as the fouling phenomenon and foulant existed in algae-related membrane processes are still not well understood.

Several studies found that TEP play important role in membrane fouling and are largely produced by diatom microalgae such as *C. vulgaris*. TEP production depends on the species of microalgae, environment conditions, and the physiological status of microalgae. According to the review by Zhang (2018), there is no known study that observed the synergy effect of combined algae-derived foulant during the filtration of algae together with its organic matter. Most of the TEP-fouling studies were performed on identification of its chemical composition and particle size on various pore size of membrane without any relation to its production during normal microalgae growth cycle.

Thus, it is very important to know the production of TEP in relation to membrane fouling. The knowledge of TEP production during the growth curve of microalgae and its relation to fouling parameter is beneficial for larger production in order to choose the suitable time for harvesting and in the same time promote the cost reduction.

CHAPTER 3: MATERIALS AND METHODS

The flow chart of methodology in relation to objectives of study is shown in Figure 3.1. Objective 1 is obtained from cultivation of *Chlorella vulgaris* and analysis of TEP concentration. Objective 2 is obtained from harvesting of C. vulgaris using microfiltration. Objective 3 is obtained from calculation of membrane fouling parameters.

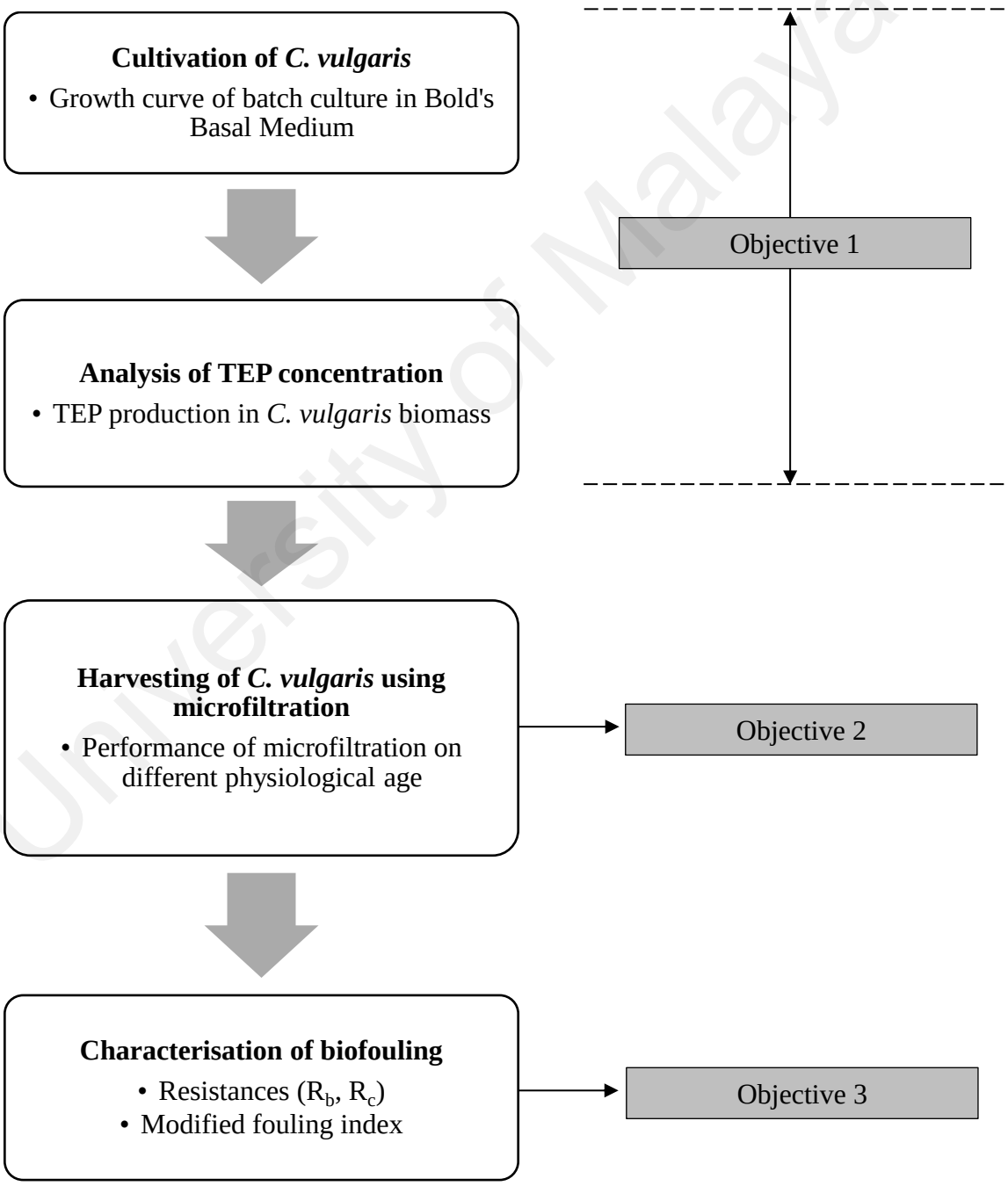

Figure 3.1: Flow chart of methodology in relation to objectives of study

3.1 Cultivation of microalgae and growth profile

The microalgae species used was *Chlorella vulgaris* Beijerinck (UMACC002) that obtained from University of Malaya Algae Culture Collection (UMACC). Figure 3.2 showed the microscopic image of *C. vulgaris* from UMACC. The culture medium used was Bold's Basal Medium (BBM) (Nichols & Bold, 1965) which contained with ten different types of chemical solutions. The composition and concentration of nutrients in medium culture are as shown in Table 3.1.

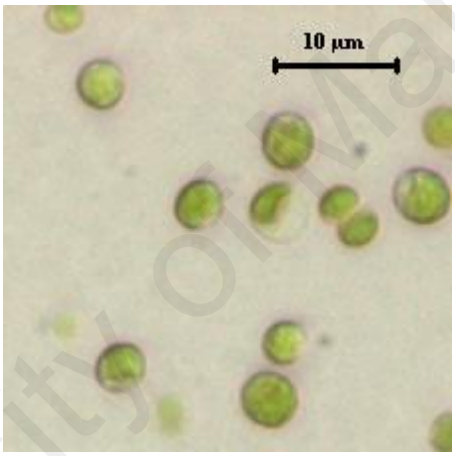

Figure 3.2: Microscopic image of *Chlorella vulgaris* from Universiti of Malaya Algae Culture Collection (Wong et al., 2011)

C. vulgaris cultivation was supplied with one time supply of BBM nutrients with pH of medium culture was 6.8±0.2. *C. vulgaris* was cultured in controlled-environment at 29±2 ℃ using 5L working volume of closed bubble column photobioreactor (PBR). The PBR consisted of dual transparent acrylic layers (3 mm thickness, 66 cm height) with outer layer diameter of 15 cm and inner layer diameter of 9 cm. Figure 3.3 showed the schematic diagram and image of PBR.

Table 3.1: Nutrient constituents in Bold's Basal Medium (Phang & Chu, 1999)

Stock	Weight	Concentration in culture medium
Main Solution	**per 400 mL**	
1. $NaNO_3$	10.0 g	2.94×10^{-3} M
2. $MgSO_4.7H_2O$	3.0 g	3.04×10^{-4} M
3. K_2HPO_4	4.0 g	4.31×10^{-4} M
4. KH_2PO_4	6.0 g	1.29×10^{-3} M
5. $CaCl_2$	1.0 g	1.70×10^{-4} M
6. NaCl	1.0 g	4.28×10^{-4} M
7. **Trace Metal Solution**	**Per L**	
$ZnSO_4$	8.82 g	7.67×10^{-5} M
MoO_3	0.71 g	1.23×10^{-5} M
$Co(NO_3)_2.6H_2O$	0.49 g	4.21×10^{-5} M
$MnCl_2$	1.44 g	1.82×10^{-5} M
$CuSO_4.5H_2O$	1.57 g	1.57×10^{-5} M
8. **Boron Solution**	**Per 100 mL**	
H_3BO_4	1.14 g	4.62×10^{-4} M
9. **Alkaline EDTA Solution**	**Per 100 mL**	
$EDTA.Na_2$	5.0 g	4.28×10^{-4} M
KOH	3.1 g	1.38×10^{-3} M
*Autoclave solutions 1-9 to dissolved		
10. **Acidified Iron Solution**	**Per L**	
$FeSO_4.7H_2O$	4.98 g	4.48×10^{-5} M
HCl	1.0 mL	
Stock solutions 1-6	10.0 mL each	
Stock solutions 7-10	1.0 mL each	
***Made up to 1L with deionized water. pH of solution was adjusted to 6.8 with 1 N KOH**		

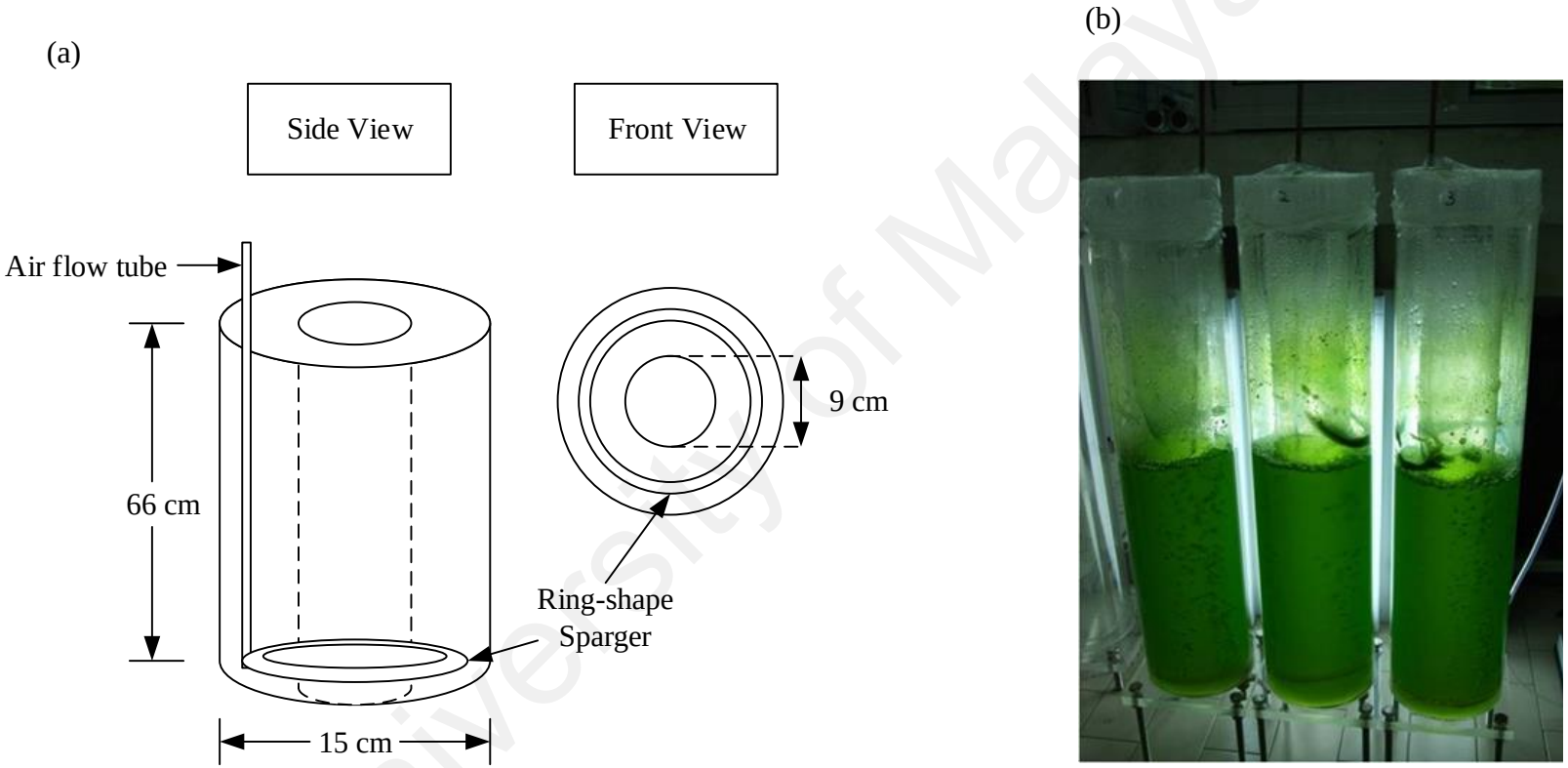

Figure 3.3: Closed bubble column PBR a) Schematic diagram, b) Image in real experiment

A mixture of CO_2 and air was supplied to PBR through 1/8" stainless steel tube connected to ring-shaped sparger made from polyethylene tube. The ambient air was pumped by Resun Air-Pump LP-100 model while adjusted and measured using Dwyer rotameter at constant rate of 10 mL/min. The mixture of 5 % v/v of pure CO_2 with ambient air was obtained by using calibrated gas mixed system.

The PBR was illuminated for 24 hours at front and back using light banks consisted of six horizontal cool white fluorescent lamps. The irradiance from the light banks is approximated to 40 µmol m^{-2} s^{-1}.

Six replicates of microalgae cultivation using PBR was performed. A sample of microalgae biomass was collected each day until it reached to senescence phase. The contamination of culture was minimal and considered negligible as the cultivation was done in axenic culture, a single species available in the culture.

3.1.1 Optical Density

The optical density of microalgae culture was measured using spectrophotometer (UV-1800, Shimadzu UV Spectrophotometer) at wavelength of 680 nm. Distilled water was used as blank for baseline of measurement. The wavelength of 680 nm was chosen due to the highest peak during the wavelength scan using UV-Vis spectrophotometer (Perkin Elmer Lambda 35). Six replicates of optical density measurement were conducted for each microalgae age.

3.1.2 Dry Weight

The concentration of microalgae biomass can be measured in dry weight. 10 - 50 mL of microalgae cultures were vacuum filtered onto glass fiber filter papers with pore size of 1.2 µm. The filter cake on filter papers were dried in oven for overnight at temperature of 80 °C. Six replicates of dry weight measurement were conducted for biomass of each microalgae age.

3.2 Analytical methods for determination of Transparent Exopolymer Particles concentration

The procedure of TEP concentration determination was adapted from (Passow & Alldredge, 1995) with minor moderation. 10 mL of microalgae culture aged 1 - 14 days were filtered through 0.45 µm polyvinylidene fluoride (PVDF) (Millipore HVLP 04700) screen filter using vacuum filtration system. 1 mL of Alcian blue (R&M Chemicals, 1% w/v solution, pH 1.20) dye solution was used as staining reagent. Alcian Blue dye is able to bond with polysaccharide substance and thus could specifically detect TEP content in the microalgae cells. The excess dye was rinsed with 1 mL of distilled water. The filter screen with Alcian Blue stain was immersed in 10 mL of 80% H_2SO_4 for 1 hour. The absorption of Alcian Blue dye into concentrated acid was measured using spectrophotometer (UV-1800, Shimadzu UV Spectrophotometer) at wavelength of 787 nm. Three replicates of Alcian Blue dye concentration measurements were conducted for each microalgae age. The concentration of TEP, C_{TEP} can be calculated from Equation (3.1).

$$C_{TEP} = (E_{787} - B_{787} - T_{787}) \times f_x \times (V_f)^{-1} \quad (3.1)$$

where C_{TEP} = concentration of TEP (µg GXeq. / L);

E_{787} = absorbance of sample (AU);

B_{787} = absorbance of blank filter (AU);

T_{787} = absorbance of sample blank (AU);

V_f = volume of filtered sample (L); and

f_x = calibration factor (µg / AU).

3.2.1 Gum xanthan standard calibration line

The weight of TEP directly proportional to the amount of Alcian Blue dye stained on the outer layer of microalgae cells (Villacorte et al., 2009b). The weight of TEP in the microalgae culture is expressed in term of Gum Xanthan equivalent (GXeq.) that derived from standard calibration line consists of Alcian Blue stain absorbance on GX against the dry weight of GX in µg. GX was selected as a standard substance due to its similarities to TEP which are dissolved in water but form small gel-like particle and also its ability to retain on >0.40 µm filter which make the weight measurement possible (Passow & Alldredge, 1995).

The standard solution was prepared by mixing 200 mg of pure GX into 200 mL of ultrapure water. The solution was stirred for 30 minutes using magnetic stirrer to break apart gel-like particle that formed. Dry weight of standard solution was determined by filtering 2 - 8 mL of standard solution onto 0.45 µm PVDF screen filter using vacuum filter. 1 mL of Alcian Blue dye was applied on the filtered 2 - 8 mL of standard solution.

Similar previous procedure from TEP determination was followed to get absorbance reading on GX. The calibration factor (f_x) can be calculated from Equation (3.2).

$$f_x = W \times \left[(GX_{787} - B_{787}) \times V_f^{-1}\right]^{-1} \quad (3.2)$$

where W = average dry weight of standard solution (µg / L);

GX_{787} = average absorbance of Gum Xanthan (AU);

B_{787} = absorbance of blank filter (AU); and

V_f = volume of filtered standard (L).

3.3 Experimental design of microfiltration

In order to identify the significance of TEP substance with regards to the fouling of membrane, a larger volume of microalgae culture needs to be filtered on membrane surface for sufficient amount of time for fouling to be established. Crossflow MF was conducted to filter 500 mL of microalgae culture using 0.45 µm pore size of titanium – zirconia (Ti-Zr) tubular ceramic membrane with 0.0047 m^2 effective membrane area. The membrane was immersed in distilled water for 6 hours to remove impurities. The operating conditions of MF processes were constant with CFV at 2.13 m/s and TMP at 0.5 bar. The filtrate was collected using a beaker and measured by electronic weight balance which directly transmitted to computer for data recording. MF processes were implemented on microalgae culture samples at the age of 2, 4, 6, 8, and 10 days. Three replicates of MF experiments were conducted on each microalgae age designated. The experimental set-up is as shown in Figure 3.4

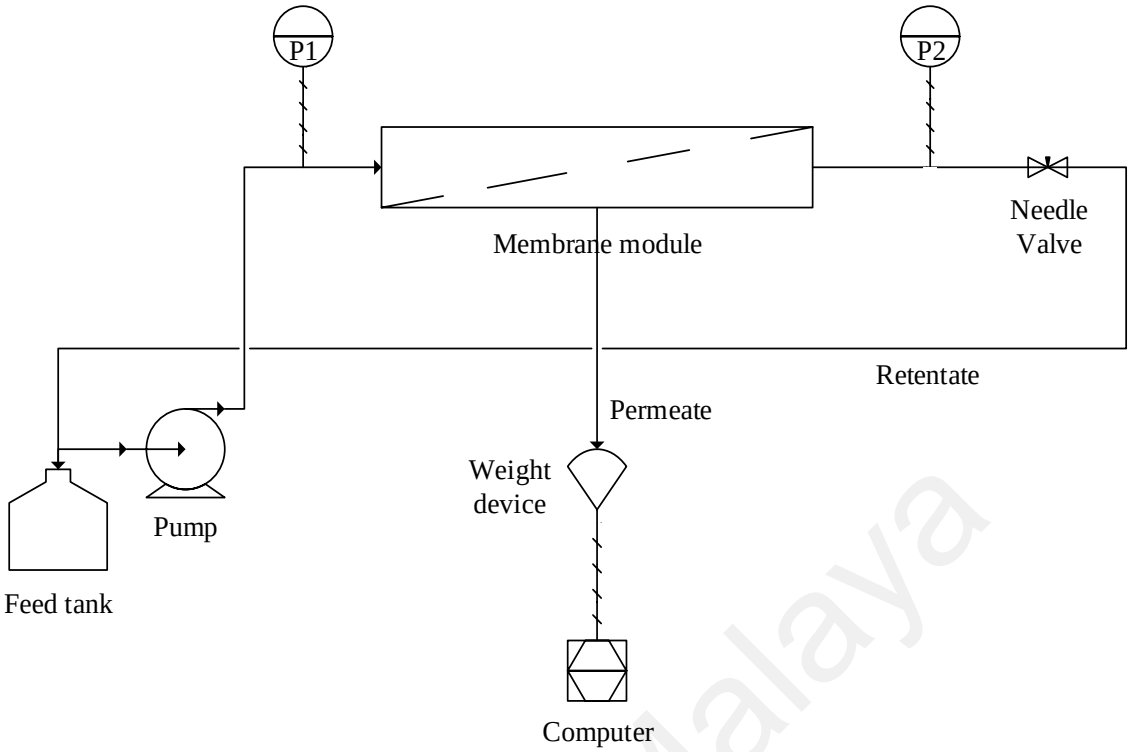

Figure 3.4: Tubular membrane experimental set-up

The performance of MF can be generally determined from the permeate flux (J) calculated from solution passed through membrane weighted over the filtration time from Equation (3.3).

$$J = \frac{Q}{A} \qquad (3.3)$$

where J = permeate flux (L m^{-2} h^{-1});

Q = volumetric flow rate ($m^3 s^{-1}$); and

A = membrane surface area (m^2).

3.4 Characterisation of fouling

3.4.1 Measurement of fouling resistance

Resistance is directly correlated to the thickness of filter cake. Fouling resistance (R_f) could be calculated quantitatively using Equation (3.4) from Darcy's law (Castaing et al., 2010):

$$J = \frac{TMP}{\eta(R_m + R_f)} \quad (3.4)$$

where J = permeate flux (L m^{-2} h^{-1});

TMP = transmembrane pressure (Pa);

η = permeate dynamic viscosity (Pa.s);

R_m = resistance of virgin membrane (m^{-1}); and

R_f = resistance of fouling (m^{-1}).

In this study, fouling resistance was caused by resistance of pore blocking (R_b) and resistance by filter cake formation on the membrane surface (R_c). Membrane resistance is always present and was influenced mainly by the thickness of membrane and pore size of membrane. Initially, pure water flux was measured on a virgin membrane. Then, filtration of microalgae culture was implemented followed by the measurement of pure water flux on filter cake to determine R_c. Lastly, 100 mL of 2 % NaOH was flushed for 30 minutes at 80 mL/min to remove filter cake or reversible fouling and pure water flux was again measured to determine R_b. The value of resistances was calculated from the following Equation (3.5), Equation (3.6), and Equation (3.7) (Ahmad et al., 2012).

$$R_m = \frac{TMP}{\eta J_{w0}} \quad (3.5)$$

$$R_b = \frac{TMP}{\eta J_{w1}} \quad (3.6)$$

$$R_c = \frac{TMP}{\eta J_{w2}} \quad (3.7)$$

where R_m = resistance of virgin membrane (m^{-1});

R_b = resistance of pore blocking (m^{-1});

R_c = resistance of cake formation (m^{-1});

TMP = transmembrane pressure (Pa);

η = permeate dynamic viscosity (Pa.s);

J_{w0} = initial pure water flux (L m^{-2} h^{-1});

J_{w1} = pure water flux before removal of filter cake (L m^{-2} h^{-1}); and

J_{w2} = pure water flux before removal of filter cake (L m^{-2} h^{-1}).

3.4.2 Modified fouling index

Modified fouling index (MFI) has been confirmed since November 2015 by American Society for Testing and Materials (ASTM) to be the best method used to predict the fouling tendency in membrane filtration. MFI was determined from the trend of filtration time against the volume of permeate. Ti-Zr membrane with pore size of 0.45 µm was used to study MFI for microfiltration of *C. vulgaris* culture. The volume of permeate was measured each 30 seconds for filtration duration of 15 minutes. MFI value can be directly calculated from the slope of t/V versus V curve line. Equation (3.8) and Equation (3.9) show the elaboration of the curve line (Castaing et al., 2010).

$$\frac{t}{V} = \frac{\eta \cdot R_m}{TMP \cdot S} + \frac{\eta \cdot C_b \cdot \alpha}{2 \cdot TMP \cdot S^2} V \quad (3.8)$$

$$MFI = \frac{\eta \cdot C_b \cdot \alpha}{2 \cdot TMP \cdot S^2} \quad (3.9)$$

MFI value in Equation (3.9) equals to tgα which can be directly calculated from the slope of t/V versus V curve line under the condition that the temperature is 20°C, the pressure applied is 207 kPa and the membrane surface area equals to 13.8 x 10^{-4} m^2. A corrected MFI value can be calculated using the following Equation (3.10) (Alhadidi et al., 2011). Equation (3.11) calculate the general water viscosity at various temperatures.

$$MFI0.45 = tg\alpha \times \frac{\eta_{20}}{\eta} \times \frac{TMP}{TMP_0} \times \left(\frac{S}{S_0}\right)^2 \quad (3.10)$$

$$\eta = 0.497 \times (T + 42.5)^{-1.5} \quad (3.11)$$

where MFI0.45 = corrected MFI value (sL^{-2});

tgα = MFI value in Equation (3.9) (sL^{-2});

η_{20} = water viscosity (Pa.s) at 20°C;

TMP_0 = reference applied pressure at 2.07 x 10^5 Pa; and

S_0 = reference membrane surface area at 13.8x10^{-4} m^2.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Growth profile of *Chlorella vulgaris*

C. vulgaris was introduced into 5L fresh liquid of 100% BBM nutrients. Figure 4.1 shows the growth phase cycle of *C. vulgaris* from the start of inoculation until its dying stage within a period of 14 days. The bar graph showed the optical density while the line graph showed the dry weight of *C. vulgaris* biomass.

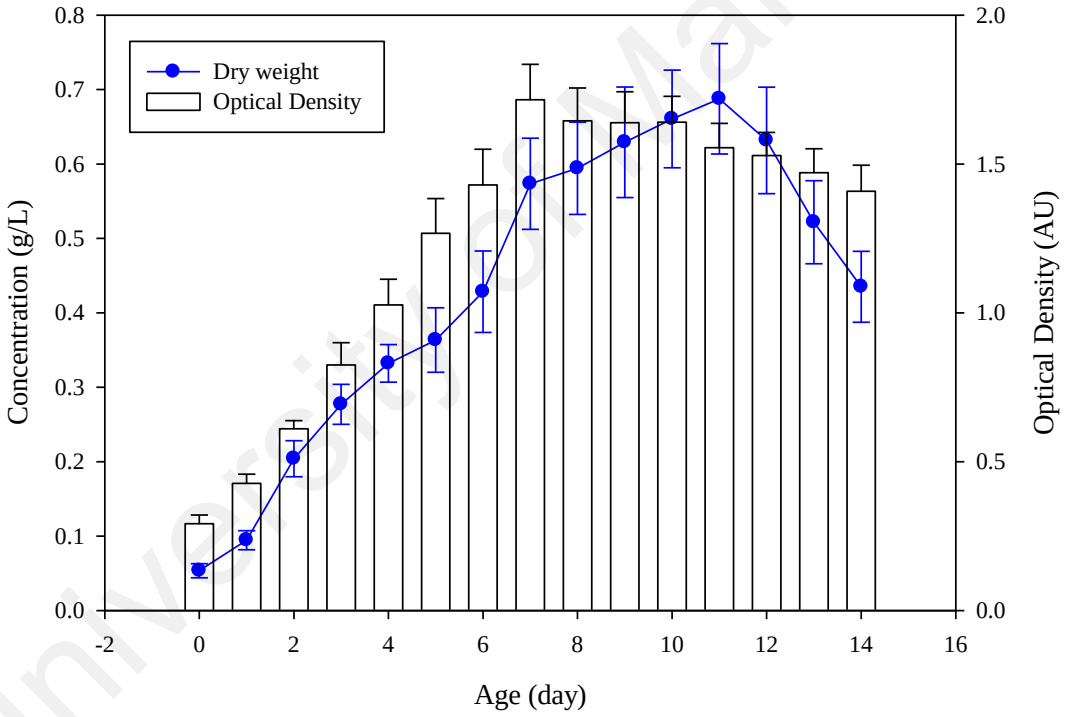

Figure 4.1: Dry weight and optical density of *Chlorella vulgaris* in batch growth cycle

The lag phase was hardly observed and the initial biomass concentration was 0.0535 g/L. *C. vulgaris* growth started straight away from exponential growth phase as such *C. vulgaris* was observed to start the cell division activities from Day 0 cultivation. This is possible due to the introduction of *C. vulgaris* into rich BBM nutrients environment which makes lag phase period brief.

Introduction of *C. vulgaris* to poor nutrients condition extended the lag phase period. A culture medium consisting of 25% of BBM nutrients prolonged *C. vulgaris* lag phase period to 4 days (Blair et al., 2014).

C. vulgaris was observed to grow exponentially since Day 0 producing a straight line until it reaches Day 7 with the biomass concentration at 0.5733 g/L. During exponential phase, no death cell was observed due to high metabolism activities and copious of nutrient. Exponential phase is the most desired phase for microalgae harvesting due to abundance of biomass concentration and high lipid content for production of products.

Biomass production during Day 7 to Day 11 increased at a lower rate indicating slower growth rate occurred until the highest biomass concentration gained was 0.6875 g/L at Day 11. Cultivation on Day 7 to Day 11 approaches stationary or stagnant phase where *C. vulgaris* cells start to die but the amount of cells generated higher than dying cells amount produced a positive slope. A stagnant biomass production will be gained if the amount of cells generated equals to amount of cell death (Novick, 1955). Reduction of optical density starts from Day 7 onwards also indicated that *C. vulgaris* cells started to die on Day 7. *C. vulgaris* has significantly changed its colour from green to slightly yellow based on physical observation on Day 9.

Biomass production of *C. vulgaris* dropped sharply started from Day 11 to Day 14. Cultivation on Day 11 to Day 14 is called the senescence or dying growth phase where the amount of cells died is higher compared to amount of cells generated. The observation of *C. vulgaris* growth cycle stopped at Day 14 when the colour of microalgae culture changed to brownish at lowest biomass concentration of 0.435 g/L.

The error bars in Figure 4.1 and Figure 4.2 consist of upper and lower standard error (± SE) from the mean (M) of measurements. The measurements of biology specimens generally are notoriously variable. SE indicates the region where the true mean (μ) of the population of *C. vulgaris* might lie. Length of SE indicates the uncertainty of the data as the wide SE indicated large error. However, SE varies inversely with the square root of sample size (n). So, the more experiments is repeated or larger the n value, the smaller SE length allowing more accurate estimation of true mean, μ by the mean, M of the results (Cumming; et al., 2007).

The large error bar in Figure 4.1 and Figure 4.2 are simply because of the six replicates of experiments is not enough to give smaller SE as the measurement of experiments varies widely. The percentage deviation of SE from M in optical density measurements are 5-10% while the dry weight measurements are 7-18%. The calculations of both measurements are shown in Appendix A and Appendix B.

4.2 TEP concentration in *Chlorella vulgaris* biomass

Transparent exopolymer particle (TEP) can be found from human debris in wastewater treatment system (Linares et al., 2012), bacteria (Vanysacker et al., 2014) and multicellular organisms but the most TEP contributor are phytoplankton due to abundant existence of TEP in the ocean during algae bloom (Passow et al., 2001). The release of TEP is a complex combination of factors from microorganism species, growth phase, physiological state and environment conditions (Discart et al., 2013; Zhang et al., 2014). The timing and amount of TEP released is very species-specific. Ecology of different species may differ which resulted different amount release of TEP and TEP precursors.

The amount of TEP in the culture is certainly important in determining the mechanism of membrane biofouling caused by TEP. In these experiments, TEP concentration released from *C. vulgaris* was observed during its growth. Figure 4.2 shows the concentration of biomass and TEP in the *C. vulgaris* culture. The concentration of TEP is expressed in dry weight of GX following the standard calibration line.

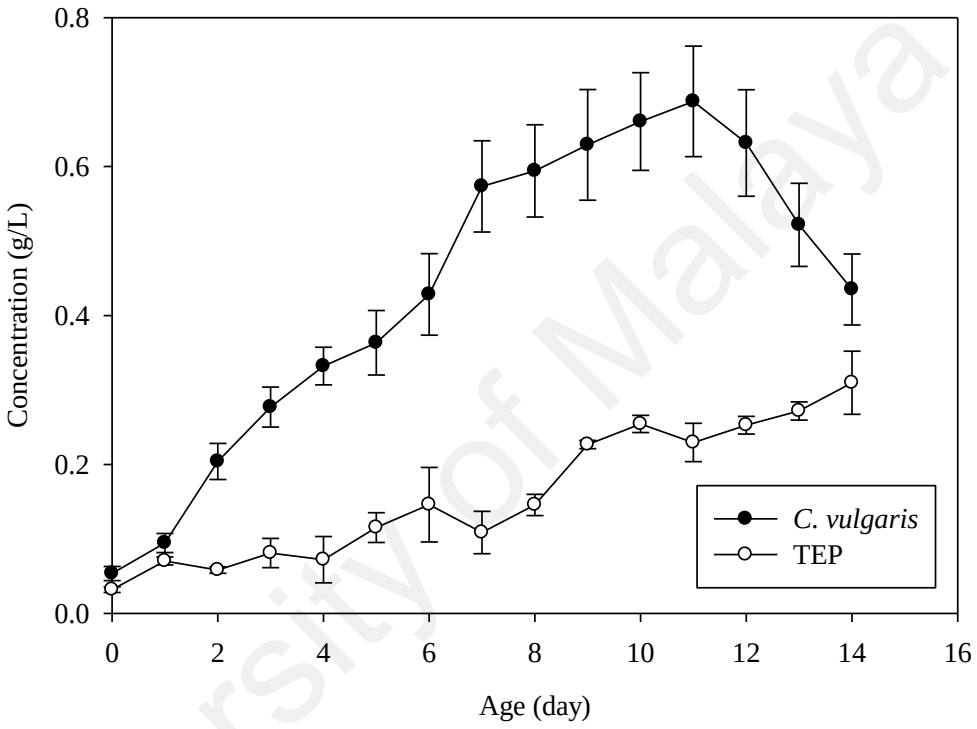

Figure 4.2: Concentration of *Chlorella vulgaris* biomass and TEP production

TEP concentration showed an increasing trend over the cultivation time. TEP production from *C. vulgaris* was slow during exponential phase then suddenly rose towards stationary phase and highly accumulated until its senescence phase. The highest amount of TEP concentration accumulated was 0.3096 g/L during microalgae cultivation of Day 14. TEP production rate was the highest during the typical harvesting time which is Day 8 at the stagnant stage. TEP concentration continuously increase as the microalgae grow from exponential stage to senescence stage.

Some microalgae has more production during exponential phase but it is very well known that majority of phytoplankton has the highest production of TEP during stationary phase (Passow, 2002a). The results showed that *C. vulgaris* cells excreted more TEP during stagnant and senescence phase. TEP precursors were secreted by free phytoplankton cells during exponential and stagnant growth stage through leakage or active excretion. However, during senescence growth stage intracellular polysaccharide substance was released into water from dead cells or rupture of cell walls (Ambrecth et al., 2014) due to bacterial infection and limited nutrient condition.

In the limited nutrient condition, more EPS was formed on the cell membrane in order to defend themselves from attachment of bacteria and virus (Ambrecth et al., 2014). The high concentration of bacteria could contribute to high generation of EPS or diatom surface mucus which also lead TEP precursors as per definition to release and highly accumulated due to abundant existence of EPS during senescence stage.

TEP productivity can also be stimulated by method of cultivation. High shear rates stimulated the growth of bacteria compared to bubbled or static (Passow, 2002b) which could enhance the TEP production from microalgae and activated their ‘defense’ mode by releasing more TEP. Light intensity and carbon dioxide concentration may also affect the amount of TEP generated by microalgae. The physical factor such as turbulence in cultivation method could increase the coagulation of TEP precursors to make larger size of TEP whereas factors such as light temperature and age of phytoplankton determines the amount of TEP generated (Passow, 2002b). Different broth culture may provide different types of nutrient and nutrient concentration that may correlate to the growth rate of microalgae.

Shammi (2017) studied the transformation of EPS to TEP upon the sunlight exposure in aquatic environment. The author found that EPS simultaneously transformed into TEP where the particle size of TEP increased significantly with increasing time and thus increase of its concentration, which the same result obtained by this study. The author claimed that TEP formed stable at first 19h and decomposed afterwards under high intensity of sunlight. The decomposed EPS/TEP formed a new protein-like substance that can reacted with free metal ions and produced complexes of metal-protein that common in membrane fouling (Shammi et al., 2017).

The dependence of TEP excretion on environment conditions suggests that production of TEP is a function of growth rate of cell. Thus, it is important to prove the impact of TEP on biofouling of membrane filtration and demonstrates the efficiency of harvesting of microalgae.

4.3 Microfiltration Performances

Microfiltration (MF) was done for 5 different physiological ages of microalgae. The fouling of membrane can be illustrated through the performance of filtration. Figure 4.3 shows the performances of filtration in terms of normalized permeate flux against the specific filtration volume for 2, 4, 6, 8 and 10d microalgae cultivation ages. Specific filtration volume shows the maximum volume filtered before the filtration reaching the steady state flux and the low value indicates a severe fouling condition.

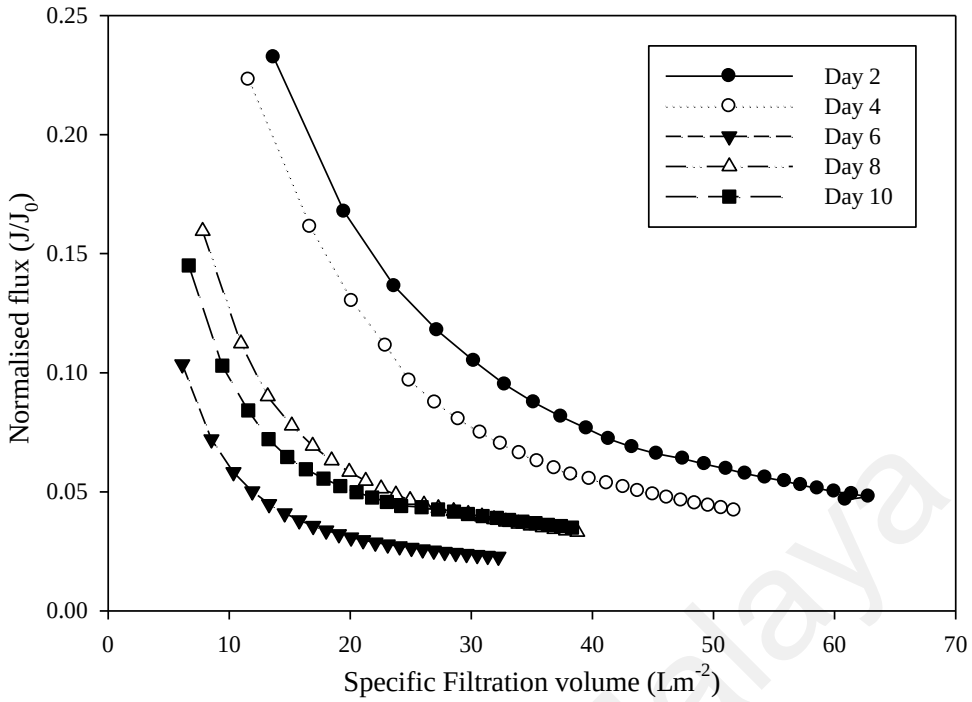

Figure 4.3: Normalized flux against specific filtration volume

The MF was evaluated until 10d which at the end of stagnant growth phase despite the highest accumulation of TEP was on 14d of microalgae cultivation is due to the condition of microalgae at 14d was in senescence phase which is unfavorable for harvesting the microalgae for commercialization purposes. The harvesting of microalgae was typically done on the end of exponential growth phase or early of the stagnant stage due to their lipid content and concentration of their biomass and in this experiment the most desired age of cultivation was between 6 to 8 days.

Filtration of Day 2 culture showed the highest membrane performance with their steady normalized flux at 0.0467 and specific filtration volume at 60.91 L/m^2 of membrane area. Filtration of Day 2 culture obtained the highest flux and the largest volume filtered in duration of 2h filtration time due to the dilute *C. vulgaris* biomass concentration gained in earlier exponential phase.

Filtration of Day 4 culture falls to second highest membrane performance with normalized flux at 0.0422 and specific filtration volume at 51.70 L/m^2 as the biomass concentration higher compared to Day 2. However, filtration of Day 6 culture has the lowest membrane performance with normalized flux at 0.0227 and specific filtration volume at 32.23 L/m^2.

Strangely, the biomass concentration on Day 8 and Day 10 were higher than that on Day 6 but their filtration performances are better. Filtration of Day 8 culture has membrane performance of normalized flux at 0.0331 and 38.75 L/m^2. Filtration of Day 10 culture has membrane performance of normalized flux at 0.0349 and specific filtration volume at 38.30 L/m^2.

Based on the observation of filtration in this study, the best performance has the largest value of specific filtration volume and normalized flux thus has the lowest fouling rate. However, the increase of *C. vulgaris* physiological age does not significantly resulted in severe membrane fouling as Day 10 has lower fouling rate compared to Day 6. MF membrane with normalized flux lower than 0.15 exhibited a higher flux decrease (Elcik et al., 2016) which similar results obtained in this study for filtration on Day 6, Day 8, and Day 10.

The growth phase of *C. vulgaris* gives a significant influence to the performance of filtration. Theoretically, severe fouling should fall on those of filtrations in the stagnant stage of cultivation due to highest biomass concentration compared to exponential and senescence growth stage. However, the performance of MF on *C. vulgaris* is the most severe during late exponential phase in this study.

Discart (2013) took *C. vulgaris* samples at different ages (2, 4, 6, 8, and 21 days) and filtered them on three different membrane pore size (0.4 µm, 0.1 µm, and 5kDa). The nutrient for sample was continuously refreshed and the results from Discart (2013) showed increasing trend for *C. vulgaris* and TEP concentration over cultivation period. However, the author claimed that the characteristics of sample to the filterability was not clear as the trend of sample permeation was not consistent. Filtration on 0.4 µm pore size, Day 4 was the lowest permeation performance while Day 6 for 0.1 µm and Day 21 for 5kDa. The author concluded that all TEP variables are highly interrelated and no sample variable and fouling parameter can solely represent the membrane performance but also claimed that soluble compound, TEP and carbohydrate to be the factors in flux decline in MF performance on *C. vulgaris* (Discart et al., 2013).

4.4 Characterisation of biofouling

Fouling is a continuous development of deposits on membrane surface depends on concentration of feed and length of time through mechanism of pore blocking, cake formation and concentration polarization before actions were taken to mitigate its effect (Pearce, 2007). Pore blocking happens when particles deposited on membrane surface, plugging and restricting the pore opening to certain degree.

The development of cake formation happens as more particles adhere on top of the initial layer of particles and controls the transport and permeability of fluid. They also act as ‘second membrane’ during this phase. A very dense cake formed after prolonged time of filtration under constant pressure drop (Babel & Takizawa, 2010). Loosely attached deposited particles typically stays in the cake layer formation phase and easily removed after the chemical cleaning.

Concentration polarization happens when the concentration of feed channel build up until the filtration and permeation becoming restricted. MF involves only physically separation that separate particle depending on the membrane pore size (0.1 - 10 mm).

A study was done on harvesting of *C. vulgaris* which cultivated in pilot-plant scale using UF. The SEM images of membrane fouling of the study is shown in Figure 4.4. Fouling phenomena will be discussed in next subtopic.

a) b)

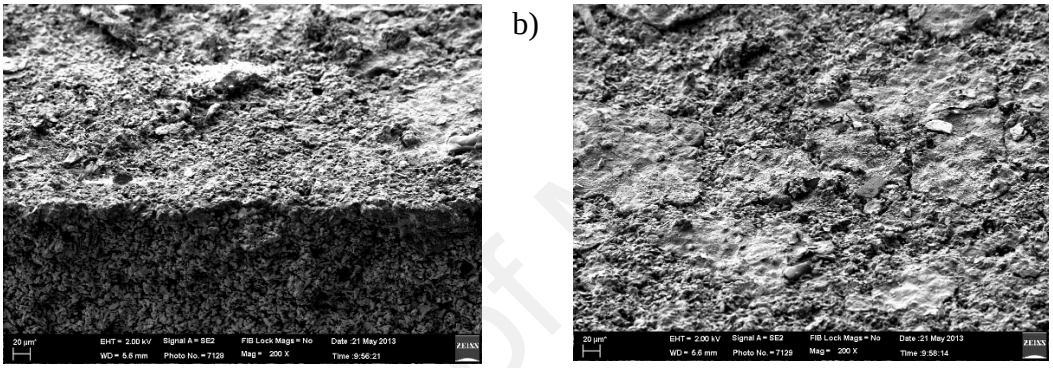

Figure 4.4: SEM images of membrane fouling by *C. vulgaris*, a) side view b) front view

4.4.1 Fouling resistance

Microfiltration is suitable to harvest fragile smaller cells, i.e., *C. vulgaris* that has the average particle size of 2 µm. *C. vulgaris* can be easily deposited on the surface of MF membranes and cause resistance to filtration. The performance of MF is also very much affected by the resistances that involved. Filtration of particle sizes smaller than the pore size causes adsorption on the membrane pore wall while particles larger than pore size tend to form a cake layer on membrane surface (Meng et al., 2009). In the study, the pore size of membrane used was 0.45µm which is smaller than *C. vulgaris* cells size; thus the resistance caused by adsorption fouling mechanism, R_a is negligible (Chiou et al., 2010).

Figure 4.5 shows R_m, R_b and R_c that are involved during MF of microalgae. Virgin membrane resistance, R_m for each MF processes was remained constant approximately at value of 2.65 x 10^{11} m^{-1} as the membrane was immersed at the same duration of 6 hours before operated. The total resistance was the highest at the lowest performance of MF, R_c and R_b were the highest at *C. vulgaris* physiological aged Day 6 with a value of 9.7343 x 10^{12} m^{-1} and 5.4288 x 10^{12} m^{-1} respectively.

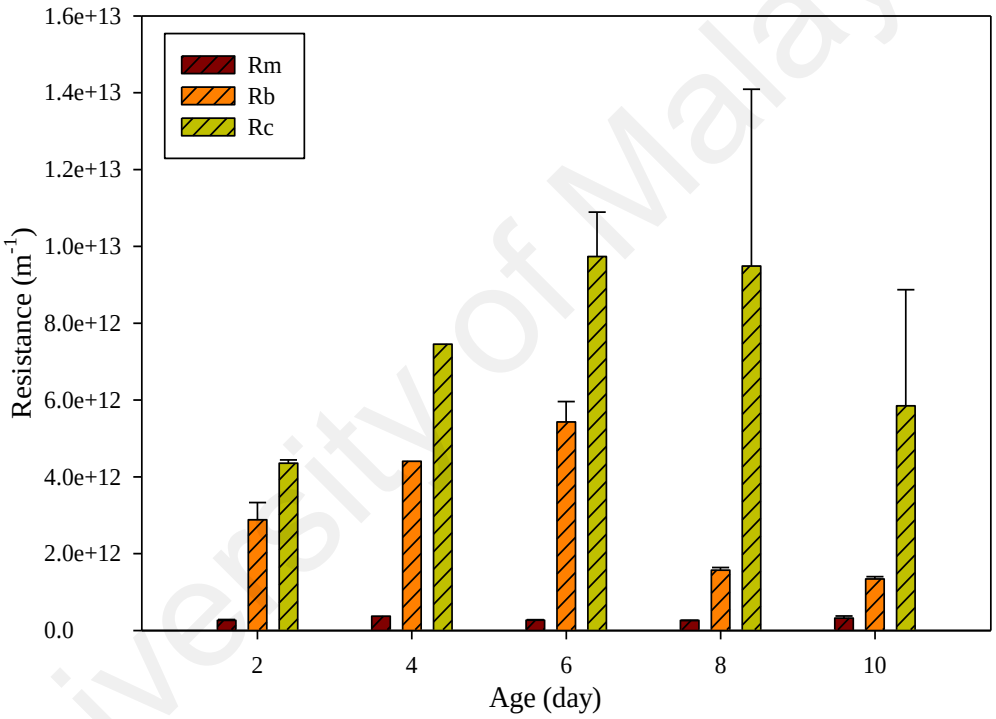

Figure 4.5: Resistance during microfiltration of *Chlorella vulgaris*

R_c is the resistance occurred when the deposited particles form a cake layer during MF of microalgae, also refers as reversible fouling. R_c is the main cause leading to total membrane fouling as shown in Figure 4.5, in contrast with a study from Elcik (2016) that claimed resistance of concentration polarization (R_{cp}) dominant in algae-MF processes. In this study, R_{cp} was in negative value after substraction of total resistance to R_m, R_b, and R_c which indicated that R_{cp} is not available in the system.

R_c constitutes more than 58% of total resistance. R_c was in high value due to microalgae cell retained by size exclusion which the cell size greater than membrane pore size (Zhang & Fu, 2018). Large error bar in R_c is probably due to large error in *C. vulgaris* biomass production especially from Day 6 onwards. A hypothesis could be said that a transition from stagnant to senescence stage differs for every batch of *C. vulgaris* cultivation.

Babel and Takizawa (2010) studied the cake layer formation on MF using *Chlorella sp.* and concluded that R_c could be increased with increase in concentration of feed solution. However, in this study the highest concentration of biomass was on Day 10 and the R_c was much lower compared to the one on Day 6 and Day 8. On Day 10 the biomass concentration may consist of low *C. vulgaris* cells together with the excretion of its organic matter content i.e., TEP as shown in Figure 4.2.

R_b is the resistance occurred when the particles plugging the membrane pore (Wu et al., 2012). R_b also refers as irreversible fouling which caused by fouling of irremovable particles after chemical cleaning which in this study 2 % NaOH solution was used. Pore blocking particulate formed and acted as a secondary membrane layer as the TMP continuously pressing the algae cells as filtration proceeded and reduced the permeation.

Zhang (2016) studied the impact of algoganic organic matter (AOM) from *Microcystis aeruginosa* and *Chlorella sp.* on fouling of ceramic membrane MF. Zhang (2016) found that AOM from stationary phase caused severe fouling compared to AOM from exponential phase. The AOM from *Chlorella sp.* caused greater fouling than *Microcystis aeruginosa* (Zhang et al., 2016).

The result of Zhang (2016) is in contrast with this study where the TEP/biomass from late exponential phase caused severe fouling compared to stationary phase. An earlier study by the same author found that high molecular weight biopolymer to be the major component determining the severity of fouling for ceramic membrane (Zhang et al., 2013). The biopolymer is said to be cell surface organic matter that could be EPS or TEP.

TEP enhance the colloidal fouling and filling the voids due to their gel-like properties. Soluble TEP decrease zeta potential of membrane due to TEP negative charge and contribute 80-90% of total fouling from cake layer formation in MBR (Wu et al., 2012). Organic matter holds particulate more tightly on the membrane and increase the difficulty of its removal by physical and chemical method. Organic matter filled in the void between cake layer and turn cake compressible during MF (Hung 2006). Microorganism cells tend to release organic substances due to oxygen limitation which causes sudden increase of EPS and TEP concentration at the bottom of cake layer (Meng et al., 2009).

Shear stress caused intensification of pore blocking and tighter porosity cake deposit (Ladner et al., 2010) involving macromolecular material such as EPS and TEP that very much correlated to the increase of resistance. Micro-particles that smaller than the size of algae cells appears to be the most fouling material that retained by small pores MF membrane. The study has found that the fouling was due to the internal deposition of micro-particles which retained mostly by 0.22 μm MF membrane which display more irreversible fouling than particle retained by 5μm MF membrane (Rickman et al., 2012).

4.4.2 Modified Fouling Index

Fouling of membrane is caused by adhesion of particle and colloids on the membrane surface that leads to severe flux decline. Estimating the fouling potential is crucial to control the membrane fouling successfully (Koo et al., 2012). American Society for Testing and Materials (ASTM) has confirmed that MFI is a more accurate fouling predictor compared to Silt Density Index (SDI) (Jin et al., 2017). MFI0.45 is developed based on the principle of cake and gel layer formation on the membrane to overcome the absence of linear relation between colloidal matter and particles in SDI.

Figure 4.6 shows the trend of t/V versus V. The slope value, tgα which obtained was corrected according to Equation (3.8) to get MFI0.45 value in Table 4.1.

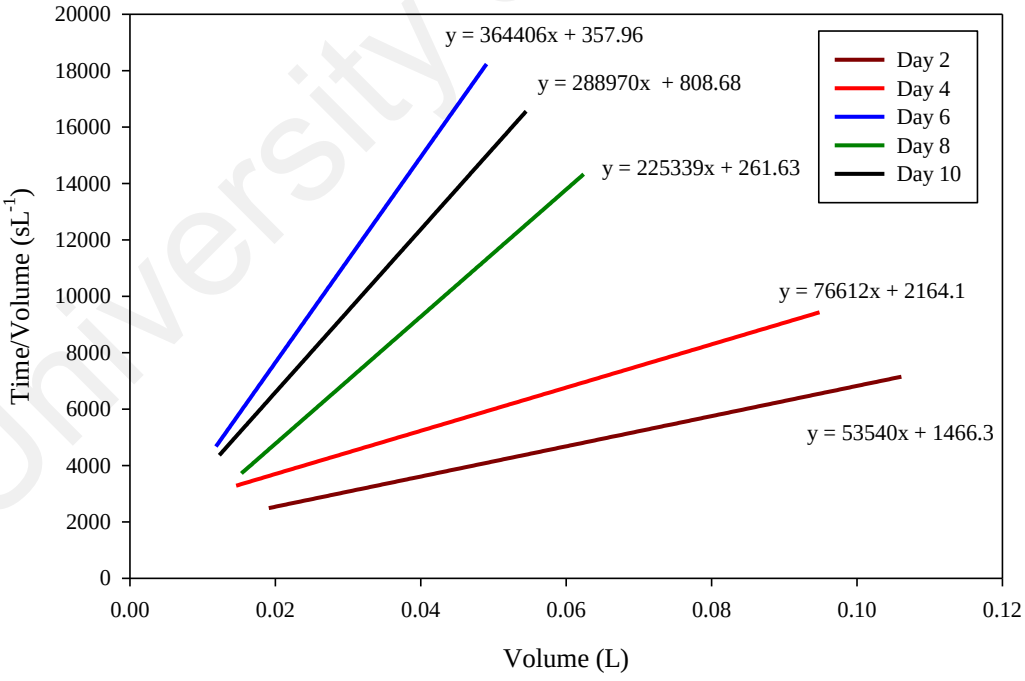

Figure 4.6: tgα value from slope line t/V versus V

The highest MFI value is 11.495 x 10^5 sL^{-2} during filtration on 6d microalgae cultivation age. The trend shows MFI value correlated to the performance of filtration. MFI0.45 has a linear relationship with the *C. vulgaris* biomass concentration as the total rejection is almost 100%.

MFI value indicates the rapidity progress of fouling in microfiltration processes as it is a function of time; showing the time required to obtain liters of permeate volume. The higher the MFI value the more rapid progress leads to severe fouling. MFI is very important in determining and predicting the suitable time for chemical cleaning or membrane replacement thus enhance the optimum process performance with minimal operational costs (Jin et al., 2017).

The minimum MFI0.45 measurement starts since the beginning of filtration where the pore blocking occurred resulted a high slope from high biomass concentration. Continuous adhesion of microalgae cells and excretes formed a cake layer continued to increase the slope (Alhadidi et al., 2011; Babel & Takizawa, 2010). MFI value could reflect on the quality of feed water to the membrane. Table 4.1 shows the corrected value of MFI0.45 from tgα value.

Table 4.1: Corrected MFI0.45 value from tgα value for different age of *C. vulgaris*

Age of microalgae (days)	Slope of t/V vs V, tgα x 10^5 (sL^{-2})	MFI0.45 x 10^5 (sL^{-2})
2	0.535	1.690
4	0.766	2.417
6	3.644	11.495
8	2.253	7.108
10	2.890	9.115

Day 6 has the highest MFI value due to the high resistances, both of R_b and R_c during 6d cultivation of *C. vulgaris*. Day 10 has the second highest tendencies to foul than Day 8 even though Day 8 has much higher R_c resistance compared to Day 10. The higher MFI value on Day 10 is probably due to the high amount of biomass and TEP content in Day 10 compared to Day 8 as shown in Figure 4.2.

Dhakal (2018) studied fouling potential using MFI-UF10 kDa for four type of marine algae and its AOM. TEP production from batch culture marine algae from all growth phase linearly related to MFI-UF10 kDa. The results obtained in this study is similar to Dhakal (2018), MFI0.45 trend linearly followed TEP production in stationary/death phase as Day 10 has higher value than Day 8 but in exponential phase *C. vulgaris* biomass heavily influences the MFI0.45 values. The TEP obtained in stationary/death phase came from the dead algae cells. Filtration of algae cells without AOM has lower fouling value compared to algae-AOM complexes indicated that TEP plays important role in fouling of membrane (Dhakal et al., 2018).

The trajectories of MFI for Day 12 and Day 14 are predicted to be higher than Day 4 but lower than Day 8. Day 12 and Day 14 are already in senescence phase, where the biomass was reduced significantly but still plentiful compared to Day 2 and Day 4. The TEP contents for Day 12 and Day 14 also were higher than Day 4. The MFI value of Day 12 is predicted to be higher than Day 14 as the biomass in Day 12 is higher than Day 14. Further explanation in next subtopic as the assessment of TEP and biomass production of *C. vulgaris* towards R_b, R_c, and MFI will be discussed.

4.5 Assessment of relationship between physiological state and biofouling

Figure 4.7 shows a 3D graph on the influence of biomass and TEP concentration on MFI and resistance values. A slight peak at the middle of 3D graph occurred due to the increased of TEP concentration temporarily and slightly decreased and then increase again when plotted against increasing value of biomass concentration. This is because the amount of TEP production dropped slightly in the transition between exponential and stationary phase.

Figure 4.7(a) shows the influence of biomass and TEP concentration to pore blocking resistance, R_b values. R_b has a negative quadratic relation with the biomass concentration. R_b values went up to the highest value of 5.4288×10^{12} m^{-1} for the increment of biomass concentration until it reached to 0.4283 g/L and then went down afterwards. Whereas R_b has a linear relation with TEP concentration. R_b values rose at a very high slope for the increment of TEP concentration.

Figure 4.7(b) shows the influence of biomass and TEP concentration on cake formation resistance, Rc values. Rc has a negative semi-quadratic relation to biomass concentration and linear relation to TEP concentration. Rc values increased with increasing of biomass and TEP concentration.

Figure 4.7(c) shows the influence of biomass and TEP concentration to MFI. MFI0.45 values slightly decreased and then stagnant with the rise of biomass concentration. MFI0.45 values rose tremendously with addition of TEP concentration.

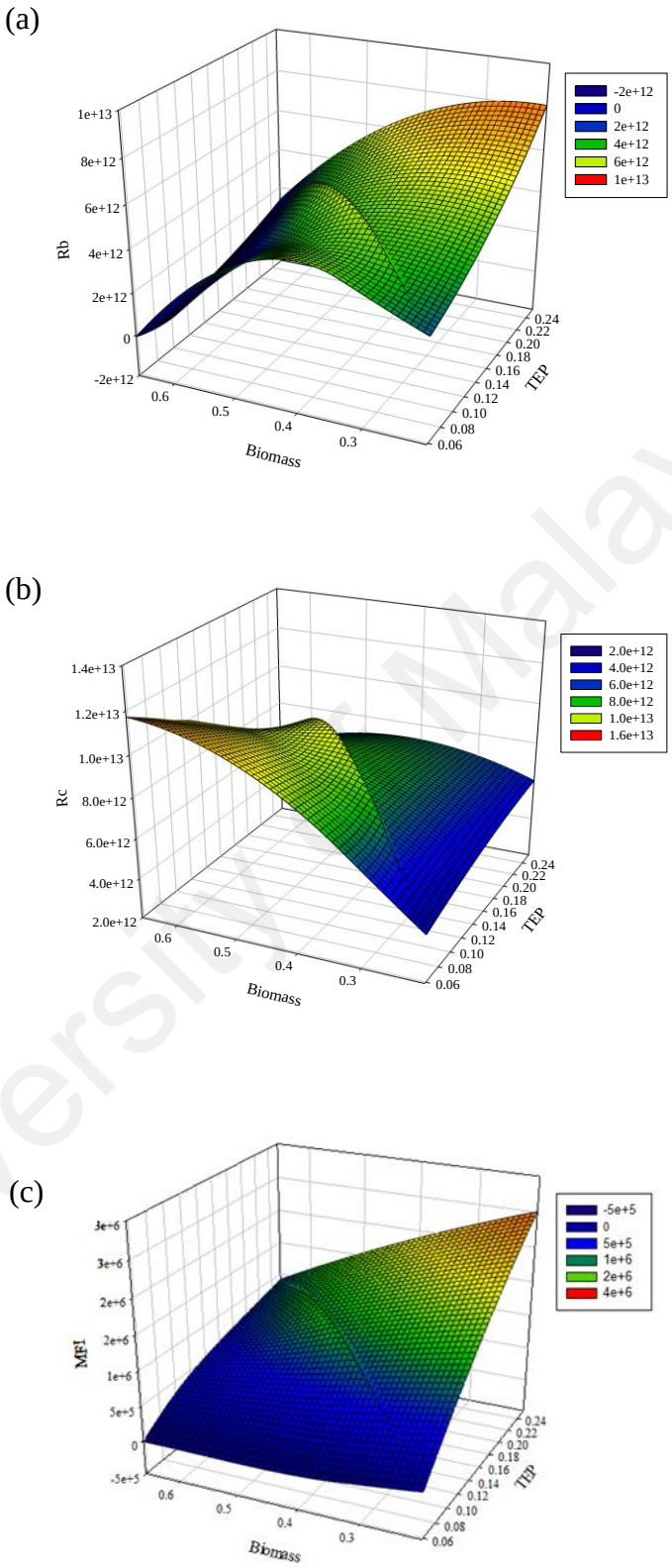

Figure 4.7 : 3D Graph a) Biomass/TEP/Rb, b) Biomass/TEP/Rc, c) Biomass/TEP/MFI

From the observation, it suggests the increment of TEP concentration increase the values of R_c, R_b, and MFI but mostly influence the latter two as biomass concentration strongly influences R_c. The higher TEP content in the microalgae cells increases the pore blocking resistance thus increase the MFI value which measured in the earlier stage of filtration.

The copious of microalgae biomass added up to the cake layer which increased the cake formation resistance. A severe irreversible fouling happened on Day 6 due to high R_b value which initiated fouling mechanisms to start from the abundance amount of microalgae biomass then bind together by TEP under pressure condition that filled the void space between cells. Continuous deposition of microalgae created a dense cake layer that can be of reversible fouling due to loose attachment of membrane.

Harvesting of microalgae at the late of exponential phase needs a clean-in-place protocol to avoid attachment of microalgae on membrane surface. Frequent backwashing at early stage of filtration of high biomass concentration factor is recommended to reduce fouling due to pore blocking that will further enhanced to dense cake after extended filtration time.

CHAPTER 5: CONCLUSIONS

This study presents the fundamental aspect of MF membrane fouling caused by TEP production in *C. vulgaris* suspension. Microalgae biomass and TEP give significant fouling to the microfiltration of microalgae. TEP production exhibits an increase linear build-up over time during the growth of *C. vulgaris* in Bold's Basal Medium (Objective 1). Accumulation of TEP was observed to be high during stationary and senescence phase in a batch culture due to cell lysis and self-protection.

The *C. vulgaris* cell size is larger than the membrane pore size thus resulting a total rejection of microalgae cells. The performance of microfiltration of *C. vulgaris* was the lowest during filtration of 6d physiological age. This indicates that fouling was the most severe at the end of microalgae exponential growth stage (Objective 2). Cake layer formation, R_c is dominant in the total fouling resistance. High concentration of TEP in addition to abundance amount of microalgae biomass advances the fouling rate and increase the severity of fouling.

The TEP formation strongly influences R_b and MFI values while the biomass concentration of *C. vulgaris* strongly influences R_c values (Objective 3). The gel-like characteristic of TEP acted as a binder for microalgae cells increase the irreversible fouling from pore blocking fouling mechanism. Developing new membrane with low affinity to hydrophobic macromolecules could help lessen the fouling with algae-derived polymeric organic matter.

Some recommendation for improvement of the results obtained are suggested. In order to avoid large error bar, more than 10 replicates should be done especially for biology experiments. More work should focused on molecular study of *C. vulgaris* such as biophysical characterization and modelling the formation kinetics of TEP. The effect of physicochemical conditions on the rate of TEP formation and fouling during microfiltration could help in improving understanding of the fouling mechanisms caused by TEP.

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LIST OF PUBLICATIONS AND PAPERS PRESENTED

Nadia Hazwani Mohd Nasir, Nik Meriam Nik Sulaiman, Phang Siew Moi (2013, Dec). *Effect of Transmembrane Pressure and Crossflow Velocity on Ultrafiltration of Microalgae*. Paper presented at the 6th AUN/SEED-Net Regional Conference on Chemical Engineering (RCCE), Manila, Philippines.