EFFECT OF TEMPERATURE TOWARDS MEMBRANE FOULING BY MICROALGAE ALGAL ORGANIC MATTER

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EFFECT OF TEMPERATURE TOWARDS MEMBRANE FOULING BY MICROALGAE ALGAL ORGANIC MATTER

by

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Nabihah Binti Mat Salleh

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LIST OF ABBREVIATION

AOM	Algae Organic Material
BCA	Bicinchoninic acid assay
bEPS	Bounded Extracellular Polymeric Substance
CA	Contact Angle
DLVO	Derjaguin, Landau, Verwey, and Overbeek theory
EOM	extracellular organic matter
EPS	Extracellular Polymeric Substance
IOM	Internal Organic Matter
MD	Membrane Distillation
NOM	Non-Organic Material
PP	Polypropylene
RO	Reverse Osmosis
SEM	Scanning Electron Microscopy
sEPS	Soluble Extracellular Polymeric Substance

KESAN SUHU TERHADAP KEKOTORAN MEMBRAN OLEH BAHAN ORGANIK MICROALGAE

ABSTRAK

Proses penyulingan membran menangani perubahan suhu dan beroperasi pada suhu rendah, membolehkan haba berlebihan atau sumber haba rendah digunakan. Walau bagaimanapun, kekotoran membran berlaku apabila bahan organik alga (AOM) terkumpul di permukaan membran, mengurangkan kebolehtelapan membran dan meningkatkan rintangan penapis. Untuk tujuan tesis ini, bahan organik alga (AOM) diasingkan kepada bahan polimer ekstraselular larut (sEPS), dan bahan polimer ekstraselular terikat (bEPS)/bahan organik dalaman (IOM) daripada dua spesies berbeza (*Chlorella sp.* dan *Navicula sp*.) dipanaskan dengan membran gentian berongga PP ke julat suhu yang direka bentuk untuk mensimulasikan proses penyulingan. Keputusan menunjukkan bahawa kedua-dua spesies sEPS dan bEPS / IOM mempunyai peratusan karbohidrat yang lebih tinggi daripada protein. Suhu mempengaruhi kepekatan karbohidrat dan protein yang dihasilkan dalam EPS. Daripada analisis sentuhan, membran gentian berongga PP paling hidrofilik dalam sEPS dan bEPS / IOM untuk Chlorella sp. pada suhu 80℃. Manakala, untuk *Navicula sp*., membran gentian berongga PP paling hidrofilik dalam sEPS dan bEPS / IOM pada suhu 70 ℃ dan 80 ℃. Analisis morfologi menunjukkan bahawa kekotoran dalaman dan kekotoran luaran berlaku untuk kedua-dua spesies dalam sEPS dan bEPS / IOM. Kekotoran dalaman dan luaran disebabkan oleh pengumpulan bahan organik, bukan organik dan biologi. *Chlorella sp.* dikenal pasti sebagai spesies yang paling berpotensi untuk kekototoran membrane berlaku berdasarkan pengeluaran EPS, analisis sudut sentuhan dan analisis pengimbasan mikroskop electron, SEM.

EFFECT OF TEMPERATURE TOWARDS MEMBRANE FOULING BY MICROALGAE ALGAL ORGANIC MATTER

ABSTRACT

Membrane distillation processes deal with the temperature changes and operate at low temperatures, allowing excess heat or inferior heat sources to be used. However, membrane fouling happens when the algal organic matter (AOM) accumulates on the membrane's surface, reducing the membrane's permeability and increasing filter resistance. For the purpose of this thesis, algae organic matter (AOM) was separated into soluble extracellular polymeric substance (sEPS), and bounded extracellular polymeric substance (bEPS) / internal organic matter (IOM) from two different species (*Chlorella sp*. and *Navicula sp*.) were heated with PP hollow fibre membrane to a temperature range that was designed to simulate the MD process. The results show that both species' sEPS and bEPS / IOM had a higher percentage of carbohydrates than proteins. The temperature affected the concentration of carbohydrates and protein produced in EPS. From contact analysis, the most hydrophilic PP hollow fibre membrane in sEPS and bEPS / IOM for *Chlorella sp.* at temperature 80℃. While, for *Navicula sp.*, the most hydrophilic PP hollow fibre membrane in sEPS and bEPS / IOM at temperatures *7*0℃ and 80℃. The morphology analysis indicates that internal fouling and external fouling occurred for both species in sEPS and bEPS / IOM. The internal and external fouling was caused by the build-up of an organic, inorganic and biological substance. *Chlorella sp*. species were identified as the most potentially foul MD based on EPS production, contact angle, CA analysis and scanning electron microscopy, SEM analysis.

CHAPTER 1

INTRODUCTION

1.1 Membrane Fouling by Microalgae Algal Organic Matter

The increase in population pressure, the production of water-intensive biofuels, and climate change cause freshwater scarcity to become a looking-forward problem in the future. The lack of freshwater has brought on the development of membrane-based desalination is widely used to extract freshwater from brackish and seawater. High-water recovery and salt rejection are higher than 99% in membrane distillation in comparison to reverse osmosis (Tilak Gulliankala et al., 2010). Membrane distillation processes operate at low temperatures, which allows the use of excess heat or inferior heat sources. Membrane distillation potentially uses inferior heat sources, a minor plant footprint, and lesser capital costs than conservative distillation processes (Eykens et al., 2017). As shown in Figure 1.1 (Daniel Gonzalez et al., 2017), the number of articles published on membrane distillation development has significantly increased in recent years. Experimental and theoretical studies, with some desalination units already operating by different businesses, are covered in this paper.

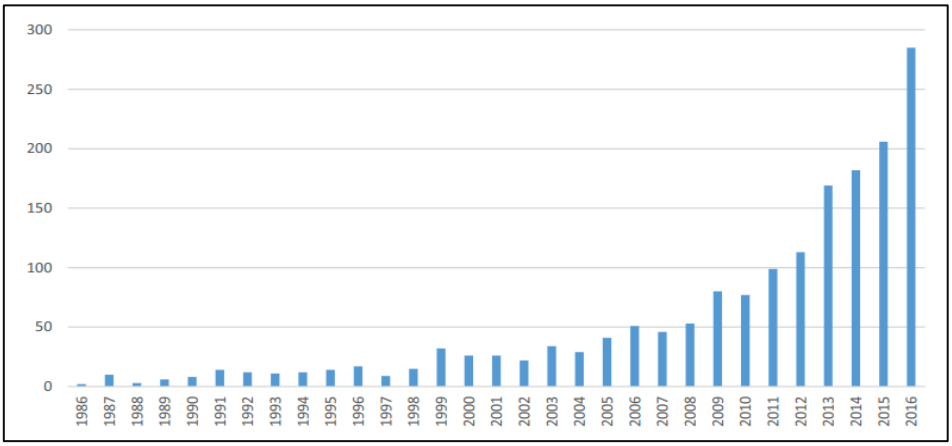

Figure 1.1: The number of papers published on Membrane Distillation development during the last 30 years (Daniel Gonzalez et al., 2017).

A key barrier in the production of clean water is the existence of algae in surface of water sources. The different species of algae present in water produce different concentrations of organic matter. An operational issue in water treatment operations is membrane fouling caused by the algae organic matter can cause. A dominant problem in the membrane distillation process is membrane fouling that can be lowering flow and shorten membrane lifespan (Chu et al., 2015). Membrane fouling happens when the algal organic matter (AOM) accumulates on the surface of the membrane, which reduces the permeability of the membrane and increases filter resistance. Usually, organic matter produced by algae might contain different types and concentrations of polysaccharides, lipids, nucleic acids, proteins, and more solvated organic matter (Pivokonsky et al., 2006; Her et al.,2004). These organic matters develop when invaded by viruses or microorganisms, degeneration of algal cells, during the growth and reproduction of algae under standard or stressful conditions.

The critical source of nutrient, light, and irrelative temperature and pH causes the algae to experience a stressful environment. Intracellular organic matters (IOM) and extracellular polymeric substances (EPS) are the components in the algae organic material. IOM secreted after cell damage or death and EPS secreted due to metabolic activity. The opposite sides of the amphipathic EPS are the bound extracellular polymer substance (bEPS) and the soluble extracellular polymer substance (sEPS). Both are considered the leading causes of membrane fouling. sEPS formed from proteins, polysaccharides, and humid-like compounds but, bEPS is from proteins and polysaccharides. Some researchers have proposed that polysaccharides and proteins cause significant membrane fouling. Hydrophilic EPS tends to build a cake film with algae cells, and hydrophobic EPS attach to the membrane and cause irreversible membrane fouling (Qu et al., 2012; Babel and Takizawa, 2010).

In recent years, biofouling in membrane-based has been an exciting topic for researchers. Biofouling of temperature-controlled MD is still poorly investigated compared to pressure-controlled membrane technology. The conduction of several studies identifies organic algae in indoor conditions (Huang et al., 2012; Qu et al., 2012a), but the composition of AOM has been affected by temperature changes. Currently, there is a gap in knowledge about the effects of temperature on changes in AOM components, so this study aims to investigate the extensiveness of fouling of the membrane when exposed to the algal organic matter of two different species which are *Chlorella sp.* dan *Navicula sp* at different temperatures. Apart from that, this study analyses concentration of protein and carbohydrate in algae organic material and the effect of temperature toward chemical and surface properties of PP hollow fibre membrane by microalgae algal organic matter. This investigation will help identify the type of fouling and the step taken to remove the fouling on the polypropylene hollow fibre membrane used where microalgae are present.

1.2 Problem Statement

Many bacteria, microalgae, and contaminants are contained in water systems. The AOM may consist of the rise of various forms and differing concentrations of polysaccharides, proteins, lipids, and small organic molecules and toxins. The MD process is a thermally driven separation process. Temperature from the process will affect the algal organic matter because some algae organic matter is expected to change when heated. Based on the literature review, the fouling happened because of the low resistance of microorganisms to high temperatures, the enzymatic activity of microbes affected by temperature, the diffusivity of organic waste, the viscosity of water, and temperature also affects the algae metabolism. Membrane fouling happens when the AOM accumulates on

the surface of the membrane, which reduces the permeability of the membrane and increases filter resistance. Thus, the major challenge in MD to produce clean water is the effect of the temperatures on membrane fouling. Apart from that, the AOM released by microalgae caused severe membrane fouling to its hydrophobic compound. Thus, investigating fouling of membranes exposed to the algal organic matter at various temperatures will aid in determining the fouling, the morphology of polypropylene hollow fibre membrane and amount of organic matter produced by *Chlorella sp.* and *Navicula sp* when it exposed to certain temperature, as well as the steps used to eliminate fouling on polypropylene hollow fibre membrane used in areas where microalgae are prevalent.

1.3 Research Objectives

The objective of this research:

1. To investigate the extensiveness of fouling of the PP hollow fibre membrane exposed to the algal organic matter at different temperatures.
2. To analysis concentration of protein and carbohydrate in algae organic material when exposed to different temperature.
3. To determine the effect of temperature toward chemical and surface properties of PP hollow fibre membrane by microalgae algal organic matter.
4. To determine the type of fouling and the steps used to eliminate fouling on polymeric membranes used in areas where microalgae are prevalent.

CHAPTER 2
LITERATURE REVIEW

2.1 Fouling of Algae Organic Material in Membrane Distillation

A leading threat to the global economy, stability, and ecosystem is freshwater scarcities. The viable method to overcome freshwater shortages is desalination by extracting fresh water from the ocean and brackish water. A new technology which is membrane distillation, makes the desalination process more cost-effective. Membrane fouling is one of the hard-pressed problems faced by MD. MD is a thermally manipulated low-pressure separation process that separates using a hydrophobic microporous membrane. In the DLVO theory, fouling can occur on any membrane surface if force interaction exists between the fouling material and the external of membrane. Fouling occurs when its charges are different and create an attractive force happened at film surface, leading to the creation of the membrane film.

The method of control fouling is by manipulating the heat and mass transfer in the MD process. Inorganic fouling, organic fouling, and biological fouling are the three types of fouling that could happen in MD, as shown in Figure 2.1. (Choudhury et al., 2019) Biological fouling, also known as biofouling shown in Figure 2.2 (Bogler and Bar-Zeev, 2018), occurs when microorganisms are triggered and build upon the membrane surface. As a result, permeation flux reduces with partial or total surface contamination. In the MD process, vapor pressure increases exponentially with the increasing temperature causes the mass transfer propulsion to enhance and reduce membrane fouling. However, high supply temperatures have a significant impact on the formation of biofouling due to the low resistance of microorganisms to high temperatures.

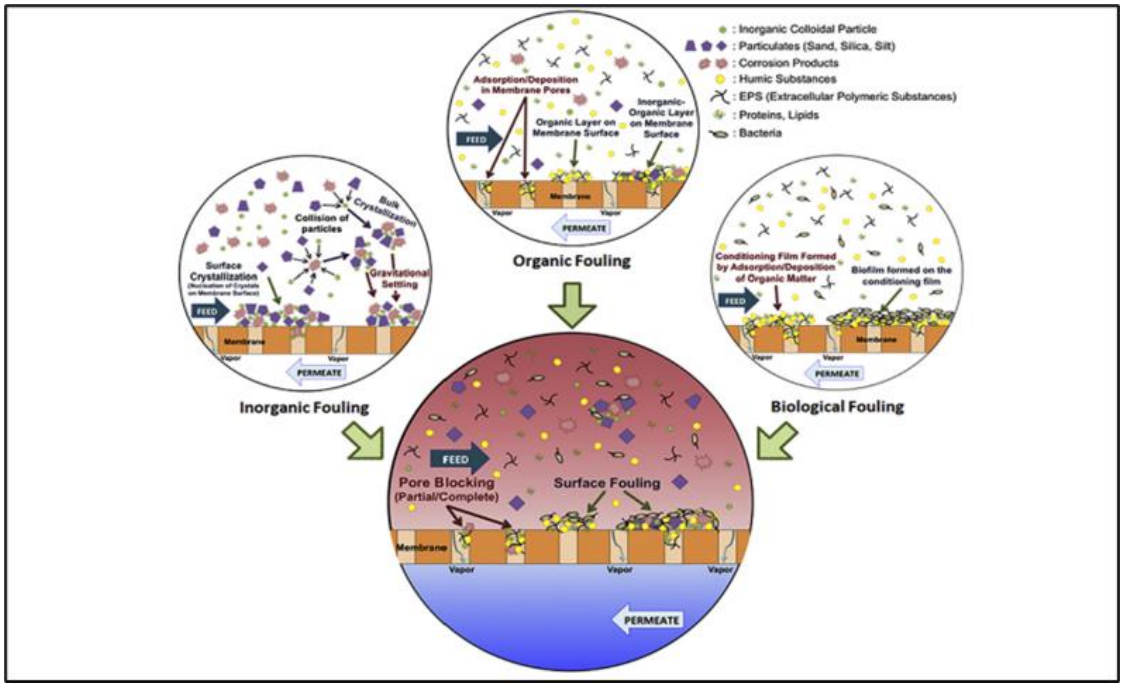

Figure 2.1: Types of fouling that occurred in membrane distillation (Choudhury et al., 2019).

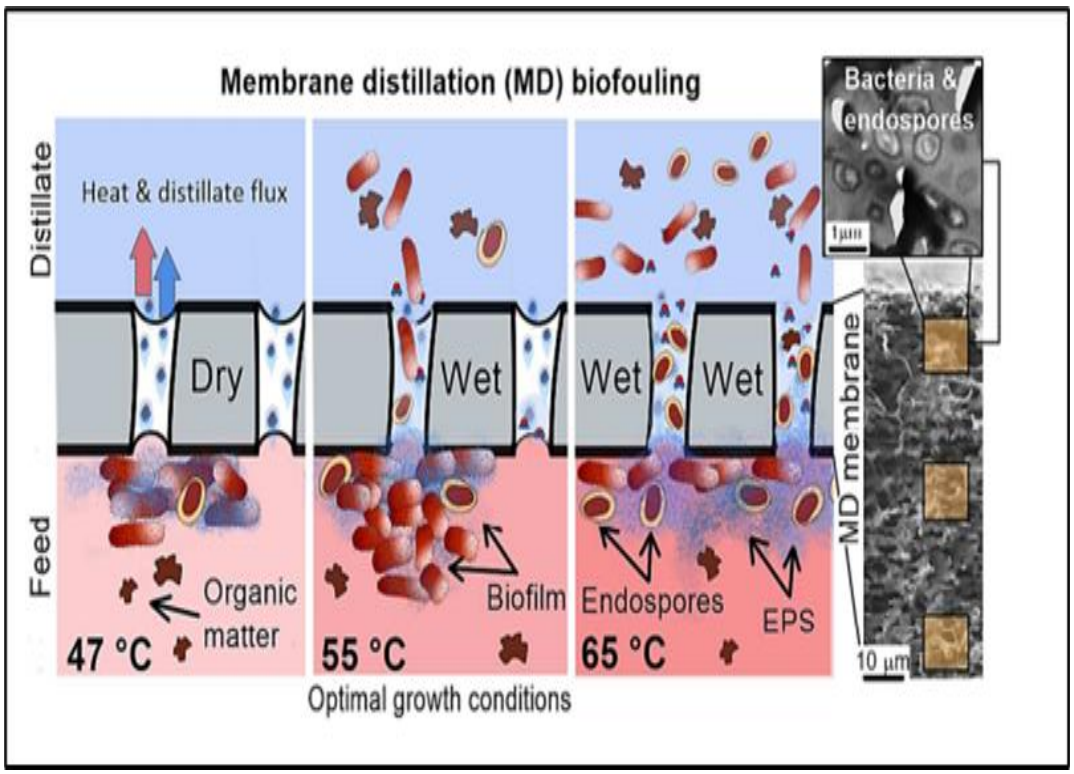

Figure 2.2: Figure of biofouling occurred in membrane distillation (Bogler and BarZeev.,2018).

Biofouling in MD to produce clean water is still less investigated because of its new technologies compared to RO. Bacteria, fungi, and biofilm investigation are part of the biofouling connected with MD. In (Warsinger et al., 2015) biofouling is categorized into bacteria and biofilm in MD and natural organic in MD. The MD process is hampered by biofouling, which wets and blocks the pores. Furthermore, the relatively porous biofouling layer slows diffusion and generates a hydrodynamically stationary water layer on the supply side (Krivorot et al., 2022). Instead of microalgae, bacteria also secrete extracellular high molecular weight substances (EPS) that adhere to the surface of the membrane.

An effective way to kill the bacteria is by chlorination, but it can be harmful to many common MD membrane materials. The colonize bacteria and secretion of EPS followed by the accumulation of organic compounds formed biofilm. Proteins, amino sugars, polysaccharides, polyhydroxy aromatic chemicals, and humid substances are all components of NOM (Babel and Takizawa., 2010). The permeability and repellent of dissolved particles in membranes can both be affected by NOM fowling. Ionic strength, pH, existing ions, membrane surface structure, molecular weight, polarity, permeate flow, and operating parameters influence membrane fouling in the presence of organic molecules. Organic contamination has less occurred on hydrophilic surfaces, but it becomes a concern when MD uses hydrophobic surfaces.

In review (Liao et al., 2018), biofouling and organic fouling are classified into two different types of fouling. The metabolism of microbial cells and the creation of biofilms at the membrane surface or within membrane holes are known as biofouling. Organic fouling such as natural organic substance NOM, proteins, polysaccharides, algae organic matter AOM, and humid substances (derived from water or microalgae secretions) play a decisive role in membrane fouling. Extracellular organic material, EOM, is a slimy

mucous material that generates cell connections in microalgae. The connection leads to an increment of cell concentration on the membrane's surface. Thus, interact with the fouling process and limiting membrane flow. The development of organic coatings on the membrane surface, micro fouling by unicellular algae colonies, and macro fouling by multicellular algae are all part of the biofouling process (Harris et al., 2013). The classification of organic fouling has an impact on biofouling.

The AOM released by microalgae caused severe membrane fouling due to its hydrophobic compound. The membrane fouling reduces permeate flow and results in high filtration defenced. During the growing stage, microalgae cells release AOM, which is an organic compound. Two subgroups of AOM are extracellular organic matter (EOM) and intracellular organic matter (IOM). The larger the quantity of EOM present in the cake layer, the stronger the filter permeability of the cake layer. Usually, EOM characterized by high molecular weight and are mostly negatively charged. AOM contains several compounds in polymers and ceramics like proteins, polysaccharides, peptides, and amino acids. It has proved to cause various membrane fouling problems. Extracellular polymer material (EPS) is an algal secretion that is divided into two parts: soluble microbial products (SMP) and extract EPS (EPS).

Furthermore, the retrieved EPS was divided into two types: loosely bound EPS and firmly bound EPS. Carbohydrates, proteins, and nucleic acids make up the majority of these compounds. It is commonly assumed that EPS protein-like molecules cause membrane fouling. Organic fouling is related to biofouling in that it is often linked with the production of a gel/cake layer of flocculation or particle organic materials. Based on (Qu et al., 2012b), another dominant element impacting membrane fouling is the amount of EOM and EPS. EOM from algae has a high molecular weight up to 100 kDa and is hydrophobic. Flux decrease, reversible and irreversible membrane fouling are all possible

effects of these substances. Distinct microalgae species may produce different forms of EPS, which can be soluble or loosely bound (Xu et al., 2016). The molecular weight distributions of EPS were discovered to be less than 1000 kDa in the nutrient-free water culture.

2.2 Effect of Temperature Toward Algae Organic Matter and Membrane Distillation

According to (Warsinger et al., 2015), the temperature can have a significant effect on biofouling due to the lack of resistance of microorganisms to high temperatures and the thermal effects on organic compounds. The experiment in hollow fibre membranes proved that most environmental organisms do not function at temperatures above 60 ° C and therefore do not grow in MD membranes (Krivorot et al., 2022). Severe protein contamination was observed at temperatures above 20-38 ° C in aqueous solutions containing typical concentrations of organic compounds such as sewage, NOM, and bovine serum albumin, but it is rarely seen at low temperatures. It is noteworthy that the hydrophobic surface is particularly prone to protein contamination. This causes problems with water-containing proteins, amino sugars, or polysaccharides in MD membranes.

Temperature impacts MD efficacy by altering microbial enzymatic activity, organic waste diffusivity, and water viscosity. High and low temperatures have an effect on algae development (Tan et al., 2014), since enzyme activity decreases at high temperatures. At temperature 25-35 ° C was more suited than a thermophilic temperature of 55 ° C (Gonzalez et al., 2015). The temperature also influences the EOM concentration and membrane filtering effectiveness. Microalgae that grow at high temperatures reduce the EOM secretion because the concentration of microalgae reduces same goes with the fouling rate. (Zhao et al., 2015) Temperature also affects

the algae metabolism, the increment of temperature faster the metabolism of microalgae, and the additional EOM secreted (Chu et al., 2016). Greater amount of EOM in the system are likely to exacerbate membrane fouling and limit permeate flow.

As a conclusion, the correct operating temperature is critical for reducing membrane fouling and maintaining high algal growth. (Chu et al, 2016) studied the impacts of heat on membrane fouling and microalgae development. Considering that, the release of EOM at various temperatures is still unspecified and requires more investigation. As algal organic matter solution is heated up, some organic matter is expected to change as well. Therefore, more research on the extensiveness of fouling on the membrane that is exposed to the algal organic matter at different temperature needs to investigate in order to identify the type of fouling and also step taken to remove the fouling on the membrane used where presences of microalgae are prominent.

2.3 Changes of organic matter in different types of algae species

According to the existing research, the composition of algae cells varies depending on the species studied. The quantity of algal AOM per cell may varied by orders of magnitude depending on the type of algae, according to the publications that have been published. This is true even during fixed development phases (for example, stationary stages). (Henderson et al., 2008a). The organic matter of algal species rose in the following order, blue green alga *M. aeruginosa* < green algal *Chlorella vulgaris* < diatom Asterionella Formosa < diatom *Melosira sp*. According to another research, diatoms and blue green algae excreted a bigger quantity of organic matter than green algae, but green algae excreted a lower amount of AOM (Nguyen et al., 2005). Table 2.1 showed changes of organic matter in different types of algae species with growth phases based on different article.

Table 2.1: Changes of organic matter in different types of algae species with growth phases

Species	Growth phase	Affinity (%)			Percentage of Carbohydrates (% or *mg/mg*)	Percentages of Protein (% or *mg/mg*)	Protein / Carbohydrate	Molecular Weight Distribution (%)		References
		Hydrophobic	Transphilic	Hydrophilic				>30 kDa	<1 kDa	
Blue green algae										
Microcystis aeruginosa	Lag	-	-	-	89.5	10.5	0.12	-	-	(Pivokonsky et al. 2006)
	Exponential	-	-	-	78.9	21.1	0.27	-	-	
	Stationary	-	-	-	69.0	30.9	0.45	-	-	
	Exponential	24	17	59	1.0	0.40	0.40	-	-	(Henderson et al. 2008b)
	Stationary	30	13	57	0.7	0.64	0.91	55	38	
	Stationary	34	4	62	-	-	-	49	32	(Qu et al. 2012c)
	Exponential	2	23	75	-	-	-	-	-	(Leloup et al. 2013)
	Stationary	20	19	61	-	-	-	-	-	
	Stationary	16	5	78	-	-	-	-	-	(Huang et al. 2014)

	Exponential	27	4	69	60.0	40.0	0.67	19	29	(Pivokonsky et al. 2014)
	Stationary	28	3	69	57.0	43.0	0.75	41	23	
Anabaena flos-aqua	Lag	-	-	-	83.3	16.7	0.20	-	-	(Pivokonsky et al. 2006)
	Exponential	-	-	-	75.2	24.8	0.33	-	-	
	Stationary	-	-	-	70.4	29.6	0.42	-	-	
Anabaena flos-aquae	Stationary	17	8	85	-	-	-	-	-	(Huang et al. 2014)
Aphanizomenon flos-aquae	Stationary	17	8	78	-	-	-	-	-	
Green algae										
Scenedesmus quadricauda	Lag	-	-	-	88.0	12.0	0.14	-	-	(Pivokonsky et al. 2006)
	Exponential	-	-	-	83.3	16.7	0.20	-	-	
	Stationary	-	-	-	80.4	19.6	0.24	-	-	
Chlorella vulgaris	Exponential	22	18	60	0.9	0.53	0.58	-	-	(Henderson et al. 2008b)
	Stationary	11	18	71	1.10	0.40	0.40	62	30	
	Exponential	-	-	-	0.69	0.81	1.17	-	-	

Chodatella sp.	Exponential	-	-	-	0.73	0.45	0.62	-	-	(Chiou et al. 2010)
Scenedesmus obliquus	Stationary	11	6	69	-	-	-	-	-	(Huang et al. 2014)
Chlamydomonas geitleri	Exponential	21	8	71	80.0	20.0	0.25	16	46	(Pivokonsky et al. 2014)
	Stationary	22	5	73	76.0	24.0	0.32	21	41	
Diatom										
Asterionella formosa	Exponential	15	12	73	-	-	-	-	-	(Henderson et al. 2008b)
	Stationary	20	10	70	1.00	0.19	0.20	9	81	
Melosira sp.	Exponential	32	4	64	0.80	0.16	0.20	30	53	
Cyclotella	Stationary	17	12	83	-	-	-	-	-	(Huang et al. 2014)
Fragilaria crotonensis	Exponential	17	9	74	68.0	32.0	0.47	12	42	(Pivokonsky et al. 2014)
	Stationary	19	7	74	64.0	36.0	0.56	20	33	

CHAPTER 3
METHODOLOGY

3.1 Overview of Research Methodology

Figure 3.1 shows a general flow chart of the research for effect of temperature towards membrane fouling by microalgae algal organic matter. The extraction of organic matter, heating process and further analysis will be explained in more detail.

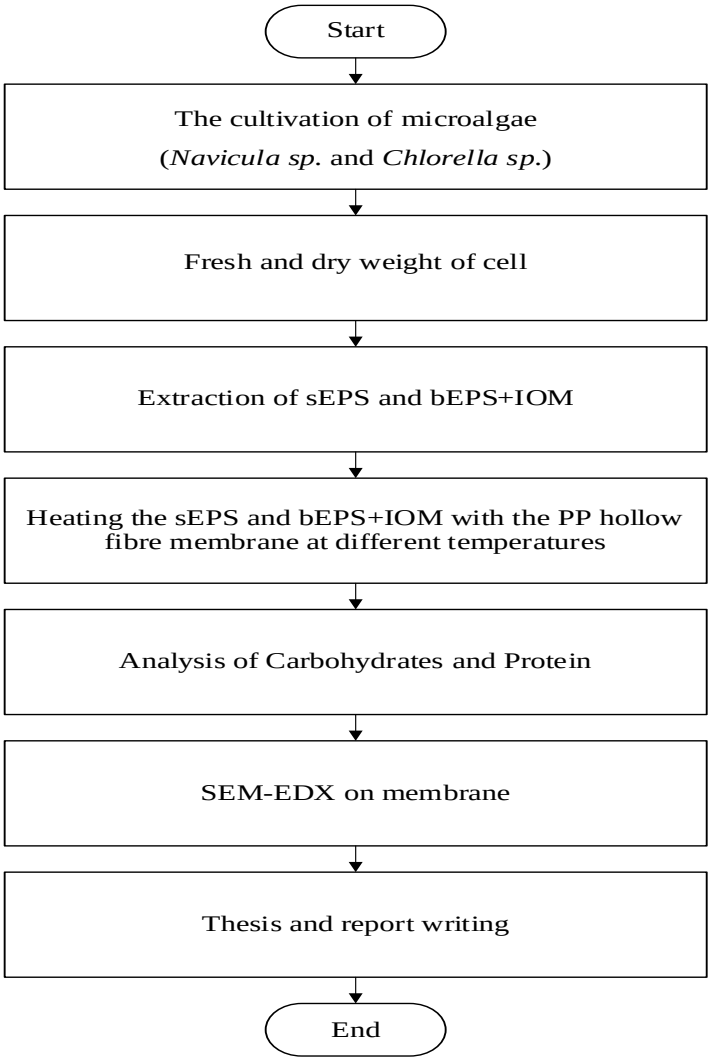

Figure 3. 1: A general flow chart of the research for this final year project

3.2 The cultivation of microalgae

Subculture and development of the *Navicula sp.* and *Chlorella sp.* are carried out. Schott flask bottles with 2 L of pure filtered air are used to grow the specified species. The cells are cultivated in fluorescent room for three weeks, keeping the light constantly at a constant temperature of 25 °C. After the growth, algal cells were collected and resuspended in media by scraping the sidewalls and shaking.

3.3 Fresh and dry weight of cell

The fresh and dry weight of cell the algal suspensions have to centrifuged twice. First, the algal suspension from the cultivation will undergo centrifugation twice to obtain the fresh and dry weight. The 50ml of the solution for each culture was centrifuged at 8500rpm for 15minutes to obtain the supernatant (soluble EPS or sEPS). Then, the supernatants are removed by pipetting it from the top fraction of the centrifuging tube. The residual algae were mixed with 50ml distilled water and centrifuged again at 8000rpm for 10minutes. The distilled water washes out any leftover sEPS in the residual algae. The distilled water fraction was then pipetted and removed to obtain the fresh weight of the sample. Before the centrifugation, the mass of centrifuging tube was measured. The fresh samples were then placed in the oven at 55°C till completely dried and constant dry mass was obtained. This method was done 3 times and the average weight was calculated.

3.4 Extraction of sEPS and bEPS+IOM

The sEPS and bEPS+IOM were extracted by using the centrifugation method. The algal suspensions were divided into 36 centrifuging tubes containing 50ml of the

solution for each culture. Similar to the weight obtaining method, the 50ml algal suspensions were centrifuged at 8500rpm for 15 minutes to extract the sEPS. Then, the remaining bEPS+IOM was mixed with 25ml of NaCl solution. These extracts were kept in the fridge at 3℃ before the heating process.

3.5 Heating the sEPS and bEPS+IOM with the PP hollow fibre membrane at different temperatures

The centrifuged sEPS and bEPS+IOM were heated with PP hollow fibre membrane (0.2 μm pore size, Membrana, Germany). to a different temperature to study the effect of AOM temperature on membrane fouling. The PP hollow fibre membrane was cut into pieces of 1.4 cm and 1 cm and placed in the beaker of the extracts. The smaller membrane was used in the contact angle analysis, whereas the more extended membrane was used for SEM analysis. sEPS extracts are separated into 4 beakers containing 450ml of the extract. One beaker of sEPS extract was left at room temperature as a control for the study. The other 3 beakers were using a hot plate magnetic stirrer for 8 hours at temperatures of 60℃, 70℃ and 80℃. Similarly, the bEPS+IOM extracts were separated into 4 beakers, each containing 225ml of the extracts. A beaker was left at room temperature as a control. The remaining were heated at temperatures similar to the sEPS extracts. After the heating, the extracts and the soaked membranes were collected back into the centrifuging tube and kept in the fridge at a temperature of 3℃ prior to any analysis. Three replicates were done for each species.

3.6 Analysis of Carbohydrates and Protein

3.6.1 Carbohydrate Analysis

The extracts were first centrifuged at 8500rpm for 15 minutes for the carbohydrate analysis. 1ml of supernatant from the centrifuged extracts was pipetted and added with 0.5ml phenol (0.5%) and 2.5 ml of concentrated sulphuric acid (97%). The solutions were left for 10 min before being placed into a water bath at a temperature of 30℃ for 15 minutes. The parafilm was used to cover the solution from evaporation. Then the solutions were cooled to room temperature, and the carbohydrate contents were read using the Ultraviolet-visible spectroscopy at a wavelength of 490nm. The readings were taken 3 times and averaged.

3.6.2 Protein Analysis

The protein analysis was done by first centrifuging the extracts at 8500rpm for 15min. Then, 0.05ml of supernatant was extracted and mixed with 1ml of BCA working reagent. Once mixed, the solutions were heated at 37℃ for 30min and let to cool to room temperature. The parafilm was used to cover the solution from evaporation. The samples were then placed in Ultraviolet-visible spectroscopy, and the data was taken at a wavelength of 562nm. The readings were taken using small and large cuvette 3 times and averaged.

3.7 SEM-EDX on membrane

After heating the PP hollow fibre membrane sample, scanning electron microscopy (SEM) imaging (TM3000, Hitachi) was used to examine the morphology and types of fouling that might occurred. The water contact angle (CA) on the membrane was determined using the static CA of the membrane and the sessile drop

method with a goniometer (Rame'-Hart Instrument Co., United States). At room temperature, a droplet of constant volume deionized (DI) water was applied to the surface of the membrane using an automated syringe. The procedure was repeated five times on the membrane's surface at various locations to determine the average value.

3.8 Writing the thesis and the report

The presentation of the data and the results were the last steps in the process of finishing this report. During this step, all the significant and essential graphs, tables, and figures were presented appropriately. After that, an appropriate discussion on the results was written, including proper and reasonable justifications for each point. In conclusion, the conclusions were justice based on the results obtained, along with a few necessary suggestions or recommendations for improvement in future works. These could be made based on the findings.

CHAPTER 4
RESULTS AND DISCUSSIONS

4.1 Fresh And Dry Weight of *Chlorella sp.* and *Navicula sp*

Table 4.1: The fresh weight and dry weight of *Chlorella sp.* and *Navicula sp.*

Species	Weight (g)	
	Fresh Weight	Dry Weight
Chlorella sp.	0.15	0.036
Navicula sp	0.238	0.0024

From Table 4.1, the fresh weight for *Navicula sp* was higher than *Chlorella sp.* In contrast, the dry weight results differed from the fresh weight because the dry weight for *Chlorella sp.* was higher than *Navicula sp.* The weight for *Chlorella sp.* reduced to 76 % and *Navicula sp.* reduced to 98%.

4.2 Effect Temperature Towards Concentration of Carbohydrate by Microalgae Algal Organic Matter

According to the result in Table 4.2, it was seen that the concentration of carbohydrates in the bEPS / IOM measures at room temperature or 25℃ were 4370.59 μg/L and 2790.57 μg/L for *Chlorella sp.* and *Navicula sp.* When both species were exposed to higher temperatures 60℃, 70℃ and 80℃, the carbohydrate released in bEPS / IOM increased. However, the concentration of carbohydrates released at

temperature 70℃ in bEPS / IOM for *Navicula sp.* was higher than 60℃ and 80℃. The amount of carbohydrate produced in bEPS / IOM was *Chlorella sp.* higher than *Navicula sp. diatom* at room temperature and 70℃. Compared to the room temperature, the carbohydrate concentration released in bEPS / IOM increased 1.6 and 3.1 times for *Chlorella sp.* and *Navicula sp* at 80℃. The carbohydrate amount in bEPS / IOM secreted by *Chlorella sp.* increased linearly with the temperature. In contrast, the carbohydrate amount in bEPS / IOM secreted by *Navicula sp.* increased with the temperature compared to room temperature. However, the carbohydrate amount at a temperature of 70℃ was lower than 60℃, as shown in Figure 4.4.

Table 4.2: The concentration of carbohydrate in the sEPS, and bEPS/IOM of *Chlorella sp.* and *Navicula sp.* at different temperatures

Species	Components	Concentration ($\mu g/L$)			
		80℃	70℃	60℃	25℃
Chlorella sp.	bEPS / IOM	7201.09	5912.04	4971.47	4370.59
	sEPS	9555.21	9704.08	14291.88	7186.20
Navicula sp.	bEPS / IOM	8705.32	5083.12	6332.92	2790.57
	sEPS	6014.89	5711.74	5945.87	13302.59

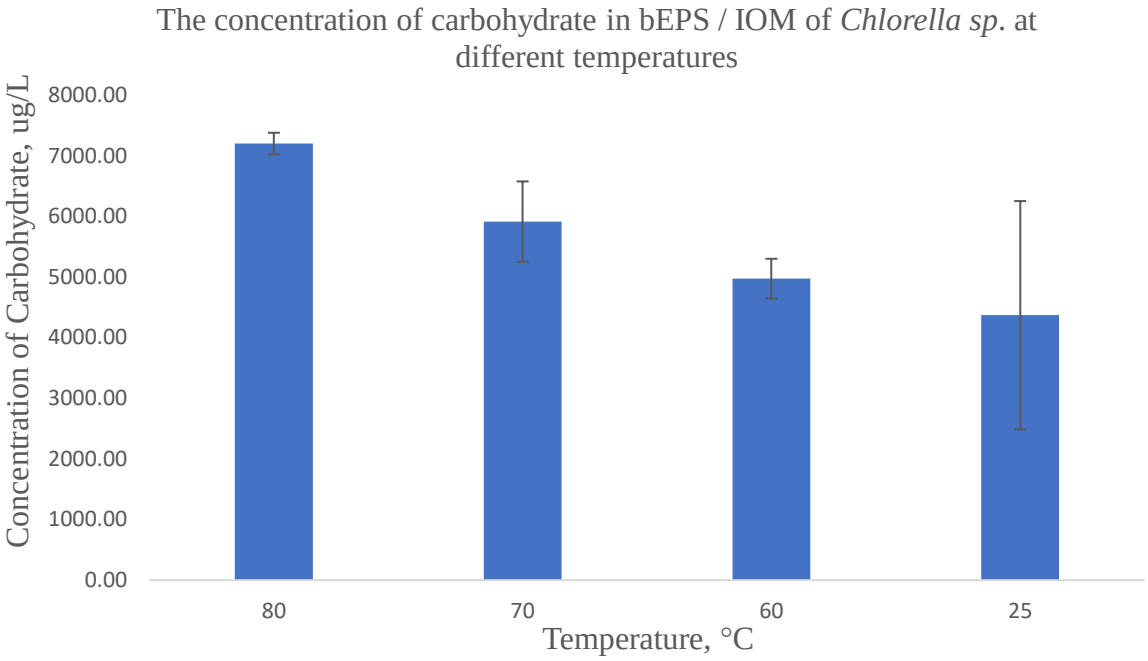

Figure 4.1: The concentration of carbohydrate in bEPS / IOM of *Chlorella sp*. at different temperatures

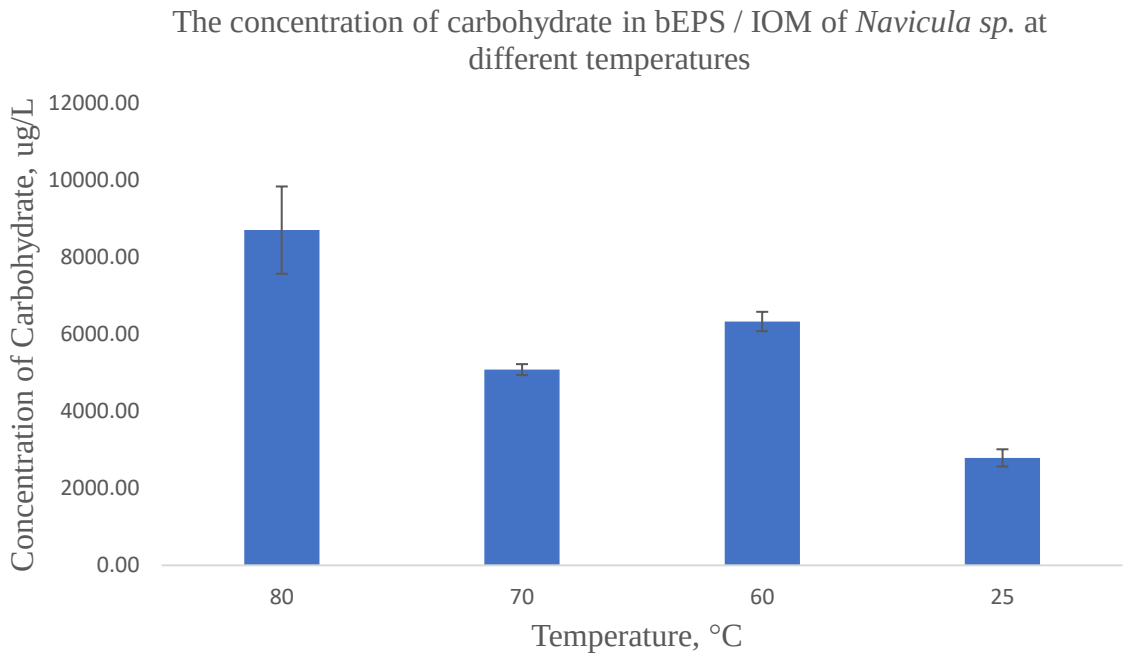

Figure 4.2: The concentration of carbohydrate in bEPS / IOM of *Navicula sp*. at different temperatures

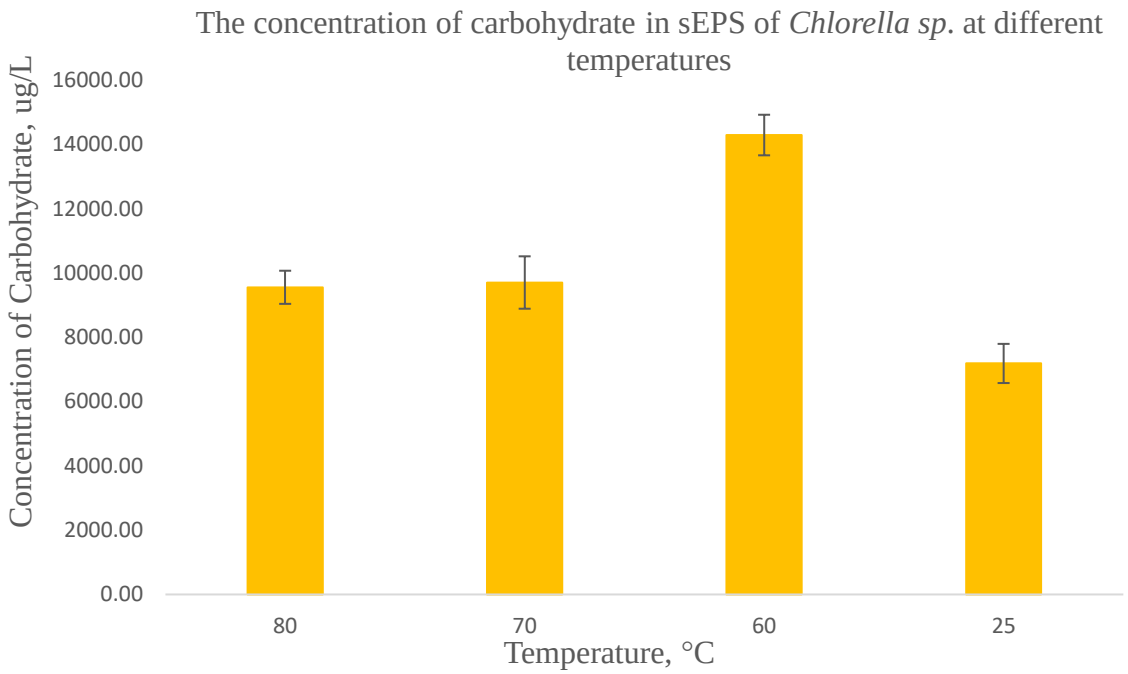

Figure 4.3: The concentration of carbohydrate in sEPS of *Chlorella sp*. at different temperatures

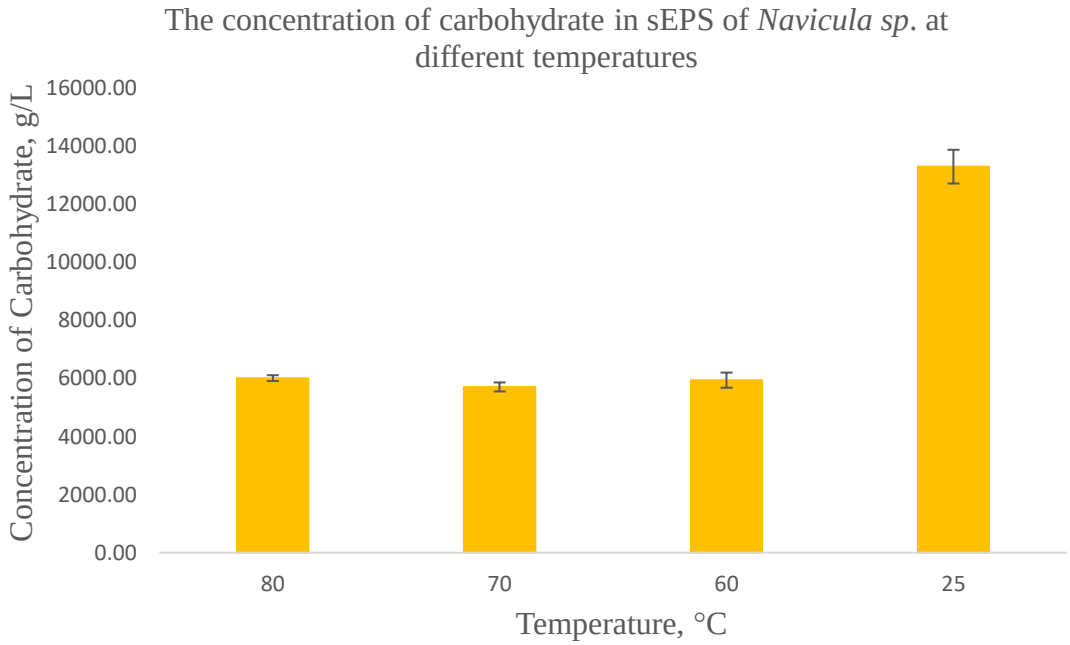

Figure 4.4: The concentration of carbohydrate in sEPS of *Navicula sp*. at different temperatures

Figure 4.3 and Figure 4.4 showed the concentration of carbohydrates in sEPS for *Chlorella sp.* and *Navicula sp.* at different temperatures. It was seen that the concentration of carbohydrates in the sEPS measures at room temperature or 25℃ were 7186.20 μg/L and 13302.59 μg/L for *Chlorella sp.* and *Navicula sp.* When *Chlorella sp* was exposed to higher temperatures of 60℃, 70℃ and 80℃, the carbohydrate released in sEPS increased compared to the room temperature. In (González-Camejo et al., 2020) obtained that a release of carbohydrates increases with temperature for *Chlorella sp.* From Figure 4.3, the results from this experiment show the amount of carbohydrate released in sEPS has the highest value at a temperature of 60℃. For *Navicula sp*, the carbohydrate in sEPS released a large amount of carbohydrate at room temperature.

Based on (Cheah et al.,2021) the amount of carbohydrate in the sEPS measured at room temperature has the lowest value compared to the amount of carbohydrate secreted when exposed to the highest temperature. The errors might be occurred during the experiment causing the results to be different from available studies. However, published research state that cell composition is influenced by culture, age, and cell wall composition. From Table 4.2, the algal organic material for both species was mainly from sEPS followed by bEPS / IOM. (McMillan et al., 2013) stated that after being exposed to higher temperatures, the algal cells' internal pressure became uncontrollable because the due to thermal pressure pushed them to rupture, and the cell wall consistency failed. Thus, the concentration of carbohydrates for EPS was highest when the temperature rose. In conclusion, changes in temperature influence the quantity of carbohydrates produced by both species.

4.3 Effect Temperature Towards Concentration Protein by Microalgae Algal Organic Matter

From Table 4.3, it was displayed that the concentration of protein in *Chlorella sp.* increased with the temperature. The highest amount of protein was produced in bEPS / IOM for *Chlorella sp.* with a concentration of 82.54 μg/L. The protein concentration in bEPS / IOM for *Navicula sp.* showed a different trend from *Chlorella sp.* The highest protein was produced in bEPS / IOM for *Navicula sp.* with a concentration of 292.22 μg/L at a temperature of 60℃. However, the concentration of protein in bEPS / IOM for *Navicula sp.* increases when the temperature increase compared to the room temperature.

Table 4.3: The concentration of protein in the sEPS, bEPS / IOM of *Chlorella sp.* and *Navicula sp.* at different temperatures

Species	Components	Concentration ($\mu g/L$)			
		80℃	70℃	60℃	25℃
Chlorella sp.	bEPS / IOM	82.54	27.17	20.79	22.20
	sEPS	96.68	134.60	91.85	98.03
Navicula sp.	bEPS / IOM	123.17	247.83	292.22	37.06
	sEPS	37.00	43.94	62.58	-3.26