EFFECT OF HIGH TEMPERATURE TOWARD MICROALGAL ORGANIC MATTER

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EFFECT OF HIGH TEMPERATURE TOWARD MICROALGAL ORGANIC MATTER

by

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# TABLE OF CONTENTS

ACKNOWLEDGEMENT ii

TABLE OF CONTENTS iii

LIST OF FIGURES vi

LIST OF ABBREVIATIONS viii

ABSTRAK ix

ABSTRACT VI

CHAPTER ONE: INTRODUCTION 1

1.1 Research background 1  
1.2 Problem statement 3  
1.3 Research objectives 4  
1.4 Research scope 4  
1.5 Organization of thesis 4

CHAPTER TWO: LITERATURE REVIEW 6

2.1 Benthic microalgae 6  
2.1.1 Environmental conditions that promote benthic microalgae growth 7  
2.1.2 External factors influencing benthic microalgae growth 7  
2.1.3 The role of benthic microalgae 8  
2.2 Components in microalgae suspension 8  
2.2.1 Algal cells 8  
2.2.2 Algal organic matter 9
4.2.2 Bounded extracellular polymeric substance 40
4.2.3 Internal organic matter 44
4.4 Protein Content 48
4.4.1 Soluble extracellular polymeric substance 48
4.4.2 Bounded extracellular polymeric substance 51
4.3.3 Internal organic matter 56

CHAPTER FIVE: CONCLUSION AND RECOMMENDATION 60
5.1 Conclusion 60
5.2 Recommendation 61

REFERENCES 62

APPENDIX 69
### LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 2.1</td>
<td>Definition of EPS structures.</td>
<td>12</td>
</tr>
<tr>
<td>Figure 2.2</td>
<td>Adsorption–desorption mechanism for humic acid through a membrane pore. (a) The humic acid is adsorbed on the membrane surface, (b) There is hydrogen bonding present between the water and humic acid, (c,d) Hydrogen bond weakens as the water vapour moves across the membrane, (e) Re-adsorption of humic acid on the membrane occurs, (f) Pore wetting occurs</td>
<td>18</td>
</tr>
<tr>
<td>Figure 4.1</td>
<td>The concentration of carbohydrate in the soluble EPS of <em>A. coffeaeformis</em>, <em>C. closterium</em> and <em>N. salinarum</em> species at different temperatures</td>
<td>25</td>
</tr>
<tr>
<td>Figure 4.2</td>
<td>The concentration of carbohydrate in the bounded EPS of <em>A. coffeaeformis</em>, <em>C. closterium</em> and <em>N. salinarum</em> species at different temperatures</td>
<td>30</td>
</tr>
<tr>
<td>Figure 4.3</td>
<td>The concentration of carbohydrate in the soluble EPS of <em>A. coffeaeformis</em>, <em>C. closterium</em> and <em>N. salinarum</em> species at different temperatures</td>
<td>33</td>
</tr>
<tr>
<td>Figure 4.4</td>
<td>MW distribution of carbohydrate in Soluble EPS at different temperatures for (a) <em>A. coffeaeformis</em>, (b) <em>C. closterium</em> and (c) <em>N. salinarum</em> species</td>
<td>36</td>
</tr>
</tbody>
</table>
Figure 4.5  MW distribution of carbohydrate in bounded EPS at different temperatures for (a) *A. coffeaeformis*, (b) *C. closterium* and (c) *N. salinarum* species

Figure 4.6  MW distribution of carbohydrate in IOM at different temperatures for (a) *A. coffeaeformis*, (b) *C. closterium* and (c) *N. salinarum* species

Figure 4.7  The concentration of protein in the soluble EPS of *A. coffeaeformis*, *C. closterium* and *N. salinarum* species at different temperatures

Figure 4.8  The concentration of protein in the bounded EPS of *A. coffeaeformis*, *C. closterium* and *N. salinarum* species at different temperatures

Figure 4.9  Protein/Carbohydrate fraction in soluble EPS and bounded EPS of (a) *A. coffeaeformis* (b) *C. closterium* (c) *N. salinarum* species

Figure 4.10  The concentration of protein in the IOM of *A. coffeaeformis*, *C. closterium* and *N. salinarum* species at different temperatures

Figure 4.11  Protein Percentage in IOM produced by *A. coffeaeformis*, *C. closterium* and *N. salinarum* species at room temperature
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOM</td>
<td>Algal organic matter</td>
</tr>
<tr>
<td>EPS</td>
<td>Extracellular polymeric substance</td>
</tr>
<tr>
<td>IOM</td>
<td>Internal organic matter</td>
</tr>
<tr>
<td>MD</td>
<td>Membrane distillation</td>
</tr>
<tr>
<td>MW</td>
<td>Molecular weight</td>
</tr>
<tr>
<td>TEP</td>
<td>Transparent exopolymer particles</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
</tbody>
</table>
KESAN SUHU TINGGI ATAS BAHAN ORGANIK MIKROALGA

ABSTRAK

Algal mekar serius boleh mempengaruhi operasi rawatan air, terutamanya proses rawatan membran terutamanya disebabkan oleh pengumpulan bahan organik alga (AOM). Kajian telah menunjukkan bahawa AOM mampu menimbulkan fouling yang teruk dalam proses membran tekanan rendah. Tahun-tahun kebelakangan ini, penyulingan membran (MD) telah mendapat perhatian yang ketara dalam industri untuk tujuan rawatan air. Walau bagaimanapun, fouling di MD berbeza daripada proses membran yang dipacu tekanan terutamanya disebabkan oleh suhu yang lebih tinggi yang digunakan dalam proses MD. Dalam kajian ini, pelbagai komponen AOM yang terdiri daripada bahan polimer ekstraselular (EPS) yang larut, EPS terikat dan bahan organik dalaman (IOM) dari tiga spesies alga bentik (Amfora coffeaeformis, Cylindrotheca closterium dan Navicula salinarum) dicirikan untuk kepekatan karbohidrat, pengagihan berat molekul karbohidrat dan kepekatan protein. Pencirian ini dilakukan dengan menggunakan analisis kolorimetrik, seperti kaedah asid fenol-sulfurik untuk mengukur kepekatan karbohidrat dan kaedah asid bicinchoninic (BCA) untuk menentukan kepekatan protein. Pencirian AOM dilakukan selepas alga dituai pada suhu bilik, dan juga selepas mereka terdedah kepada suhu yang lebih tinggi pada 60ºC, 70ºC dan 80ºC selama 1 jam. Ini adalah untuk membandingkan ciri-ciri AOM pada keadaan biasa dan juga pada suhu yang lebih tinggi semasa proses penyulingan membran. EPS yang dihasilkan oleh tiga spesies alga terdiri daripada kepekatan karbohidrat yang lebih tinggi berbanding dengan protein, di mana terdapat 93.04% karbohidrat untuk mikroalga A. coffeaeformis, 78.35% untuk mikroalga N. salinarum dan 82.83% karbohidrat untuk mikroalga C. closterium. Sebaliknya, peratusan protein dalam mikroalga A. coffeaeformis ialah 51.77%, untuk mikroalga C. closterium ialah 40.97%, dan mikroalga N. salinarum adalah 72.25%. Oleh
itu, kandungan karbohidrat dan protein berbeza dalam spesies yang berlainan, dan tidak dapat diperkatakan untuk setiap spesies alga. Apabila suhu yang terdedah kepada sel-sel algal meningkat, didapati kepekatan karbohidrat dan protein dalam EPS bertambah, sementara kepekatan karbohidrat dan protein dalam IOM menurun. Selain itu, didapati bahawa komposisi karbohidrat berat molekul yang lebih tinggi dalam AOM berkurangan pada suhu yang lebih tinggi, manakala komposisi karbohidrat berat molekul yang lebih rendah dalam AOM meningkat pada suhu yang lebih tinggi. Secara keseluruhan, komposisi karbohidrat, protein dan pengagihan berat molekul dalam EPS larut dalam EPS dan IOM pada suhu yang berbeza akan menentukan keparahan fouling di MD semasa alga mekar.
EFFECT OF HIGH TEMPERATURE TOWARD MICROALGAL ORGANIC MATTER

ABSTRACT

Algal blooms can seriously affect the operation of water treatment especially membrane treatment processes mainly due to accumulation of algal organic matter (AOM). Studies have shown that AOM is capable of causing severe fouling in low pressure membrane processes. In the recent years, membrane distillation (MD) has gained significant regard in the industry for water treatment purpose. However, fouling in MD differs from pressure-driven membrane processes mainly due to the higher temperature used in MD process. In this study, the different components of AOM, which are soluble extracellular polymeric substance (EPS), bounded EPS and internal organic matter (IOM) were extracted from three common species of benthic algae (Amphora coffeaeformis, Cylindrotheca closterium and Navicula salinarum) and they were characterized for the concentration of carbohydrate, the molecular weight (MW) distribution of carbohydrate and the concentration of protein. These characterization was done by employing colorimetric analysis, such as phenol-sulphuric acid method to measure the carbohydrate concentration and bicinchoninic acid (BCA) method to determine the protein concentration. The characterization of the AOM was done after the algal were harvested at room temperature, and also after they were exposed to higher temperature at 60°C, 70°C and 80°C for 1 hour. This is to compare the characteristics of the AOM at normal conditions and also at higher temperature during MD process. The EPS produced by the three algal species comprised of higher concentration of carbohydrate compared to protein, where there is 93.04 % of carbohydrate for A. coffeaeformis diatoms, 78.35 % for N. salinarum diatoms and 82.83 % of carbohydrate for C. closterium diatoms. On the
other hand, the protein percentage in \textit{A. coffeaeformis} diatoms was 51.77 \%, for \textit{C. closterium} diatoms was 40.97 \%, and for \textit{N. salinarum} diatoms was 72.25 \%. Hence, the carbohydrate and protein content varies in different species, and cannot be generalized for every algal species. When the temperature exposed to the algal cells were increased, it was seen that the concentration of carbohydrate and protein in EPS increases, while the concentration of carbohydrate and protein in IOM decreases. Apart from that, it was found that the composition of higher MW of carbohydrate in AOM decreases at higher temperature, while the composition of lower MW of carbohydrate in AOM increases at higher temperature. Overall, the compositions of carbohydrate, protein and the MW distributions in the soluble EPS, bounded EPS and IOM at different temperatures will likely dictate the severity of fouling in MD during algal blooms.
CHAPTER ONE
INTRODUCTION

1.1 Research background

Most of the microalgae produces algal organic matter (AOM), composed of organic compounds that are present in waters. These organic matters are usually produced by enzyme-catalyzed reaction for normal growth, or other stressful environment, such as nutrient limitation, unfavorable temperature, light or pH and attack by viruses or bacteria through the invasion or decay of algal cells (Xu et al., 2013). Cell rupture due to autolysis or external action can cause the concentration of AOM to be increased to a large extent (Pivokonsky et al., 2006). AOM produced by algae may comprise of various forms and differing concentrations of polysaccharides, proteins, lipids, small organic molecules such as low molecular weight (MW) acids (~1000 Da) and toxins (Villacorte et al., 2015a). AOM consists of extracellular polymeric substance (EPS) released by metabolic activity, and internal organic matter (IOM) generated due to cell rupture. The AOM content are different among different algal species but the trend of AOM concentration increases with the increase of age for all observed algal species (Henderson et al., 2008).

A fraction of AOM are released as EPS or also known as slime. They are formed by aggregation of dissolved precursors and exists as discrete particles (Passow, 2002). EPS is usually released during normal metabolic activity of algal cells or can also be released during cell lysis (Passow et al., 2001). EPS consists of mainly sticky polysaccharides and glycol-proteins (Alldredge et al., 1993). As the name “slime” implies, EPS is highly flexible and sticky organic material that constitutes biofilms and allows cellular attachment to surfaces (Passow, 2002). EPS also consists of different sizes from 0.4 to 200 μm (Alldredge et al., 1993). The amount of EPS also increases as the
algae growth, which means that it increases as AOM increases. Since EPS is a very sticky component of AOM, it has a very strong capability to absorb other AOM components, trace metals and other microbes in the algal suspension to stimulate the formation of aggregates (Mopper et al., 1995). Therefore, EPS acts as nutritious substrate for bacteria that are attached to it. (Van Nevel et al., 2012).

The increase of eutrophication, blooms of algae in aquatic systems have caused huge amounts of AOM and EPS to enter water treatment processes and cause water quality to drop and complicate the water treatment processes. In the recent years, membrane distillation (MD) has gained significant regard in the industry for water treatment purpose. However, the major drawback of using membrane processes for treatment of algal-rich water is due to the fouling of the membrane. Algal cells and AOM have been identified as the major components of algal suspensions. Many studies have shown that EPS is the main contributor to biological fouling during membrane filtration of AOM (Zhang and Fu, 2018). The accumulation of these algal cells and their metabolic products on the membrane surface and in the membrane pores reduces the membrane permeability. This causes the reduction in filtration efficiency, which increases the treatment cost (Babel and Takizawa, 2010).

Studies have shown that AOM is capable of causing severe fouling in low pressure membrane processes. This membrane fouling are related to the high MW organic matter (Lee et al., 2006). One study showed that the fouling behavior of microfiltration membrane caused by AOM extracted from five different algae that cause blooms, cyanobacteria, green algae and diatoms. They found out that AOM from the cyanobacterium Aphanizomenon flosaquae cause the greatest flux decline (Huang et al., 2014). The fouling of membrane is also contributed by the combined effect of AOM characteristics such as molecular size and hydrophobicity (Huang et al., 2014). Another
study showed the effect of hydrophilic and hydrophobic characteristics of AOM on fouling reversibility on the membrane. The AOM extracted from *Chlorella zofingiensis* consists mainly of carbohydrates, had the highest values of total and irreversible resistance. These observations are due to the role of hydrogen bonding between carbohydrates and the membrane which resulted in the formation of a dramatically compact cake layer (Zhang *et al.*, 2013a).

1.2 Problem statement

MD separation process requires the use of porous membranes to produce distilled water or concentration of wastewaters to recover and recycle valuable compounds. However, the efficiency of this technology is hampered by the fouling phenomena, which is caused by the accumulation of organic and inorganic residues that also includes biological matter on the membrane surface and in the membrane pores. This reduces the permeability and separation performance of the membrane. Also, fouling in MD differs from pressure-driven membrane processes such as microfiltration and ultrafiltration due to the different temperatures used and different driving forces. (Laqbaqbi *et al.*, 2017).

There are many studies that have been carried out to determine the effect of algae and AOM in pressure-driven membrane processes (Zhang *et al.*, 2013a, Chiou *et al.*, 2010, Ladner *et al.*, 2010). However, very few studies has been done to determine the effect of AOM in MD separation process. Previous studies have been done to identify the components of EPS and IOM of algal (Henderson *et al.*, 2008, Her *et al.*, 2004, Huang *et al.*, 2012). However, there is minimal literature that studied the effect of temperature towards the components of AOM (Singh and Singh, 2015). In this paper, the effect of temperature towards these components will be studied.
1.3 Research objectives

The aims of the research are as follows;

1. To identify the components of EPS and IOM.

2. To study the effect of temperature towards all these components

1.4 Research scope

In this study, were grown based on F/2 formulation and prepared accordingly. After the algal species were grown up to the exponential phase, soluble EPS, bounded EPS and IOM were separated. These samples were then taken for characterization of the amount of carbohydrate and protein contents in the samples. The carbohydrate characterization was done using phenol-sulphuric acid method, while the protein characterization was done using bicinchoninic acid (BCA) assay. These samples were then analyzed using UV-Vis Spectrophotometer. Lastly, these samples were also characterized after heating the algal suspensions to three different high temperatures, which are 60°C, 70°C and 80°C for 1 hour. The changes in the amount of EPS and IOM were compared among the different temperatures and different species.

1.5 Organization of thesis

This thesis consists of five main chapters and each chapter contributes to the sequence of this study. The following are the contents for each chapter in this study:

Chapter 1 introduces the overview of this research and the significance of effect of EPS and IOM on membrane fouling, problem statement, research objectives, research scope and the organization of thesis.
Chapter 2 discusses the literature review of this study. An insight into Benthic microalgae, a discussion on properties of microalgae suspension that consists of algal cells and AOM, which consists of soluble EPS, bounded EPS and IOM and the influence of different temperatures to AOM and the effect of AOM to membrane fouling are included as well.

Chapter 3 covers the experiment materials required for this study and the details of methodology from the start of this research project. It discusses on the description of equipment and materials used, characterization steps and the experimental procedures.

Chapter 4 refers to the experimental results and discussions of the data obtained. Further elaboration on the characterization results, comparison of effect of AOM at different temperatures are provided in this chapter.

Chapter 5 concludes all the findings achieved in this research study. Recommendations or future studies on this research topic are included as well.
CHAPTER TWO  
LITERATURE REVIEW

2.1 Benthic microalgae

Benthic microalgae, which is also known as estuarine microphytobenthos usually covers the hard bottoms from the seashore down up to 40 metres in depth. Below that level, inadequate sunlight hinders their growth. (Sullivan and Moncreiff, 1988). Benthic microalgae are vastly found in the intertidal sediments. This microphytobenthic community has to adapt themselves to various stressful factors such as temperature, nutrient concentrations, sediment transport and steep gradients in light for maximum productivity (Barranguet et al., 1998). However, certain species are able to be motile, which means they could migrate vertically up or down during environmental changes due to their ability to adapt to changes in their habitat, compared to those that are non-motile and bounded to the sediment (Conley et al., 1997).

Due to their dependence for light to grow, microphytobenthic species are usually confined to the uppermost few millimetres of sediment. However, they have also been found in substantial numbers in depths of 10 cm or more. At these depths, they are shown to play a role in limited reproduction due to the absence to light. Once they are exposed to light again, these organisms will resume their normal production and photosynthetic activities (Pinckney and Zingmark, 1993). The interactions of microphytobenthos with light, nutrient availability and grazing pressure hugely control the temporal and spatial patterns in its composition and abundance (Bennett et al., 2000). The changes in the abundances of microphytobenthos most likely effects the food webs in the coastal systems. This is because they are important food source for other benthic invertebrates (Bennett et al., 2000).
2.1.1 Environmental conditions that promote benthic microalgae growth

Microalgae is usually found in well-mixed sandy sediments within high-energy environments up to a depth of 10 cm. Microphytobethos can only photosynthesis to a depth of 0.2-2mm, since light only penetrates the sediment to this depth. Benthic microalgae usually live and grow in the top few millimetres of these bare and shallow ecosystem. Microphytobenthos uses light energy to fix CO$_2$ into organic matter. The extent of the currents, sediment mixing by waves and tidal actions, as well as the abundance of benthic macro fauna effects the depth distribution of microphytobenthos. This species is limited at the upper layer or oxygenated sediment because of the low energy organic rich environment (MacIntyre et al., 1996).

2.1.2 External factors influencing benthic microalgae growth

Microphytobenthic species are usually found at the uppermost surface layers of the sediments right at the sediment-water interface. This is because they are usually limited by the availability of light and nutrients. For microphytobenthos to photosynthesize, the layers for light should be good enough and it varies with both the granulometry of the sediment and its organic content. Benthic microalgae are usually known for their mobility, moving to and away from the surface in response to a multitude of factors, such as light, desiccation, predation and resuspension tide cycles. (Paterson et al., 1998)

The velocities of the cells to migrate vertically are usually low, ranging from 10 to 27 mm. However, their ability to move is important because the top layers of the sediment represent a region with strong physical and chemical gradients. Generally, the growth of benthic microalgae is not limited by nutrients as the nutrient concentrations in the pore water are generally high. However, the diatoms at the sediment surface biomass may become very concentrated, and therefore causing the nutrients to become depleted.
(Admiraal, 1977). When there are abundant of nutrients, the microphytobenthos can stabilize the sediment surface against resuspension and erosion. This is done by secreting mucilaginous films and forming thin, brownish mats or carpets (De Brouwer and Stal, 2001). These biofilms are mainly formed by diatoms excreting extracellular polymeric substances, also known as EPS (Cadée and Hegeman, 1974).

2.1.3 The role of benthic microalgae

Microphytobenthos plays an important role for the carbon source for the local benthic food webs, and also provides an important link for organic and inorganic compounds which are available to higher trophic levels and top predators (Mortazavi et al., 2000). The microphytobenthic community influences the nutrient fluxes and stabilization of the sediment processes by forming highly active bio-film, which significantly impacts the exchange of dissolved and particulate matter between the water column and sediment (Reay et al., 1995). Microphytobenthos are also important food source in estuaries due to their availability all year round and also their constant re-suspension of huge number of cells that are in the water column that enhance their accessibility to filter, suspension feeders and benthic grazers (Adams et al., 1999).

2.2 Components in microalgae suspension

2.2.1 Algal cells

Microalgae have variety of shapes, which are spheres, ellipsoids, and needles. Their cell sizes usually vary from several micrometers to one hundred micrometers (Ozkan and Berberoglu, 2013). All microalgae is able to change their locations and move towards a suitable microhabitat with better illumination or more nutrients for growth and reproduction. Flagellated cells usually swim through the medium by beating their organs of locomotion, known as flagella, while cyanobacteria and some diatoms, which do not contain flagella, can glide over the surface of a substrate using secreted mucilage. The
passage of microalgae cells is usually retained by microfiltration and ultrafiltration membranes and then forms a layer of cake on the surface of the membrane. The size and shape of the algal cells can influence the degree of flux decline and the morphology of the cake layer. Apart from the cells, there are some organic matter secreted by the cells that are attached to the outer cell walls of the algal, which will determine the chemical properties of the cell surface and changes the cell growth and forms (Castaing et al., 2011). The fouling of the membranes can be increased by the secreted materials by the cells.

2.2.2 Algal organic matter

Most of the microalgae is able to produce organic matters, which are known as AOM, produced by enzyme catalyzed reaction for normal growth or produced in response of extreme conditions, such as nutrient limitation, unfavorable temperature and attack by bacteria or viruses (Fogg, 1983). The concentrations of these organic matter is usually increased when cell ruptures during autolysis or due to external reactions (Pivokonsky et al., 2006). AOM widely consists of polysaccharides, proteins, humic-like substances and other small organic molecules such as low-MW acids and toxins (Zhang et al., 2013b). AOM is divided to EPS, which are usually released during metabolic activities of algae cells and IOM that are released during cell lysis. EPS were defined as “extracellular polymeric substances of biological origin that participate in the formation of microbial aggregates” (Sheng et al., 2010).

For different algal species, different amount of AOM are produced, ranging from several milligrams per liter to around 100 mg/L in the culture media (Pivokonsky et al., 2006). The AOM in algal species increases in concentration as they increase in age. The growth of algal species are divided into four stages, which are, the lag, exponential, stationary, and death phases (Huang et al., 2007). In the early phases of their growth,
most microalgal cells releases mainly EPS and very little IOM, as they have good integrity when they are young and the operating conditions are normal. However, the amount of EPS released by per cell in the exponential phase is much higher than the amount released during the stationary phase (Huang et al., 2007). During the death phase, the cells undergoes autolysis and rupture, which causes the IOM to be released into the culture media (Pivokonsky et al., 2006). This causes the concentration of AOM to exponentially increase. The amount of IOM in a cell is always higher compared to the amount of EPS released by the cell. The dissolved organic content of IOM in the exponential phase is around six times higher than the EPS released for Microcystis aeruginosa (Ou et al., 2011). Hence, to control membrane fouling, it is important to avoid the algal cells from rupturing. Therefore, the AOM concentration is an important parameter to be studied when using membrane technologies for the treatment of algae-rich water (Zhang et al., 2012).

AOM has properties such as organic nitrogen/carbon concentration, MW distributions and hydrophilicity/hydrophobicity, which can influence the tendency of membrane fouling. The properties of AOM obtained from culture of algal species is usually important for membrane fouling experiments. In AOM, the hydrophilic fractions are about 57%–80% during the exponential and stationary growth phases, for algal species of Chlorella vulgaris, M. aeruginosa (Henderson et al., 2008). On the other hand, the MW distribution varies among different microalgae species. The MW distributions of AOM in M. aeruginosa and C. vulgaris were typically bimodal, which were 55 and 62% was more than 30 kDa, and 38 and 30% was less than 1 kDa (Henderson et al., 2008). Generally, the large molecules exist in AOM are biopolymers, which consists of proteins and polysaccharides, while the small molecules consists of low-MW acids and humic-like substances (Li et al., 2008; Hong et al., 2008). For the size distribution of
polysaccharides that are present in AOM, the lower MW portion was higher than the high MW portion (Henderson et al., 2008). Different species of microalgae shows different properties of AOM.

AOM in microalgal are constitutes mainly of EPS and IOM. In IOM, for species such as M. aeruginosa and A. flosaquae, the amount of proteins that are present are up to 66.4 and 50.7%. For EPS, the polysaccharide compounds were higher compared to protein content. However, as the cell age, the protein content in EPS is increased (Pivokonsky et al., 2006). Therefore, it can be said that the amount of protein content is higher in IOM compared to EPS, as can be seen in species such as M. aeruginosa and Fragilaria crotonensis (Pivokonsky et al., 2014). The proteins and polysaccharides that were present in IOM has higher portions of high-MW (>100 kDa) compared to EPS (Pivokonsky et al., 2006). The average MW of IOM present in species such as M. aeruginosa is greater compared to the amount present in EPS, where for IOM the MW is greater than 30 kDa, where else for EPS the MW was widely distributed between 0.3-300 kDa (Li et al., 2012). This is why when the cell undergoes autolysis in the later growth phases, the AOM will consist of a higher MW components since more IOM is being released from the cell (Pivokonsky et al., 2014).

For the hydrophilic and hydrophobic property of EPS and IOM, some results has shown that the hydrophilic properties of proteins present in microalgae has higher proteins compared to polysaccharides. That is why the amount of hydrophilic compounds in EPS, which is around 69% or more is lower compared to that which is present in IOM, which is around 87% or more (Li et al., 2012). Meanwhile, there are some studies that shows that EPS is more hydrophilic compared to IOM, due to protein being classified as stronger hydrophobic compounds compared to polysaccharides (Zhou et al., 2014). The difference in these results may be due to the difference in their cultivation time.
Due to their fouling properties, more attention is usually paid to EPS compared to IOM. There are two categories of EPS, which are soluble EPS that consists of soluble macromolecules, colloids, and slimes and bounded EPS that consists of sheaths, capsular polymers, condensed gels, loosely bound polymers, and attached organic materials (Sheng et al., 2010). Soluble EPS are soluble cellular components that released and dissolved into surrounding medium (Laspidou and Rittmann, 2002). On the other hand, bounded EPS can be categorized as tightly bounded EPS, which are tightly adhered to the cell surface and stay in a fixed shape in the inner layers of EPS matrix, and loosely bounded EPS, where it is the outer layers of EPS that are loosely bound to the cells without certain edge (Sheng et al., 2010). Soluble EPS and bounded EPS can be separated by centrifugation, where the polymers remained in the supernatant are defined as soluble EPS, while pellets formed by polymers are considered as bounded EPS (Nielsen and Jahn, 1999). Figure 2.1 shows the definition of EPS structures (Sheng et al., 2010, Nielsen and Jahn, 1999).

![Figure 2.1: Definition of EPS structures (Sheng et al., 2010)](image)

Soluble EPS and bounded EPS are rich in polysaccharides and protein. They are also mainly composed of hydrophilic organic materials distributed in the high MW fraction, which are greater than 100 kDa and the low MW fractions, which are less than 1 kDa, similar to the properties of EPS (Qu et al., 2012a). However, soluble EPS has
much lower protein to polysaccharide ratio compared to bounded EPS. Soluble EPS also has a larger proportion of low MW and a greater hydrophilic content compared to bounded EPS. The proteins in the EPS is considered as hydrophobic materials with higher ratio of large MW fraction than polysaccharides (Qu et al., 2012a). For *M. aeruginosa* species, soluble EPS takes up a total of 80% of the total amount of EPS produced (Qu et al., 2012a). Hence, it can be concluded that soluble EPS has a higher influence of membrane fouling compared to bounded EPS.

Many environmental and nutritional factors such as temperature, radiation, shear stress, nutrient salts, and pH effects the algae growth apart from the type of algal species and the growth phases. Hence, the amount and properties of algal cells and AOM in algal suspension should be carefully evaluated, as they have great association with the propensity to foul membranes during water treatment processes.

2.3 Effect of algal organic matter on membrane fouling

AOM is one of the critical foulants of membranes that are used in algal suspension treatment compared to algal cells (Zhang et al., 2013a). Protein and polysaccharides that are present in the IOM are found to be the main foulants components of the membrane during membrane treatment processes (Hung and Liu, 2006). The fouling on the membrane caused my AOM can be a reversible process, which means deposition of foulants on the membrane surface and an irreversible process, which is the adsorption of foulants inside the membrane pores. AOM properties such as molecular distribution, hydrophilicity and hydrophobicity, and charge plays an important role in membrane fouling (Zhang et al., 2013a). For ultrafiltration process of AOM solution, algal AOM caused much rapid decline of flux at the initial stage, but a lesser total flux decline when compared with algal cells (Zhang et al., 2013a). AOM that consists of portions of low MW components could cause immediate and irreversible plugging and narrowing. On the
other hand, biopolymers are usually retained on the membrane surface by size exclusion and they slowly forms a layer of cake with time. This will alter the properties of the original membranes, and sieve or adsorb more biopolymers and low MW components in the AOM by serving as a dynamic barrier layer. Therefore, a cake layer is formed which is less compressible compared to that formed by cells (Zhang et al., 2011). Compared to pore plugging and narrowing, cake formation is more responsible for membrane fouling by algal AOM.

For *C. zofingiensis*, hydrophobic acid, hydrophilic acid, and hydrophilic non-acid fractions of the AOM was extracted. It was found that the hydrophilic non-acid fractions, which consist of mainly carbohydrates, had the highest value for the irreversible fouling resistance (Zhang et al., 2013a). This is because there were hydrogen bondings formed between the carbohydrate and hydrophilic polyvinyl chloride (PVC) membranes that caused the formation of a very compact cake layer. Another study showed that IOM consists of larger hydrophilic molecules. These molecules causes greater total and irreversible fouling compared to EPS. This is due to the IOM-membrane adhesion and IOM-IOM cohesion energy are higher compared to the EPS and the membrane surface. Cake formation was the main mechanism for IOM fouling, whereas EPS fouling caused pore plugging due to their differences in MW distributions (Li et al., 2014).

In a flux behavior of an ultrafiltration study, soluble EPS from *Microcystis aeruginosa*, had higher organic content and stronger hydrophilicity, which lead to a severe flux decline with higher reversible fraction. On the other hand, the bounded EPS caused a slower and irreversible decline in flux due to less electrostatic repulsion and greater hydrophobic adhesion (Qu et al., 2012a). The trend for microfiltration membrane fouling and ultrafiltration membrane fouling is very much similar, where after 90 minutes, the initial flux can be reduces to more than 80 % due to pore blocking and cake layer
formation by the foulants present in the AOM (Zhang et al., 2013a). For one experiment, when using the original large pore microfiltration membrane, large AOM molecules, more than 20,000 Da could be rejected, forming a cake layer. This also enhanced the removal of smaller molecules. Hence, cake formation is the main cause of AOM fouling in microfiltration systems (Zhang et al., 2013a). The electrostatic interaction between AOM and the membrane surface can also contribute membrane fouling by AOM. When comparing the AOM in *M. aeruginosa* and *C. vulgaris*, the AOM in *C. vulgaris* had lower negative charge and caused it to adsorb to the negatively charged membrane better. Hence, a higher irreversible fouling resistance took place on the membrane (Zhang et al., 2016).

The electrostatic repulsion and attraction, hydrophobic, hydrophilic and transphilic properties, and other specific interactions between the membrane and AOM depend greatly on the physicochemical properties of the AOM when the applied membrane used is the same. However, a thorough characterization of AOM samples is still difficult to be achieved. The most important constitute of AOM matrix on the membrane surface are carbohydrates. They consist of complex biochemical properties due to their diverse individual components and structures (Leppard, 1995). Moreover, each carbohydrates type has its own intrinsic properties and these properties changes accordingly as they age and due to environmental conditions (Mopper et al., 1995). Therefore, the characteristics of AOM should be study thoroughly to understand its effect on membrane fouling.

### 2.4 Fouling in membrane distillation

Fouling in MD is still relatively less studied and poorly understood when compared to fouling in other membrane separation process such as pressure driven membrane processes such as microfiltration, nanofiltration and reverse osmosis. In MD,
fouling can be divided into three types, which are organic fouling, inorganic fouling and biological fouling. AOM usually causes organic fouling in MD (Gryta, 2008). Therefore, in this paper, we will be discussing more on organic fouling in MD. Generally, foulants reacts with one another and also with the membrane surface to form deposits. This causes partial or total blockage of the membrane pores, which causes decline in the permeate flux of the membrane, and this causes the available evaporation area to decrease. Hence, a formation of fouling area on the membrane surface occurs which leads to the formation of a new resistance to the mass transfer. Therefore, the membrane tends to become prone to wetting, particularly when used for long term MD operations. Most of the studies done on MD is for direct contact membrane distillation using different membrane materials and membrane types.

Organic fouling is occurred due to the deposition of natural organic matter (NOM) on membrane surface. Example of NOM are carbohydrates, protein, humic acid, and carboxylic acid. The main components of NOM are humic substances, followed by carbohydrates, proteins and then variety of acidic and low MW components (Naidu et al., 2014). Fouling in MD occurs due to several factors which includes the membrane surface characteristics. This is because a more hydrophobic membrane with smaller pore size can (Khayet et al cause the fouling to increase substantially al., 2004). Apart from that, fouling is dependent on the nature of organic matter, the operating conditions of MD, such as the temperature, transmembrane pressure and the flowrate, and the characteristics of the feed solution, such as the pH and ionic strength of the feed components. An example of adsorption- desorption mechanism where the humic acid migrates through a membrane pore can be seen in Figure 2.2. First, the humic acid is adsorbed on the membrane surface (a). There is hydrogen bonding present between the water and humic acid (b) and this
bond weakens as the water vapour moves across the membrane (c,d). Then, re-adsorption of humic acid on the membrane occurs (e) and causes pore wetting (f).

In a MD process where humic acid solutions are used with concentrations of 10 to 50 mg/L, it showed that the permeate flux declines for less than 8 % after 30 hours of operation (Khayet et al., 2004). Another study showed that very limited fouling occurred and a flux decline of less than 6% occurred when using solutions contain humic acid with concentrations from 20 to 100 mg/L (Srisurichan et al., 2005). In a study fouling on MD by using humic acid, amino acid and bovine serum albumin (BSA), it was seen that BSA and humic acid caused very high fouling on the membrane, where a permeate flux decline of 50% occurred. However, due to its hydrophilic nature, amino acid showed very little fouling of the membrane. When BSA and humic acid feed solutions were compared, it was seen that there were higher deposits for BSA feed solution, which is about 35.2% higher carbon mass (Naidu et al., 2014).
Figure 2.2: Adsorption–desorption mechanism for humic acid through a membrane pore. (a) The humic acid is adsorbed on the membrane surface, (b) There is hydrogen bonding present between the water and humic acid, (c,d) Hydrogen bond weakens as the water vapour moves across the membrane, (e) Re-adsorption of humic acid on the membrane occurs, (f) Pore wetting occurs.

(Laqbaqbi et al., 2017)
2.5 Colorimetric analysis of AOM

AOM is mainly composed of carbohydrates and protein. Therefore, conventional colorimetric analysis has been used to test the quantity of carbohydrate and protein in the AOM (Sheng et al., 2010). Carbohydrates are usually measured using the anthrone method or the phenol-sulfuric acid method, where glucose is used as the standard. The measured results using these two methods gave quite similar results (Gaudy, 1962, Dubois et al., 1956). However, coefficient of variation for the phenol-sulfuric acid method was higher than that for the anthrone method (Fr et al., 1995). Nevertheless, phenol-sulfuric acid method is a simple and rapid colorimetric method usually used to determine the amount of carbohydrates in a sample. This method is capable to detect virtually all classes of carbohydrates, which includes mono-, di-, oligo-, and polysaccharides (Dubois et al., 1956).

Proteins can generally be tested using the Lowry method (Lowry et al., 1951) and bicinchoninic acid (BCA) method (Smith et al., 1985), where using Bovine serum albumin (BSA) is used as the standard. BCA method is similar to the Lowry method. However, it is more stable in alkaline conditions, and it can be carried out in a one-step compared to the Lowry method, which is a two-step process. Hence, BCA is a four times easier and faster process compared to classical Lowry method (Smith et al., 1985).
CHAPTER THREE
MATERIALS AND METHOD

3.1 Organism and culture Conditions

First, F/2 medium was prepared and sterilized in an autoclave at 121°C for 15 minutes to kill any bacteria, spores and germs. *Amphora coffeaeformis, Cylindrotheca closterium* and *Navicula salinarum* diatoms were then subcultured and grown in the sterilized F/2 medium. The F/2 medium composition is attached in the Appendix A. These diatoms were cultivated in 2 L Erlenmeyer flasks at 25°C in a laminar flow cabinet, to prevent contamination. A total of 4 L of cultures for each species were prepared. The cultures were then aerated using pumps. Finally, all cultures were then incubated under the fluorescent tubes (1500 lux) with a 16 h light: 8h dark photoperiod for 3 weeks. Cells were then harvested during their stationary growth phase. The culture are gently shaken by hand, until all cells are in suspension, to harvest the cells.

3.2 Effect of high temperature towards all these components

500 mL of harvested cells were transferred into four glass jars, for each species. The samples were taken in duplicate (n=2) from one species. One of the glass jar containing the harvested cells were left at room temperature for 1 hour, while the harvested cells in the other three glass jars were heated to 60°C, 70°C and 80°C for 1 hour in a water bath. Then, it was left to cool at room temperature. Once the cells were cooled down, the extraction of soluble EPS, bounded EPS and IOM were carried out, and then these samples were takes for characterizations of carbohydrate content, MW distribution and protein content, as stated in following subsections. The comparisons of the results were made for different species at different temperatures.
3.3 Extracellular polymeric substance (EPS) & internal organic matter extraction (IOM) isolation

3.3.1 Soluble extracellular polymeric substance (EPS)

500 mL of the harvested cells were centrifuged at 4500 rpm for 15 minutes. Then, the supernatant were collected as samples of soluble EPS, while the precipitate cells were used for extraction of bounded EPS and IOM. 0.2 mL of the soluble EPS sample was checked for carbohydrate, 5 mL of the sample was used for MW distribution and 0.1 mL of the sample was used to check for protein contents.

3.3.2 Bounded extracellular polymeric substance (EPS)

The precipitated cells obtained after centrifuging were rinsed thoroughly 3 times with a total of 135 mL of 1.5 M NaCl to extract the bounded EPS from the cells, and also to ensure that all the bounded EPS were removed from the cells. Then, the suspension was centrifuged at 4500 rpm for 15 minutes. After centrifugation, the supernatant were collected as samples of bounded EPS, while the precipitate cells were used for extraction of IOM. 0.2 mL of the bounded EPS sample was checked for carbohydrate, 5 mL of the sample was used for MW distribution and 0.1 mL of the sample was used to check for protein contents.

3.3.3 Internal organic matter (IOM)

After removal of the bounded EPS, the precipitated cells obtained after centrifugation were washed three times with seawater salt water. The cells were then re-suspended in 20 mL of distilled water. The cells were then exposed to sonication at 37 kHz for 30 minutes to break the cell walls of algal cells and release the intercellular materials. The suspension were then centrifuged at 4500 rpm for 15 minutes. The organic matter in the supernatant were referred to as IOM. 0.2 mL of the IOM sample was checked
for carbohydrate, 5 mL of the sample was used for MW distribution and 0.1 mL of the sample was used to check for protein contents.

3.4 Characterization of extracellular polymeric substance (EPS) and internal organic matter (IOM)

3.4.1 Carbohydrate

The carbohydrate content of the EPS and IOM was determined using the colorimetric phenol-sulphuric acid methods described by Dubois et al. (1956). The sample (0.2 mL) was transferred into a test tube and distilled water was added to make up a 2 mL solution. Then, 1 mL 5% phenol was added to the solution followed by 5 mL concentrated sulphuric acid (97%). The test tube were carefully vortexed and allowed to stand for 10 minutes at room temperature (25 °C) before being placed in water bath at 30 °C for 15 minutes. The absorbance of the solution was read at 490 nm. The amount of carbohydrate present was determined by reference to a standard curve constructed using glucose through a series of dilutions as attached in Appendix D. (Dubois et al., 1956)

3.4.2 Molecular weight distributions of carbohydrate

The samples initially prepared were taken to analyze for their MW distribution of carbohydrate. First, 5 mL of the soluble EPS, insoluble EPS and IOM samples were dialyzed in 100 mL of distilled water through dialysis membrane with a MW cut off of 12000-14000 Daltons overnight at 5°C. Then, 0.2 mL of the collected solution in the dialysis membrane was tested for carbohydrate content using phenol-sulphuric acid method. The sample (0.2 mL) was transferred into a test tube and distilled water was added to make up a 2 mL solution. Then, 1 mL 5% phenol was added to the solution followed by 5 mL concentrated sulphuric acid (97%). The test tube were carefully vortexed and allowed to stand for 10 minutes at room temperature (25 °C) before being placed in water bath at 30 °C for 15 minutes. The absorbance of the solution was read at
490 nm. The amount of carbohydrate present was determined by reference to a standard curve constructed using glucose through a series of dilutions. The concentration obtained from this data would indicate the concentration of carbohydrate with MW greater than 12000- 14000 Daltons, while the remaining would indicate the concentration of carbohydrate with MW less than 12000- 14000 Daltons.

3.4.3 Protein

The samples initially prepared were taken for protein analysis. A standard working solution (SWR) is formed by mixing 50 vol of Reagent A (0.1 g of sodium bicinchoninate, 2.0 g of Na2CO3·H2O, 0.16 g of sodium tartrate (dihydrate), 0.4 g of NaOH and 0.95 g of NaHCO3, made up to 100 mL) with 1 vol of reagent B (0.4 g of CuSO4·5H2O in 10 mL of water). The solution formed is apple green in color and is stable at room temperature for 1 week. 2 mL of SWR is added to the 100 µL of sample. The solution let to incubate at 37°C for 30 min. The sample is then cooled to room temperature, and the absorbance was measured at 563 nm using a spectrophotometer. A calibration curve is constructed using dilutions of stock of 2 mg/mL of bovine serum albumin (BSA). A standard curve of absorbance as a function of initial protein concentration is plot and used to determine the unknown protein concentrations as attached in Appendix E. (Walker, 1996).
CHAPTER FOUR
RESULT AND DISCUSSION

4.1 Carbohydrate Content

The cells were harvested 3 weeks after they were cultured. This experiment was carried out to determine the amount of carbohydrate that is present in the soluble EPS, bounded EPS and IOM produced by *A. coffeaeformis*, *C. closterium* and *N. salinarum*. Therefore, phenol-sulphuric acid method was used to determine the carbohydrate content in the sample. This method was used as it detects all classes of carbohydrates, including mono-, di-, oligo-, and polysaccharides present.

4.1.1 Soluble extracellular polymeric substance (EPS)

Based on the Figure 4.1, it was seen that *A. coffeaeformis* diatoms released the highest amount of soluble EPS when they were harvested at room temperature, with concentration of carbohydrate of 3859.21 ± 79.52 μg/g. Then, it was followed by *N. salinarum* diatoms that released 2868.00 ± 56.16 μg/g of carbohydrate in the soluble EPS. The least amount of carbohydrate released in the soluble EPS among these three species was *C. closterium* diatoms that released 758.82 ± 34.32 μg/g of carbohydrate in the soluble EPS.
Soluble EPS is dissolved in the algal suspension. The chemical characteristics of EPS, based on laboratory cultures of diatoms, shows that it consists of mainly heteropolysaccharides, which contain significant amounts of uronic acid and sulphate residues (Myklestad, 1995, Magaletti et al., 2004). In addition, there are other polysaccharide components that are released as a result of the damage or destruction of diatom cells. (Santschi et al., 1998).

Based on Figure 4.1, *A. coffeaeformis* diatoms, it was seen that when the amount of carbohydrate in the soluble EPS was measured at room temperature, the amount of carbohydrate present in the cells were 3859.21 ± 79.52 μg/g. When the cells were heated to 60°C, the concentration of carbohydrate present in the soluble EPS increased to 3913.63 ± 43.80 μg/g. As the cells were further heated to 70°C, the concentration of carbohydrate present in the soluble EPS increased to 3984.3 ± 37.75 μg/g. At 80°C, the concentration of carbohydrate increased to 4072.28 ± 63.98 μg/g. When the cells were
immersed at a higher temperature for 1 hour, it was observed that the concentration of carbohydrate present in the soluble EPS increases.

Similar trend was observed for *C. closterium* diatoms, where when the amount of carbohydrate in the soluble EPS was measured at room temperature, the amount of carbohydrate present in the cells were 758.82 ± 34.32 μg/g. When the cells were heated to 60°C, the concentration of carbohydrate present in the soluble EPS increased to 866.00 ± 17.95 μg/g. As the cells were further heated to 70°C, the concentration of carbohydrate present in the soluble EPS further increased to 1044.19 ± 25.55 μg/g. At 80°C, the concentration of carbohydrate increased to 1223.13 ± 57.08 μg/g. This data is observed in Figure 4.1. When the cells were immersed at a higher temperature for 1 hour, it was observed that the concentration of carbohydrate present in the soluble EPS increases.

*N. salinarum* diatoms also shows a similar trend of the amount of carbohydrate present in the soluble EPS as the other diatoms mentioned earlier, as observed in Figure 4.1. When the concentration of carbohydrate in the soluble EPS was measured at room temperature, the carbohydrate present in the cells were 2868.00 ± 56.16 μg/g. When the cells were heated to 60°C, the concentration of carbohydrate present in the soluble EPS increased to 2938.27 ± 37.33 μg/g. As the cells were further heated to 70°C, the concentration of carbohydrate present in the soluble EPS further increased to 3093.45 ± 146.83 μg/g. When the cells were heated to 80°C, the concentration of carbohydrate increased to 3226.22 ± 330.67 μg/g.

When the algal cells were immersed in the hot water bath, it has caused cell disruption by thermally induced pressure, leading to cells rupturing as they fail to contain their elevated internal pressure. The majority of disrupted cells led to the production of large debris from cracked and split cells. There are some exceptions to the large debris, where in some cases the cells exploded in a very uniform manner releasing all of the cell
contents in a wide radius (McMillan et al., 2013). Based on this study, it can be seen that there is an increase in the concentration of carbohydrate in the soluble EPS as the temperature the cells increases, and this is caused by the disruption of cells that caused the IOM in the cell to be released. When the temperature increases, the disruption on the number of cells also increases, which causes the release of the IOM to the surroundings to increase. This increases the carbohydrate concentration in the soluble EPS.

Available literature suggests that cell disruption depends on the microalgae species, age of the culture and the composition of cell wall. Therefore, the results obtained from the three species varies and these results obtained from one species cannot be generalized to all other species (Rickman et al., 2012). In addition, depending on the algal species, the polysaccharide fraction is also present in the form of structural cell walls and cellulose. These polysaccharides usually depolymerise under higher temperatures to produce monomers (Toor et al., 2013). This might also lead to the additional increase of carbohydrate in the soluble EPS at higher temperature.

There is a special class of EPS that are described as mucopolysaccharides, called transparent exopolymer particles (TEP). It is usually defined based on being retained by a filter with a pore size of greater than 0.4 μm (Alldredge et al., 1993). Hence, TEP are defined as gel particles. TEP exists in the water column suspended in colloidal form, likely formed by the aggregation of smaller EPS molecules (Engel et al., 2004). It is also possible that the majority of these TEP components may have originated from cell wall material and intracellular sources as compromised and deteriorating cells during the stationary-death phase may have released more TEP-like material (Villacorte et al., 2015a). Therefore, based on this study, TEP components would have been formed from the deteriorating cells when exposed to high temperature, as well as may be released during cell lysis.
The main components of TEP are polysaccharides (Villacorte et al., 2015a). The distinctive components of TEP exhibit the properties of high flexibility and stickiness (Passow, 2002). As the major component of the sticky materials in EPS, TEP have a strong tendency to promote the formation of aggregates by absorption of other EPS components, trace metals, and other microbes in the algal suspension (Mopper et al., 1995, Jackson, 1990). There are many studies that identified TEP as the main cause to biological fouling during membrane treatment processes of algal organic matter (Villacorte et al., 2015a, Discart et al., 2013).

Based on a study carried out, it was found that when the EPS was extracted from *C. zofingiensis*, most of the carbohydrates were hydrophilic components (Zhang et al., 2013a). These hydrophilic components had the highest values of total and irreversible hydrophilic resistance. This was due to the hydrogen bonding between carbohydrates and hydrophilic polyvinyl chloride (PVC) membranes, where it caused the formation of a dramatically compact cake layer. However, when hydrophobic polyethersulfone membranes were used, polysaccharides, which contained major hydrophilic organics in the soluble EPS proved to only contribute to a fraction of the irreversible fouling. This is due to the smaller MW fractions of polysaccharides that caused clogging of the membrane pores. They were still responsible for the total fouling by cake layer formation because of their strong affinity toward water (Qu et al., 2012b).

Therefore, during MD process, when the operating temperature used is higher, the carbohydrate released to the surrounding as soluble EPS will be increased. This will increase the fouling of the membranes used. However, if a suitable membrane is chosen, where minimal irreversible fouling is caused, then the lifespan of the membrane used will be longer. Based on the three algal species used in this study, *A. coffeaeformis* diatoms is
most likely to cause the most fouling of membrane, as it contain the highest amount of carbohydrate in the soluble EPS.

Each carbohydrate type has its own intrinsic properties, these properties can vary substantially at different temperatures when being operated in MD process. (Zhang et al., 2011, Mopper et al., 1995). For example, TEP formed can enhance the growth of biofilm on the membrane surface (Berman and Holenberg, 2005, Berman et al., 2011), which could also alter the foulant matrix on the membrane surface (Zhang et al., 2011). Therefore, additional attention should be given to study the characteristics of EPS at higher temperature and the structural properties of the EPS matrix on the membrane surface.

4.1.2 Bounded extracellular polymeric substance (EPS)

Figure 4.2 shows the concentration of carbohydrate in bounded EPS released by the three different algal diatoms. It was seen that A. coffeaeformis diatoms released the highest amount of carbohydrate, which is 1638.89 ± 90.32 μg/g. Then, it was followed by N. salinarum diatoms that released 868.99 ± 91.18 μg/g of carbohydrate in the bounded EPS. The least amount of carbohydrate released in the bounded EPS among these three species was C. closterium diatoms that released 398.62 ± 19.84 μg/g of carbohydrate in the soluble EPS.
Based on Figure 4.2, the concentration of carbohydrate in the bounded EPS increases when *A. coffeaeformis* diatoms were heated from room temperature to 60°C, 70°C and 80°C. At 25°C, the concentration of carbohydrate in bounded EPS was 1638.89 ± 90.32 μg/g. When the cells were heated to 60°C, the concentration of carbohydrate in bounded EPS increased to 1907.6 ± 162.33 μg/g. At 70°C and 80°C, the concentration of carbohydrate in bounded EPS increased to 1952.52 ± 61.80 μg/g and 2128.60 ± 96.41 μg/g.

For *C. closterium* diatoms, the concentration of carbohydrate in the bounded EPS also increases when the cells were heated to higher temperature, as can be seen in Figure 4.2. At room temperature, the concentration of carbohydrate in the bounded EPS was 398.63 ± 19.84 μg/g. At 60°C, the concentration of the carbohydrate in the bounded EPS increased to 433.85 ± 22.65 μg/g. When the cells heated to 70°C and 80°C, the
concentration of carbohydrate in bounded EPS increased to 446.59 ± 8.79 μg/g and 566.20 ± 23.77 μg/g.

Based on Figure 4.2, *N. salinarum* diatoms also shows an increase in the amount of carbohydrate present in the bounded EPS as the temperature increase. When the amount of carbohydrate in the bounded EPS was measured at room temperature, the amount of carbohydrate present were 868.99 ± 91.18 μg/g. When the cells were heated to 60°C, the concentration of carbohydrate present in the bounded EPS increased to 998.44 ± 44.74 μg/g. When the cells were heated to 70°C, the concentration of carbohydrate present in the bounded EPS further increased to 1139.37 ± 8.79μg/g. At 80°C, the concentration of carbohydrate increased to 1285.01 ± 23.77 μg/g.

Bounded EPS is the EPS produced by algal that coats the cell surfaces. When a higher temperature was exposed to the algal cells, the concentration of carbohydrate in the bounded EPS increases. This is due to cell lysis caused by the thermally induced pressure on the cell, causing the IOM in the cells to be released to the surrounding, as explained in Section 4.1.1 earlier. The carbohydrate released from the cells then sticks on the cell surface, due to the stickiness caused by TEP present in the EPS, causing it to be bounded to the cells. When the temperature increases, the cell lysis also increases, causing the release of more AOM. In addition, polysaccharide fraction is also present in the form of structural cell walls and cellulose. The depolymerization of these carbohydrate under higher temperatures to produce monomers might also be the cause of the increase in carbohydrate in bounded EPS (Toor *et al.*, 2013). TEP released also will be increased due to the deteriorating cells and cell lysis, as previously mentioned. Therefore, the carbohydrate concentration in the bounded EPS increases when the temperature the algal cells exposed to increases.
Based on this study, soluble EPS accounted 70.19% of the total EPS (soluble EPS + bounded EPS) content for *A. coffeaeformis* diatoms at 25°C. For *N. salinarum* diatoms, soluble EPS accounted of 76.75% of the total EPS content, and for *C. closterium* diatoms, soluble EPS accounted of 65.56% of the total EPS content. Other studies also found that the soluble EPS of *M. aeruginosa* accounted for 80% of the total EPS content (Qu et al., 2012b). Hence, we can deduce that soluble EPS has a more important correlation with membrane fouling compared to bounded EPS.

The impact of bounded EPS on membrane fouling has not been clearly identified. Some studies shown that bounded EPS were not directly associated with membrane fouling (Geng and Hall, 2007). However, bounded EPS can be subdivided into tightly bounded EPS, which are closely adhered to the cell surface, and loosely bounded EPS, which are the outer layers of EPS that are loosely bounded to the cells. A study showed that loosely bounded EPS had a more significant impact on membrane fouling compared to tightly bounded EPS (Wang *et al.*, 2009).

However, in MD process, the carbohydrate concentration in the bounded EPS increases since higher temperature is used, as shown in this study. This may also increase the carbohydrate content in the loosely bounded EPS, which causes a higher rate of fouling of membrane to occur. The fouling of membrane caused by carbohydrate in EPS has been discussed in Section 4.1.1. Comparing the three algal species studied, *A. coffeaeformis* diatoms is most likely to cause the most fouling of membrane, as it contain the highest amount of carbohydrate in the bounded EPS.

### 4.1.3 Internal organic matter (IOM)

Figure 4.3 shows the concentration of carbohydrate in the IOM that is present in the three different algal diatoms. In *A. coffeaeformis* diatoms, the amount of carbohydrate in the IOM was 556.79 ± 20.33 μg/g, which was the highest. Then, it was followed by *N.*
**salinarum** diatoms that released 450.33 ± 12.92 μg/g of carbohydrate in the IOM. The least amount of carbohydrate released in the IOM among these three species was *C. closterium* diatoms that released 436.56 ± 24.30 μg/g of carbohydrate in the IOM.

![Graph showing concentration of carbohydrate in IOM of different diatoms at different temperatures](image)

**Figure 4.3**: The concentration of carbohydrate in the IOM of *A. coffeaeformis*, *C. closterium* and *N. salinarum* species at different temperatures

The trend of IOM of all three diatoms shows a decrease in the concentration of the carbohydrate in the IOM. Based on Figure 4.3, the concentration of carbohydrate in the IOM decreases when *A. coffeaeformis* diatoms were heated to 60°C, 70°C and 80°C from room temperature. At 25°C, the concentration of carbohydrate in IOM was 556.79 ± 20.33 μg/g. When the cells were heated to 60°C, the concentration of carbohydrate in the IOM decreased to 282.25 ± 17.94 μg/g. At 70°C and 80°C, the concentration of carbohydrate in IOM decreased to 236.40 ± 5.99 μg/g and 103.40 ± 7.01 μg/g.

When *C. closterium* diatoms were heated to high temperature, the concentration of the carbohydrate in IOM decreases, as seen in Figure 4.3. At room temperature, the
concentration of carbohydrate in the IOM was 436.56 ± 24.30 μg/g. When the cells were heated to 60°C, the concentration of carbohydrate in the IOM decreased to 295.70 ± 11.61 μg/g. At 70°C and 80°C, the concentration of carbohydrate in the IOM further decreased to 143.80 ± 2.26 μg/g and 100.76 ± 5.66 μg/g.

Based on Figure 4.3, *N. salinarum* diatoms also shows a decrease in the amount of carbohydrate present in the IOM as the temperature increase. When the amount of carbohydrate in the IOM was measured at room temperature, the amount of carbohydrate present were 450.33 ± 12.92 μg/g. When the cells were heated to 60°C, the concentration of carbohydrate present in the IOM decreased to 310.54 ± 16.88 μg/g. When the cells were heated to 70°C, the concentration of carbohydrate present in the IOM further decreased to 154.82 ± 11.00 μg/g. At 80°C, the concentration of carbohydrate decreased to 74.77 ± 19.43 μg/g.

When the temperature to the algal cells were increased, the concentration of carbohydrate in the IOM decreases rapidly for the three diatoms. This is caused by cell lysis due to thermal pressure on the cell that caused the IOM in the cell to be released. When the temperature increases, the number of cells that undergoes cell lysis due to the higher thermal pressure increases, which causes the amount of the IOM to be released to the surrounding to increase, as discussed in sections 4.1.2 and 4.1.3. This might also release some TEP that is present in the IOM. Hence, the amount of IOM remaining in the cells decreases, which causes the carbohydrate content in the cell to decrease as well.

Some studies showed that the polysaccharides in *M. aeruginosa*’s IOM, contains carbohydrates, which are larger and hydrophilic compared to EPS. (Li *et al.*, 2014, Zhang *et al.*, 2013). Since hydrophobic membrane will be used in MD, only cake formation will occur, which is reversible and can be recovered by hydraulic backwashing.
Also, when high temperature is used during MD process, the carbohydrate tend to undergo depolymerization to form monomers, which reduces the MW. Hence, irreversible fouling of membrane might occur due to the pore plugging of the membrane during MD process, as have been discussed in Section 4.1.1 (Li et al., 2014). Based on the three algal species studied, A. coffeaeformis diatoms is most likely to cause the most fouling of membrane, as it contain the highest amount of carbohydrate in the IOM.

4.2 Molecular weight distribution of carbohydrate

This experiment was carried out to determine the amount of carbohydrate with MW that is greater than 12,000- 14,000 Daltons and less than 12,000- 14,000 Daltons present in the soluble EPS, bounded EPS and IOM produced by A. coffeaeformis, C. closterium and N. salinarum. Phenol-sulphuric acid method was used to determine the amount of carbohydrate present as this method is able to detect all classes of carbohydrates, including mono-, di-, oligo-, and polysaccharides present. The MW was distributed by dialysis the sample in distilled water overnight using a dialysis membrane with a MW cut off of 12000- 14000 Daltons.

4.2.1 Soluble extracellular polymeric substance (EPS)

Figure 4.4 (a), (b) and (c) shows the concentration of carbohydrate with MW greater and less than 12,000- 14,000 Daltons in the soluble EPS that is released by the three different algal diatoms. At 25°C, it was seen that C. closterium diatoms released higher amount of carbohydrate with MW greater than 12,000- 14,000 Daltons in the soluble EPS and lesser amount of carbohydrate with MW less than 12,000- 14,000 Daltons in the soluble EPS. On the other hand, A. coffeaeformis and N. salinarum diatoms released lesser amount of carbohydrate with MW greater than 12,000- 14,000 Daltons in the soluble EPS and higher amount of carbohydrate with MW less than 12,000- 14,000 Daltons in the soluble EPS. Some studies have shown that shown that for the size
distribution of polysaccharides in algal organic matter, the lower MW portion was greater compared to the higher MW portion (Qu et al., 2012b, Henderson et al., 2008). This study is in sync with the results of *A. coffeaeformis* and *N. salinarum* diatoms. However, different microalgae produce different amounts of AOM, which may also have varying properties.

![MW distribution of carbohydrate in Soluble EPS at different temperatures for (a) *A. coffeaeformis*, (b) *C. closterium* and (c) *N. salinarum* species](image)

Figure 4.4: MW distribution of carbohydrate in Soluble EPS at different temperatures for (a) *A. coffeaeformis*, (b) *C. closterium* and (c) *N. salinarum* species
Figure 4.4: Continued
Based on Figure 4.4 (a), it was seen that the carbohydrate with MW greater than 12,000-14,000 Daltons in soluble EPS released by A. coffeaeformis diatoms decreases as the temperature increase. At room temperature, the concentration of carbohydrate with MW greater than 12,000-14,000 Daltons in soluble EPS was 1748.33 μg/g, and as the temperature increased to 60°C, 70°C and 80°C, the concentration if the carbohydrate with MW greater than 12,000-14,000 Daltons also decreased to 1017.97 μg/g, 572.86 μg/g and 425.05 μg/g. On the other hand, the carbohydrate with MW less than 12,000-14,000 Daltons in soluble EPS increases as the temperature increases. At room temperature, the concentration of carbohydrate with MW less than 12,000-14,000 Daltons in soluble EPS was 2167.11 μg/g, and as the temperature increased to 60°C, 70°C and 80°C, the concentration of the carbohydrate with MW less than 12,000-14,000 Daltons also increases to 2864.69 μg/g, 3438.18 μg/g and 3692.47 μg/g.

Similar trend was observed for C. closterium diatoms as seen in Figure 4.4 (b), where the concentration of carbohydrate with MW greater than 12,000-14,000 Daltons in soluble EPS decreases as the temperature increase. At room temperature, the amount of carbohydrate with MW greater than 12,000-14,000 Daltons in soluble EPS was 448.30 μg/g, and as the temperature increased to 60°C, 70°C and 80°C, the concentration if the carbohydrate with MW greater than 12,000-14,000 Daltons also decreases to 577.13 μg/g, 390.11 μg/g and 315.33 μg/g. On the other hand, the carbohydrate with MW less than 12,000-14,000 Daltons in soluble EPS increases as the temperature increases. At room temperature, the concentration of carbohydrate with MW less than 12,000-14,000 Daltons in soluble EPS was 286.25 μg/g, and as the temperature increased to 60°C, 70°C and 80°C, the concentration if the carbohydrate with MW less than 12,000-14,000 Daltons also increases to 301.56 μg/g, 672.14 μg/g and 948.16 μg/g.
The results of *N. salinarum* diatoms can be seen in Figure 4.4 (c). The concentration of carbohydrate with MW greater than 12,000-14,000 Daltons in soluble EPS also decreases as the temperature increase. At room temperature, the amount of carbohydrate with MW greater than 12,000-14,000 Daltons in soluble EPS was 1253.23 μg/g. When the cells were heated to 60°C, the concentration decreased to 1004.53 μg/g, and it further decreased to 863.51 μg/g and 784.10 μg/g, when heated to 70°C and 80°C. On the other hand, the carbohydrate with MW less than 12,000-14,000 Daltons in soluble EPS increases as the temperature increases. At room temperature, the amount of carbohydrate with MW less than 12,000-14,000 Daltons in soluble EPS were 1575.05 μg/g, and as the temperature increased to 60°C, 70°C and 80°C, the concentration if the carbohydrate with MW less than 12,000-14,000 Daltons also increases to 1960.14 μg/g, 2333.77 μg/g and 2675.93 μg/g.

Experiment have been carried out for cultures of *A. coffeaeformis, C. closterium* and *N. salinarum* diatoms under conditions of saturation with nutrients to determine the monosaccharide compositions in the EPS of the diatoms. The results showed that their cells produce a complex of EPS containing ramnose, fucose, xylose, mannose, galactose, glucose, and uronic acids (Underwood *et al.*, 2004). The low MW components produced by the diatoms at normal conditions in this study most likely consists of these monosaccharides.

Generally, the MW of EPS components are widely distributed in the range of 0.3–300 kDa, while for IOM components was dominated by molecules with MW greater than 30 kDa (Li *et al.*, 2014, Li *et al.*, 2012). Therefore, in the later growth phases of the cells, the EPS consists of higher MW products as more IOM is released due to cell autolysis (Pivokonsky *et al.*, 2014, Huang *et al.*, 2009). However, for this case, as the temperature exposed to the cells increase, the higher MW products in the soluble EPS decreases, while
the lower MW products in the soluble EPS increases. There are polysaccharides present in the EPS and IOM of the diatoms. Algal species also consists of polysaccharide fraction that is present in the form of structural cell walls and cellulose. When these cells were exposed to high temperature, the polysaccharides may have depolymerize to produce monomers, such as ramnose, fucose, xylose, mannose, galactose, glucose, and uronic acids (Toor et al., 2013). Therefore, as the temperature is increased, more polysaccharides might depolymerize to form monomers having lower MW, which increases the lower MW of carbohydrate.

In MD process, the low MW carbohydrate could cause immediate and irreversible plugging and narrowing of membrane. Meanwhile, the biopolymers can be retained on the membrane surface by size exclusion and gradually form a cake layer with time (Zhang et al., 2011, Villacorte et al., 2015b). Thus, the cake formation on the membrane would be less as at high temperature, the MW of the carbohydrates decreases.

4.2.2 Bounded extracellular polymeric substance (EPS)

Figure 4.5 (a), (b) and (c) shows the concentration of carbohydrate with MW greater and less than 12,000-14,000 Daltons in the bounded EPS that is released by the three different algal diatoms. At normal conditions, it was seen that *C. closterium* diatoms released higher concentration of carbohydrate with MW greater than 12,000-14,000 Daltons in the bounded EPS and lesser amount of carbohydrate with MW less than 12,000-14,000 Daltons in the bounded EPS. On the other hand, *A. coffeaeformis* and *N. salinarum* diatoms released lesser amount of carbohydrate with MW greater than 12,000-14,000 Daltons in the bounded EPS and higher amount of carbohydrate with MW less than 12,000-14,000 Daltons in the bounded EPS.
Figure 4.5: MW distribution of carbohydrate in bounded EPS at different temperatures for
(a) A. coffeaeformis, (b) C. closterium and (c) N. salinarum species
Based on Figure 4.5 (a), when *A. coffeeaeformis* diatoms were heated to higher temperature, it was seen that the carbohydrate with MW greater than 12,000- 14,000 Daltons in bounded EPS decreases. At room temperature, the amount of carbohydrate with MW greater than 12,000- 14,000 Daltons in bounded EPS was 602.39 μg/g, and as the temperature increased to 60°C, 70°C and 80°C, the concentration of the carbohydrate with MW greater than 12,000- 14,000 Daltons decreases to 296.61 μg/g, 164.11 μg/g and 92.08 μg/g. On the other hand, the carbohydrate with MW less than 12,000- 14,000 Daltons in bounded EPS increases as the temperature increases. At room temperature, the amount of carbohydrate with MW less than 12,000- 14,000 Daltons in bounded EPS was 972.63 μg/g, and as the temperature increased to 60°C, 70°C and 80°C, the concentration if the carbohydrate with MW less than 12,000- 14,000 Daltons also increases to 1496.21 μg/g, 1744.71 μg/g and 2104.70 μg/g.
For *C. closterium* diatoms, similar trend was observed, as seen in Figure 4.5 (b), where the concentration of carbohydrate with MW greater than 12,000-14,000 Daltons in bounded EPS decreases as the temperature increase. At room temperature, the concentration of carbohydrate with MW greater than 12,000-14,000 Daltons in bounded EPS was 219.26 μg/g, and as the temperature increased to 60°C, 70°C and 80°C, the concentration if the carbohydrate with MW greater than 12,000-14,000 Daltons also decreases to 76.63 μg/g, 60.96 μg/g and 53.20 μg/g. On the other hand, the carbohydrate with MW less than 12,000-14,000 Daltons in bounded EPS increases as the temperature increases. At room temperature, the amount of carbohydrate with MW less than 12,000-14,000 Daltons in bounded EPS was 165.34 μg/g, and as the temperature increased to 60°C, 70°C and 80°C, the concentration if the carbohydrate with MW less than 12,000-14,000 Daltons also increases to 341.20 μg/g, 391.84 μg/g and 496.19 μg/g.

For *N. salinarum* diatoms, the concentration of carbohydrate with MW greater than 12,000-14,000 Daltons in bounded EPS decreases as the temperature increase, as seen in Figure 4.5 (c). At room temperature, the concentration of carbohydrate with MW greater than 12,000-14,000 Daltons in bounded EPS was 384.32 μg/g. When the cells were heated to 60°C, the concentration decreased to 372.46 μg/g, and it further decreased to 297.48 μg/g and 190.37 μg/g, when heated to 70°C and 80°C. On the other hand, the carbohydrate with MW less than 12,000-14,000 Daltons in bounded EPS increases as the temperature increases. At room temperature, the amount of carbohydrate with MW less than 12,000-14,000 Daltons in bounded EPS was 420.204 μg/g, and as the temperature increased to 60°C, 70°C and 80°C, the concentration of the carbohydrate with MW less than 12,000-14,000 Daltons also increases to 657.60 μg/g, 830.30 μg/g and 1143.71 μg/g.

It was seen that as the temperature exposed to the algal cells increase, the higher MW products in the bounded EPS decreases, while the lower MW products in the
bounded EPS increases. At high temperature, the cells went through cell lysis and the higher MW substance from the IOM expelled out of the cell, and some of the components would have bounded on the cell surface. However, the polysaccharides which are present in the EPS and IOM of the diatoms and also the polysaccharide fraction that is present in the form of structural cell walls and cellulose would have depolymerize to produce monomers of carbohydrate, when they were exposed to high temperature. Hence, it can be seen that as the temperature is increased, more polysaccharides depolymerize to form monomers having lower MW, which increases the lower MW of carbohydrate that is contained in the EPS, as has been previously discussed in Section 4.2.1.

However, only loosely bounded EPS had a more significant impact on membrane fouling compared to tightly bounded EPS, as discussed earlier in section 4.1.2. Hence, it is more likely when more IOM is released at higher temperature, more loosely bounded EPS will be formed, which will lead to the cause of membrane fouling. However, at higher temperature, most of the fouling would be caused by monosaccharides. Hence, most of the fouling would be caused by immediate and irreversible plugging and narrowing of membrane of the low MW components, as discussed earlier in Section 4.2.1.

4.2.3 Internal organic matter (IOM)

Figure 4.6 (a), (b) and (c) shows the concentration of carbohydrate with MW greater and less than 12,000- 14,000 Daltons in the IOM that is produced by the three different algal diatoms. At normal conditions, it was seen that C. closterium and N. salinarum diatoms produced higher amount of carbohydrate with MW greater than 12,000- 14,000 Daltons in the IOM and lesser amount of carbohydrate with MW less than 12,000- 14,000 Daltons in the IOM. On the other hand, A. coffeaeformis diatoms produced lesser amount of carbohydrate with MW greater than 12,000- 14,000 Daltons in the IOM and higher amount of carbohydrate with MW less than 12,000- 14,000 Daltons
in the IOM. However, different species releases different amounts of organic matter depending on the type of species, cell wall structure, age of culture and more. Therefore, the results obtained from one species cannot be generalized for all the species.

Figure 4.6: MW distribution of carbohydrate in IOM at different temperatures for (a) *A. coffeaeformis*, (b) *C. closterium* and (c) *N. salinarum* species
Based on Figure 4.6 (a), *A. coffeaeformis* diatoms shows that the carbohydrate with MW greater than 12,000-14,000 Daltons and less than 12,000-14,000 Daltons in IOM decreases when they were heated to higher temperature, as shown in figure 28. For the concentration of carbohydrate with MW greater than 12,000-14,000 Daltons, the concentration at room temperature was 241.76 μg/g, and as the temperature increased to 60°C, 70°C and 80°C, the concentration decreased to 130.79 μg/g, 107.28 μg/g and 82.66 μg/g. For the amount of carbohydrate with MW less than 12,000-14,000 Daltons, the concentration at room temperature was 300.66 μg/g, and as the temperature increased to 60°C, 70°C and 80°C, the concentration decreased to 138.77 μg/g, 124.88 μg/g and 15.77 μg/g.

Similar trend was shown for *C. closterium* diatoms, as shown in Figure 4.6 (b). For the concentration of carbohydrate with MW greater than 12,000-14,000 Daltons, the
concentration at room temperature was 223.80 μg/g, and as the temperature increased to 60°C, 70°C and 80°C, the concentration decreased to 111.15 μg/g, 70.27 μg/g and 37.29 μg/g. For the amount of carbohydrate with MW less than 12,000-14,000 Daltons, the concentration at room temperature was 195.58 μg/g, and as the temperature increased to 60°C, 70°C and 80°C, the concentration decreased to 176.34 μg/g, 75.12 μg/g and 59.47 μg/g.

Based on Figure 4.6 (c), it can be seen that N. salinarum diatoms also showed similar trend as the previous diatoms. For the amount of carbohydrate with MW greater than 12,000-14,000 Daltons, the concentration at room temperature was 321.03 μg/g, and as the temperature increased to 60°C, 70°C and 80°C, the concentration decreased to 208.31 μg/g, 79.48 μg/g and 35.39 μg/g. For the amount of carbohydrate with MW less than 12,000-14,000 Daltons, the concentration at room temperature was 120.17 μg/g, and as the temperature increased to 60°C, 70°C and 80°C, the concentration decreased to 90.29 μg/g, 67.56 μg/g and 53.12 μg/g.

When the temperature exposed to the algal cells increase, the higher MW products and the lower MW products in the IOM decreases. This is caused by cell lysis due to thermal pressure on the cell that caused both the higher MW and lower MW IOM in the cells to be released to the surrounding. When the temperature increases, higher number of cells undergoes cell lysis, which causes higher amount of the IOM to be released. However, as the temperature was further increased, some of the polysaccharides which was still remained in the cells may have depolymerize to form monomers having lower MW carbohydrate.

It was found in some studies that the average polysaccharides in IOM consists of higher portion of MW which are greater than 100 kDa than in EPS for most species tested, such as M. aeruginosa (Pivokonsky et al., 2006, Pivokonsky et al., 2014, Fang et al.,
A study found that the average MW of *M. aeruginosa*’s IOM was greater than that of EPS, which makes the results consistent with the results obtained for *C. closterium* and *N. salinarum* diatoms obtained in this study (Li et al., 2014, Li et al., 2012). However, in this study, most of the larger MW components in the IOM of the three diatoms studied would have depolymerized to form monosaccharides, as discussed in the previous sections.

Thus, in MD process, monosaccharides and low MW components that is contained in the IOM would cause immediate and irreversible plugging and narrowing of membrane. At the same time, the larger polysaccharides can be retained on the membrane surface by size exclusion and gradually form a cake layer at a slower rate, as less higher MW components are present at high temperature. (Zhang et al., 2011, Villacorte et al., 2015b)

### 4.4 Protein Content

This experiment was carried out to determine the amount of protein that is present in the soluble EPS, bounded EPS and IOM produced by *A. coffeaeformis, C. closterium* and *N. salinarum* at different temperatures. BCA protein assay was used for quantitation of total protein in the samples. The method causes proteins to reduce Cu²⁺ to Cu⁺ in an alkaline solution (the biuret reaction) and result in a purple color formation by bicinchoninic acid.

#### 4.4.1 Soluble extracellular polymeric substance (EPS)

Based on the Figure 4.7, it was seen that *N. salinarum* diatoms released the highest amount of protein in the soluble EPS when they were harvested, with concentration of 410.71 µg/g. Then, it was followed by *A. coffeaeformis* diatoms that released 223.21 µg/g of protein in the soluble EPS. The least amount of protein released
in the soluble EPS among these three species was \textit{C. closterium} diatoms that released 215.66 μg/g of protein in the soluble EPS.

![Graph showing the concentration of protein in the soluble EPS at different temperatures for A. coffeaeformis, C. closterium, and N. salinarum](image)

**Figure 4.7:** The concentration of protein in the soluble EPS of \textit{A. coffeaeformis}, \textit{C. closterium} and \textit{N. salinarum} species at different temperatures

For \textit{A. coffeaeformis} diatoms, it was seen in Figure 4.7 that when the amount of protein in the soluble EPS was measured at room temperature, the amount of protein present in the cells were 223.21 μg/g. When the cells were heated to 60°C, the concentration of protein present in the soluble EPS increased to 470.05 μg/g. As the cells were further heated to 70°C, the concentration of protein present in the soluble EPS increased to 608.97 μg/g. At 80°C, the concentration of protein increased to 719.11 μg/g. When the cells were immersed at a higher temperature for 1 hour, it was observed that the concentration of protein present in the soluble EPS increases.

Similar trend was observed for \textit{C. closterium} diatoms in Figure 4.7, where when the amount of protein in the soluble EPS was measured at room temperature, the amount
of protein present in the cells were 215.66 μg/g. When the cells were heated to 60°C, the concentration of protein present in the soluble EPS increased to 418.86 μg/g. As the cells were further heated to 70°C, the concentration of protein present in the soluble EPS further increased to 474.79 μg/g. At 80°C, the concentration of protein increased to 638.10 μg/g. When the cells were immersed at a higher temperature for 1 hour, it was observed that the concentration of protein present in the soluble EPS increases.

Based on Figure 4.7, *N. salinarum* diatoms also shows a similar trend of the amount of protein present in the soluble EPS as the other diatoms mentioned earlier. When the amount of protein in the soluble EPS was measured at room temperature, the amount of protein present in the cells were 410.71 μg/g. When the cells were heated to 60°C, the concentration of protein present in the soluble EPS increased to 700.46 μg/g. As the cells were further heated to 70°C, the concentration of protein present in the soluble EPS further increased to 1004.93 μg/g. When the cells were heated to 80°C, the concentration of protein increased to 1248.68 μg/g.

The increase in the amount of protein in the soluble EPS as the temperature increase is due to the release of the IOM present in the algal cells is due to the environmental stress which causes disruption of algal cells. When the algal cells were immersed in the hot water bath, it has caused cell disruption by thermally induced pressure, leading to cells rupturing as they fail to contain their elevated internal pressure. The majority of disrupted cells led to the production of large debris from cracked and split cells, and also caused the release of IOM to the surrounding. When the temperature increases, more algal cells undergoes cell lysis, and which causes the release of the IOM to the surroundings to increase. This increases the protein concentration in the soluble EPS. Furthermore, the cell wall and cell debris of algal that consist of membrane bound protein, which consists of peptide bond linking amino acids. At high temperatures, these
bonds might undergo hydrolysis to produce amino acids (Peterson et al., 2008). As the temperature increases, the hydrolysis of protein to amino acids also increase. This can also be a reason for the increase of protein content in soluble EPS as the temperature increase.

Some studies showed that proteins contain in EPS were identified as more hydrophobic materials with a higher ratio of large MW fraction compared to polysaccharides (Qu et al., 2012a, Chu et al., 2015). In membrane processes, the hydrophobic fraction with high protein content in the soluble EPS is capable of causing an irreversible fouling due to its strong hydrophobic interaction with the hydrophobic membranes (Qu et al., 2012b). On the other hand, the high ratio of MW can cause the formation of cake on the membrane. This is most likely the case for membrane distillation since hydrophobic membrane is used. (Zhang et al., 2013c).

Some studies have shown that EPS was more hydrophilic than IOM because they classified proteins as stronger hydrophobic compounds than polysaccharides (Fang et al., 2010, Qu et al., 2012a). Therefore, in MD process, when as the temperature used increases, the irreversible fouling of hydrophobic membrane increases, as the protein content in the soluble EPS increases. Comparing the three algal species in this study, N. salinarum diatoms is most likely to cause the highest hydrophobic fouling since it consists of the highest amount of protein in the soluble EPS.

### 4.4.2 Bounded extracellular polymeric substance (EPS)

Based on the Figure 4.8, it was seen that N. salinarum diatoms released the highest amount of protein in the bounded EPS when they were harvested, with concentration of 363.72 μg/g. Then, it was followed by A. coffeaeformis diatoms that released 188.20 μg/g of protein in the bounded EPS. The least amount of protein released in the bounded EPS
among these three species was *C. closterium* diatoms that released 104.11 μg/g of protein in the bounded EPS.

Based on Figure 4.8, the concentration of protein in the bounded EPS increases when *A. coffeaeformis* diatoms were heated to 60°C, 70°C and 80°C from room temperature. At 25°C, the concentration of protein in bounded EPS was 188.20 μg/g. When the cells were heated to 60°C, the concentration of protein in bounded EPS increased to 277.23 μg/g. At 70°C and 80°C, the concentration of protein in bounded EPS increased to 316.66 μg/g and 390.93 μg/g.

For *C. closterium* diatoms, the concentration of protein in the bounded EPS also increases when the cells were heated to higher temperature, as can be seen in Figure 4.8. At room temperature, the concentration of protein in the bounded EPS was 104.11 μg/g. At 60°C, the concentration of the protein in the bounded EPS increased to 217.46 μg/g.
When the cells heated to 70°C and 80°C, the concentration of protein in bounded EPS increased to 263.38 μg/g and 307.52 μg/g.

Based on Figure 4.8, *N. salinarum* diatoms also shows an increase in the amount of protein present in the bounded EPS as the temperature increase. When the amount of protein in the bounded EPS was measured at room temperature, the amount of protein present were 363.72 μg/g. When the cells were heated to 60°C, the concentration of protein present in the bounded EPS increased to 460.59 μg/g. When the cells were heated to 70°C, the concentration of protein present in the bounded EPS further increased to 728.03 μg/g. At 80°C, the concentration of protein increased to 864.66 μg/g.

When a higher temperature was exposed to the algal cells, the concentration of protein in the bounded EPS increases. This is due to cell lysis caused by the thermally induced pressure on the cell, causing the IOM in the cells to be released to the surrounding, as explained in Section 4.4.1 earlier. When the temperature increases, the cell lysis also increases, causing the release of more AOM. The hydrolysis of peptide bonds in the in the cell wall and cell debris depolymerize to form amino acids might also lead to the increase in protein in the bounded EPS. These protein released from the cells then sticks on the cell surface, due to the stickiness caused by EPS. Therefore, the protein concentration in the bounded EPS increases when the temperature the algal cells exposed to increases.

Figure 4.9 (a), (b) and (c) shows the protein/carbohydrate ratio in the soluble EPS and bounded EPS for *A. coffeaeformis, C. closterium, N. salinarum* diatoms. For *A. coffeaeformis* diatoms, it was seen that the protein/carbohydrate ratio is similar in the bounded EPS and the soluble EPS at room temperature. Also, it was seen that when the cells exposed to high temperature, the protein/carbohydrate ratio in the soluble and bounded EPS also increase, which means that the amount of protein in the soluble and
bounded EPS increases with temperature. For *C. closterium* diatoms, a higher ratio of protein/ carbohydrate in the bounded EPS was seen compared to soluble EPS when the cells were extracted at room temperature. When the temperature exposed to the cells increase, it was seen that the ratio increased for soluble EPS but there was only mild increase for bounded EPS. This means the amount of protein in the soluble EPS increased when the cells were heated. This may be because when the cell ruptures, most of the IOM might be released to the soluble EPS compared to the bounded EPS. For *N. salinarum*, it was seen that at 25°C, the ratio of protein/ carbohydrate in bounded EPS was higher compared to soluble EPS. Also, this ratio increases as the cells were exposed at higher temperature.

Other studies also have shown that compared to bounded EPS, soluble EPS has a lower protein/polysaccharide ratio at normal conditions. (Chu *et al.*, 2015, Qu *et al.*, 2012a). Also, the increase in the ratio of protein/ carbohydrate is caused by cell lysis, which released the protein-rich IOM. Protein is considered as a more hydrophobic materials with a higher ratio of large MW fraction compared to the polysaccharides (Chu *et al.*, 2015, Qu *et al.*, 2012a). Hence, for *N. salinarum* and *C. closterium* diatoms, the bounded EPS can be considered to be more hydrophobic and consist of larger MW components compared to soluble EPS at room temperature (Chu *et al.*, 2015, Qu *et al.*, 2012a). Also, as the temperature exposed to the cells at higher temperature, the soluble EPS and bounded EPS is considered to become more hydrophobic and consist of larger MW components as the protein content increases, due to the release of IOM for the three species.

For membrane distillation process, at high operating temperature, the soluble and bounded EPS has a higher hydrophobicity and consists of higher MW components due to the increase in protein, it is most likely to cause an increased rate of irreversible fouling
of hydrophobic membrane and reversible fouling in hydrophilic membranes, as discussed previously in Section 4.4.1.

(a)

(b)
Figure 4.9: Protein/Carbohydrate fraction in soluble EPS and bounded EPS of (a) *A. coffeaeformis* (b) *C. closterium* (c) *N. salinarum* species

### 4.3.3 Internal organic matter (IOM)

Figure 4.10 shows the concentration of protein in the IOM that is present in the three different algal diatoms. In *N. salinarum* diatoms, the amount of protein in the IOM was 1172.62 μg/g, which was the highest. Then, it was followed by *A. coffeaeformis* diatoms that released 597.74 μg/g of protein in the IOM. The least amount of protein released in the IOM among these three species was *C. closterium* diatoms that released 302.97 μg/g of protein in the IOM.
The trend of IOM of all three diatoms shows a decrease in the concentration of the protein in the IOM. Based on Figure 4.10, the concentration of protein in the IOM decreases when *A. coffeaeformis* diatoms were heated to 60°C, 70°C and 80°C from room temperature. At 25°C, the concentration of protein in IOM was 597.74 μg/g. When the cells were heated to 60°C, the concentration of protein in the IOM decreased to 343.76 μg/g. At 70°C and 80°C, the concentration of protein in IOM decreased to 278.90 μg/g and 195.42 μg/g.

As seen in Figure 4.10, when *C. closterium* diatoms were heated to high temperature, the concentration of the protein in IOM decreases. At room temperature, the concentration of protein in the IOM was 302.97 μg/g. When the cells were heated to 60°C, the concentration of protein in the IOM decreased to 95.97 μg/g. At 70°C and 80°C, the concentration of protein in the IOM further decreased to 53.66 μg/g and 27.40 μg/g.
N. salinarum diatoms also shows a decrease in the amount of protein present in the IOM as the temperature increase in Figure 4.10. When the amount of protein in the IOM was measured at room temperature, the amount of protein present were 1172.62 μg/g. When the cells were heated to 60°C, the concentration of protein present in the IOM decreased to 803.95 μg/g. When the cells were heated to 70°C, the concentration of protein present in the IOM further decreased to 501.25 μg/g. At 80°C, the concentration of protein decreased to 315.98 μg/g.

When the temperature to the algal cells were increased, the concentration of protein in the IOM decreases rapidly for the three diatoms. This is caused by cell lysis due to thermal pressure on the cell that caused the IOM in the cell to be released. When the temperature increases, the number of cells that undergoes cell lysis due to the higher thermal pressure increases, which causes higher amount of the IOM to be released to the surrounding, as discussed in previous sections. Hence, the amount of IOM remaining in the cells decreases, which causes the protein content in the cell to decrease as well.

Figure 4.11 shows the percentage of protein content in the IOM at normal conditions based on this study. The portions of proteins in IOM were 51.77%, 40.97% and 72.25% for A. coffeaeformis, C. closterium and N. salinarum diatoms. Other studies have shown that for M. aeruginosa and A. flosaquae, the portions of proteins in IOM can be up to 66.4 and 50.7%, respectively. For EPS, the main components are polysaccharides, while the protein content increased with cell age (Pivokonsky et al., 2006). IOM are much richer in proteins compared to EPS. This results have been proved by other authors that the protein content in IOM for M. aeruginosa and F. crotonensis is higher compared to polysaccharides. Studies also found that proteins IOM exhibited a higher portion of MW greater than 100 kDa compared to the MW contained in the EPS.
for all the species tested, such as *M. aeruginosa* (Pivokonsky *et al.*, 2006, Pivokonsky *et al.*, 2014, Fang *et al.*, 2010, Li *et al.*, 2012).

During MD process, the high temperature causes unfavorable conditions for algal cells, which cause cell lysis, which releases large MW protein component. However, at higher temperature, the large MW components might depolymerize to form smaller components. Studies have shown that IOM can lead to greater total and irreversible fouling than EPS, due to the IOM-membrane adhesion and IOM-IOM cohesion energy were much higher than those between EPS and the membrane surface or EPS-foulants themselves (Li *et al.*, 2014, Zhang *et al.*, 2013a). Also, cake formation was the main mechanism of IOM due to the large MW protein components present (Li *et al.*, 2014). Comparing the three algal species in this study, *N. salinarum* diatoms is most likely to cause the highest membrane fouling since it consists of the highest amount of protein in the IOM and releases them at high temperature.

![Figure 4.11: Protein Percentage in IOM produced by A. coffeaeformis, C. closterium and N. salinarum species at room temperature](image)

Figure 4.11: Protein Percentage in IOM produced by *A. coffeaeformis, C. closterium* and *N. salinarum* species at room temperature
CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Based on this study, we can see that the soluble EPS and bounded EPS extracted from algal cultures contains a higher fraction of carbohydrate compared to IOM for all three species. For IOM, the fraction of protein was higher than polysaccharides for \( A. \) \textit{coffeaeformis} and \( N. \) \textit{salinarum} diatoms. However, \( C. \) \textit{closterium} diatoms showed a lower fraction of protein compared to polysaccharides.

In addition, for \( A. \) \textit{coffeaeformis} and \( C. \) \textit{closterium} diatoms, it was seen that the concentration of carbohydrate with MW smaller than 12,000-14,000 Daltons in the soluble and bounded EPS was higher compared to concentration of carbohydrate with MW greater than 12,000-14,000 Daltons. However, for \( N. \) \textit{salinarum} diatoms, the concentration of carbohydrate with MW smaller than 12,000-14,000 Daltons in the soluble and bounded EPS was less compared to concentration of carbohydrate with MW greater than 12,000-14,000 Daltons. In IOM, \( A. \) \textit{coffeaeformis} diatoms showed higher amount of concentration of carbohydrate with MW less than 12,000-14,000 Daltons, while \( C. \) \textit{closterium} and \( N. \) \textit{salinarum} showed a higher amount of concentration of carbohydrate with MW greater than 12,000-14,000 Daltons. Thus, the results obtained from the three species varies and these results obtained from one species cannot be generalized to all other species.

Other than that, the experimental results shows that when the algal cells were exposed to high temperature, the concentration of carbohydrate in the soluble EPS and bounded EPS increases, while the concentration of carbohydrate in the IOM decreases. Similar trend was observed for the concentration of protein in the soluble EPS, bounded EPS and
IOM. This shows that as the temperature increase, the fouling caused on the membrane is probably higher.

As the temperature increase, the larger MW of carbohydrate decreases, while the smaller MW of carbohydrate increases in the soluble EPS and the bounded EPS. However, both the larger and smaller MW of carbohydrate decreases in the IOM as the temperature increases due to cell lysis, which caused the IOM to be expelled out. Hence, during MD process, most of the fouling would be caused by immediate and irreversible plugging and narrowing of membrane of the low MW components.

5.2 Recommendation

Some recommendations for future study are;

i. The fouling caused by AOM on membranes results from complex set of phenomena which involves reversible and irreversible processes that is strongly dependent on AOM properties such as molecular distribution, hydrophilicity and hydrophobicity, and charge. Therefore, these properties of the AOM should be further studied to understand the cause of fouling of the membrane better.

ii. Even though each carbohydrate and protein type has its own intrinsic properties, such as the structural properties, physico-chemical interactions and the interaction with the membrane surface of the particular organic molecules these properties can vary substantially due to environmental conditions, such as the high temperature used during MD process. Therefore, the characteristics of AOM and also the structural properties of the AOM matrix on the membrane surface should be further studied using scanning electron microscope (SEM).
REFERENCES


and Nannochloropsis salina under subcritical and supercritical water conditions. *Bioresource Technology, 131*, 413-419.


APPENDIX

Directions

For 1 L Total,

1. To approximately 950 mL of non-pasteurized seawater (30-35 ppt), add each of
the components in order specified (except vitamins) while stirring continuously.

2. Bring total volume to 1 L with non-pasteurized seawater.

3. Cover and autoclave medium.

4. When cooled add sterile vitamins.

5. Store at refrigerator temperature.

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
<th>Stock Solution Concentration</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. NaNO3 (Fisher BP360-500)</td>
<td>1 mL</td>
<td>7.5 g/100 mL dH2O</td>
<td>880 μM</td>
</tr>
<tr>
<td>2. NaH2PO4.H2O (MCIB 742)</td>
<td>1 mL</td>
<td>0.5 g/100 mL dH2O</td>
<td>36 μM</td>
</tr>
<tr>
<td>3. Na2SiO3.9H2O (Sigma 307815)</td>
<td>1 mL/L</td>
<td>3 g/100 mL dH2O</td>
<td>106 μM</td>
</tr>
<tr>
<td>4. Trace Metals Solution</td>
<td>1 mL/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Vitamin B12</td>
<td>1 mL/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Biotin Vitamin Solution</td>
<td>1 mL/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Thiamin Vitamin Solution</td>
<td>1 mL/L</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Appendix A: F/2 Medium Recipe
<table>
<thead>
<tr>
<th>Materials/Chemicals</th>
<th>Molecular Formula</th>
<th>Supplier</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>F/2 Medium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Sodium Nitrate</td>
<td>NaNO₃</td>
<td>Fisher BP360-500</td>
<td>To prepare medium for culture</td>
</tr>
<tr>
<td>2. Sodium Dihydrogen Phosphate Monohydrate</td>
<td>NaH₂PO₄.H₂O</td>
<td>MCIB 742</td>
<td></td>
</tr>
<tr>
<td>3. Sodium Metasilicate Nonahydrate</td>
<td>Na₂SiO₃.9H₂O</td>
<td>Sigma 307815</td>
<td></td>
</tr>
<tr>
<td>1.5 M Sodium Chloride</td>
<td>NaCl</td>
<td>EMSURE</td>
<td>To extract bounded EPS from algal cells</td>
</tr>
<tr>
<td>1.5 M Seawater salt water</td>
<td>R &amp; M Chemicals</td>
<td></td>
<td>To rinse the algal cells</td>
</tr>
<tr>
<td>5% Phenol</td>
<td>C₆H₅OH</td>
<td>R &amp; M Chemicals</td>
<td>For carbohydrate characterization</td>
</tr>
<tr>
<td>97% Sulphuric acid</td>
<td>H₂SO₄</td>
<td>QReC</td>
<td>For carbohydrate characterization</td>
</tr>
<tr>
<td>BCA Reagent A</td>
<td></td>
<td>Thermo Scientific</td>
<td>For protein characterization</td>
</tr>
<tr>
<td>containing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. sodium carbonate</td>
<td>Na₂CO₃</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. sodium bicarbonate</td>
<td>NaHCO₃</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. bicinechominic acid</td>
<td>(HO₂CC₉H₅N)₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. sodium tartrate</td>
<td>C₄H₄O₆Na₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. 0.1M sodium hydroxide</td>
<td>NaOH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCA Reagent B</td>
<td></td>
<td>Thermo Scientific</td>
<td>For protein characterization</td>
</tr>
<tr>
<td>containing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. 4% cupric sulfate</td>
<td>CuSO₄</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Appendix B: List of chemical used
<table>
<thead>
<tr>
<th>Equipment/Facility</th>
<th>Brand/Model</th>
<th>Usage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vortex mixer</td>
<td>Fisher Scientific</td>
<td>For mixing F/2 solution and algal suspensions to homogenize the mixture</td>
</tr>
<tr>
<td>Weight balance</td>
<td>SHIMADZU</td>
<td>For measuring the weight of chemicals</td>
</tr>
<tr>
<td>Water bath</td>
<td>Memmert</td>
<td>For heating the algal suspension</td>
</tr>
<tr>
<td>Centrifuge</td>
<td>Thermo Scientific</td>
<td>For separation of supernatant and cells</td>
</tr>
<tr>
<td>Ultrasonic sonicator</td>
<td>Fisherbrand</td>
<td>To break the algal cells and release the IOM</td>
</tr>
<tr>
<td>UV-Vis Spectrophotometer</td>
<td>Agilent Technologies</td>
<td>To determine the unknown concentration of the sample</td>
</tr>
<tr>
<td>Refrigerator</td>
<td>Panasonic</td>
<td>To store the sample at low temperature to prevent contamination</td>
</tr>
<tr>
<td>Laminar Flow cabinet</td>
<td>ESCO</td>
<td>To culture algal cells in a clean environment to prevent contamination</td>
</tr>
<tr>
<td>Fume hood</td>
<td>AAL</td>
<td>To carry out phenol- sulphuric acid method safely</td>
</tr>
<tr>
<td>Autoclave</td>
<td>Tuttnuer</td>
<td>To sterilize the medium and equipments before culturing the algal cells.</td>
</tr>
</tbody>
</table>

Appendix C: List of equipment used
Instrument: Cary 60
Instrument version no.: 2.00
Wavelength (nm): 490.0
Calibration eqn: $\text{Abs} = 1.71482\times\text{Conc}$
Correlation Coefficient: 0.98024

Appendix D: Calibration curve of carbohydrate
Appendix E: Calibration curve of protein

\[ y = 0.0014x + 0.0053 \]

\[ R^2 = 0.9724 \]