# Freely-Suspended *Marine Microalgae Nannochloropsis oculata* and *Isochrysis galbana* for Palm Oil Mill Effluent Remediation

By

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14233

Dissertation report in partial fulfillment of the

requirement for the Bachelor of Engineering (Hons)

(Chemical)

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# CERTIFICATION OF APPROVAL

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Chemical Engineering Programme

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(CHEMICAL)

Approved by,

(Dr. Azizul Bin Buang)

CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my own except as specified in the references and acknowledgement, and that the original work contained herein have not been undertaken or done by unspecified sources or persons.

(DEREK LAI CHAI ZERN)

# ABSTRACT

POME having the characteristics of high chemical oxygen demand (COD) and biochemical oxygen demand (BOD), mineral content such as nitrogen and phosphorus, that can lead to a serious pollution to the environment. COD level determines the amount of organic compounds in water while BOD level is the amount of dissolved oxygen that are present in the water. POME remediation in POME using microalgae is a sustainable and cost effective way. Algae, with their photosynthesis abilities, are able to produce lipids and hydrocarbons. Immobilization is among the techniques that could lead to continued use of algae over prolonged period. A combination of wastewater treatment and renewable bioenergy production will act as a benefit to the palm oil industry and renewable energy sector. In this study, palm oil mill effluent (POME) was used as an alternative medium for algal biomass and lipid filtered and centrifuged POME in seawater (1, 5, 10, 15%) on microalgal cell growth. Both *N. oculata* and *I. galbana* had enhanced cell growth after 16 days of flask cultivation. Algae cultivation with POME media also enhanced the removal of COD (93.6-95%), BOD (96-97%) and TOC (71-75%) from POME.

# CHAPTER 1

# INTRODUCTION

## 1.1 BACKGROUND OF STUDY

Palm oil industry is the main agro-based industry in Malaysia that contributes towards the country's economy. However, the industry generates massive amount of wastes in the form of oil palm trunks (OPT), fronds (OPF), empty fruit brunches (OPEFB), palm pressed fibres (PPF), shells and palm oil mill effluent (POME) (Hassan et al, 2004). POME is effluent water containing soluble materials with high amount of BOD, COD, and other minerals, that are harmful to the environment. These generate $CH_4$, $SO_2$, $NH_3$, halogens or soluble liquids or solids which contain ions of either organic or inorganic origin and with their concentration above the threshold value. Palm oil mill effluent (POME) is considered one of the most polluting agro-industrial effluents due to its high value of COD and BOD concentrations ranging from 50,000 to 90,000 mg $L^{-1}$ (Damayanti et al. 2010).

In general, wastewater contains substantial amounts of beneficial nutrients and toxic heavy metals, which are creating both opportunities and problems for agriculture production (Chen et al., 2005; Singh et al., 2004). Without proper treatment, excess nitrogen and phosphorus in discharged wastewaters can lead to downstream eutrophication and ecosystem damage (Correll et al., 1998). Heavy metals are ubiquitous in the environment, as a result of both natural and anthropogenic activities andhumans are exposed to them through various pathways (Wilson and Pyatt., 2007). Wastewater irrigation, solid waste disposal, sludge applications, vehicular exhaust and industrial activities are the major sources of soil contamination with heavy metals. Excessive accumulation of heavy metals in agricultural soils through wastewater irrigation, may not only result in soil contamination, but also lead to elevated heavy metal uptake by crops and thus affect food quality and safety (Muchuweti et al., 2006; Khan et al., 2007).

The effluent treatment currently used by the Malaysian palm oil industry include anaerobic/facultative ponds, tank digestion and mechanical aeration, tank digestion

and facultative ponds, decanter, physicochemical and biological treatment. Treatment of POME has also been tried using membrane technology, up-flow anaerobic filtration, up-flow anaerobic sludge blanket and up-flow anaerobic sludge fixed film bioreactor. At present, 85% of POME treatment is based on anaerobic and facultative pond system, followed by open tank digester attached with extended aeration in a pond (Vijayaraghavan et al., 2007). Since POME contains high level of organic matters, implementation of anaerobic digestion in the first stage of the treatment process is a necessity to alter the bulk of the wastes to biomethane. The treated effluent is further exposed to aerobic treatment in order to meet the required discharge standards. These treatment steps have been applied either as an open pond or open digesting tank systems in Malaysian palm oil mills [Subramanian et al, 2008]. Microalgae have received great attention because of their capability of using carbon dioxide as carbon source and wastewater components as nutrients while producing biomass. There has also been significant interest on microalgal utilization as an advanced energy feedstock for bioethanol production (Rosenberg et al. 2008). Algae contain 75% complex carbohydrates which could be hydrolyzed into a fermentable hexose monomer with 80% theoretical yield of fermentated ethanol (Huntley and Redalje 2007). Algae adds an advantage to effluent treatment by increasing performance of degradation, improving $CO_2$ balance and lowering energy demand for oxygen supply in aerobic treatment stage. The role of algae is both to assimilate plant nutrients and to support bacteria with oxygen. Bacteria, in turn, are involved in degradation of organic material in wastewater, the same process utilized in activated sludge (Kirkwood et al., 2003).

Microalgae-based treatment disrupts the socio-ecological principles to a lesser degree than the other treatment methods, where the use of microalgae biomass could narrow the cost gap and make the process cost-effective (Kryder et al., 2007).Compared to physical and chemical treatment processes, algae based treatment can achieve nutrient removal in an ecologically safer way with the added benefits of resource recovery and recycling (Graham et al., 2009; Oswald, 2003). Large scale methods of producing and harvesting algae can be used in wastewater treatment for biofuel and other bio-product applications (Hoffman, 1998; Oswald, 2003). Microalgae could reduce the eutrophication potential with a more environmentally sound approach (Orpez et al. 2009)

Sustainable energy management in palm oil mill has entered a new dynamic era with the opportunity of culturing microalgae using POME (Ahmad et al., 2014). Most palm oil millers favor the culture of microalgae as a tertiary treatment before POME is discharged due to practically low cost and high efficiency. Therefore, most of the nutrients such as nitrate and ortho-phosphate that are not removed during anaerobic digestion will be further treated in microalgae pond. The cultured microalgae will then be used as a diet supplement for live feed culture (Li et al, 2008). The U.S Department of Energy has recognized the potential synergy of wastewater treatment and biofuel production from algae, stating that "Inevitably, wastewater treatment and recycling must be incorporated with algae biofuel production (U.S. DOE, 2010).

The aim of this work was to investigate the centrifuged POME at different composition in seawater for culturing the green marine microalgae *N.oculata* and *I.galbana*. The removal of the nutrient, BOD, COD and TOC from POME.

## 1.2 PROBLEM STATEMENT

With million tonnes of crude palm oil production, palm oil industries produce huge amount of wastes in the form of POME with high COD and BOD which can cause severe water pollution to the environment. The conventional treatment system consisting of aerobic and anaerobic pond treatment is not cost effective. The application of immobilized microalgae can solve the problem of POME remediation and heavy metal removal. For continued and prolonged use, immobilization of microalgae must be implemented. An understanding of interaction between factors is important to provide the basis to formulate cultivation strategies and for the prediction of optimum conditions for heavy metals removal, cell growth, lipid and carbohydrates accumulation.

## 1.3 OBJECTIVES AND SCOPE OF STUDY

1. To develop suitable technique to the treatment of POME using Nannochloropsis oculata and Isochrysis galbana.
2. To compare the performance of freely-suspended of Nannochloropsis oculata and Isochrysis galbana cells for POME remediation.

Selections of specific microalgae (*Nannochloropsis oculata* and *Isochrysis galbana*) will be evaluated based on high growth rates. The comparative study of freely suspended for POME remediation will be carried out.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 POME

POME is a viscous brown liquid with fine suspended solids at pH ranging between 4-5 (Poh et al, 2009) composed of high organic content mainly oil and fatty acids, carbohydrates (29.55%), proteins (12.75%), nitrogenous compounds, lipids, and a considerable amount of cellulose and non-toxic minerals which provide a good source for microbial fermentation (Wu et al, 2007). POME contains high chemical oxygen demand (COD) and biological oxygen demand (BOD) and oil and grease, due to the lignocelluloses and hemicelluloses components of the material. This can cause considerable environmental problems if discharged without proper treatment by polluting land, water and eco-system. Ecosystems are destroyed by the rising temperature in water, as coral reefs are affected by the bleaching effect due to warmer temperatures. Metals can bio-accumulate in living organisms and the soil organic layer; contaminate the ground and surface water (Shavandi et al., 2012).

The oil palm industry has always been linked to the environment because it is a land intensive industry. Any unplanned disposal of POME could lead to severe pollutions. POME is generated from various points during processing in an oil palm mill. These include clarification sludge, sterilization condensates, fruit washing water, hydro-cyclone drain-off and various boiler blows down, tank and decanters drain. The compositions of POME are mainly water, oil, solids and sand. The compositions of total POME are water(93%-95%), solid (3-4%) and oil (0.5-2%) (Hassan et al,2004). POME also contains different metals at critical levels such as iron (Fe), Zinc (Zn) and Manganese (Mn). These heavy metals will contaminate the water and eventually enters into the human body, causing health effect.

## 2.2 WASTES-WATER TREATMENT METHODS

Various treatments methods have been used to treat POME including: - (a) anaerobic/facultative ponds (Wong et al, 19980, Andreasen et al, 1982); (b) tank digestion and mechanical aeration; (c) tank digestion and facultative ponds; (d) decanter and facultative ponds; (e) physico-chemical and biological treatment [28]; (f) evaporation (Ma et al, 1993) and clarification pond coupled with filtration and aeration (UNEP et al, 1994). The conventional treatment is the ponding system and biological treatment. In most mills, the under-sized biological treatment system is unable to cope with increasing POME volume (Cheng et al, 2010, Singh et al, 2011). Cost effective treatment is needed to ensure sustainable economic growth of palm oil industry whilst protecting the environment. Anaerobic treatment of POME is widely used because of its low operational cost. Most palm oil mills and refineries have their own treatment systems for POME.

## 2.3 ALGAL GROWTH

Under favorable conditions, unicellular algae grow continuously by a process known as cell division (Baptist et al., 1993). Cell division happens when each cell enlarges and divides itself into two daughter cells that subsequently grow and divide yielding a culture that increases exponentially. Growth slows as the algal population becomes more crowded. The amount of nutrients will decrease, metabolites build, and light penetration decreasing because of self-shading. Thus, the cultures have reached their stationary phase at the cultivation conditions and will not increase in density and dry weight. The amount of time for algae to divide and the maximum density attained depend upon factors such as species, temperature, salinity, light intensity, nutrients, $CO_2$ aeration, vessel size and any presence of microscopic, predaceous contaminants (Shah et al., 2014)

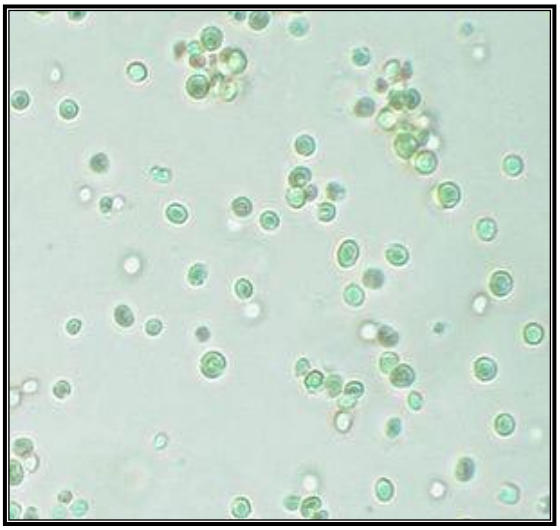

**Figure 1:** Nannochloropsis oculata

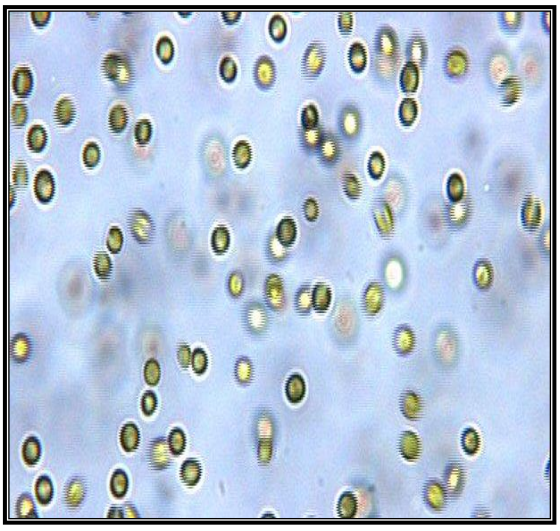

**Figure 2:** Isochrysis galbana

# CHAPTER 3

# METHODOLOGY

## 3.1 MATERIALS AND METHODS

Marine microalgal strains, such as (*N.oculata* and *I. galbana*) will be selected based on high sequester of $CO_2$ and generated high biomass, fastest growing abilities and resistant to extreme temperatures. Chemical Oxygen Demand (COD) measurement will be carried out by using spectrophotometer DR2800 and 5000-Reactor Digested Method.

## 3.2 SAMPLE PREPARATION

### 3.2.1 POME

The fresh POME will be collected from FELCRA Nasaruddin, a palm oil mill in Bota, Perak. The POME sample will be kept into cooled room at $4^oC$ to avoid microbial contamination.

POME will be filtered to remove sand and dust particles and then centrifuged (Avanti J-251 Centrifuge). The supernatant of the effluent which contains nutrient will be taken for algal culture and the pellet will be removed for other uses. The supernatant will be diluted with sea-water to various range of POME and ground water, and autoclaved at $121^oC$ for 30 minutes to eliminate bacterial and other contaminations. pH level of POME medium will be adjusted to pH 7-8 and filtered again before use.

### 3.2.2 Culturing of Microalgae

Conway and POME with different combination of sea water will be tested for microalgae culturing and heavy metals removal. Two species of marine microalgae (*N.oculata* and *I.galbana*) used in the present study, were collected from Fisheries Research Institute (FRI), Pulau Sayak, Sungai Petani, Kedah, Malaysia. All chemicals and solvents were obtained from Merck (Darmstadt, Germany).

### 3.2.3 Investigation of POME and sea water composition

POME will be filtered to remove sand and dust particles and then centrifuged (Avanti J-251 Centrifuge). The supernatant of the effluent which contains nutrient will be taken for algal culture and the pellet will be removed for other uses. The supernatant will be diluted with sea-water to various range of POME and sea-water ratio, and autoclaved at 121$^{o}$C for 30 minutes to eliminate bacterial and other contaminations. pH level of POME medium will be adjusted to pH 7–8 and filtered again before use.

### 3.2.4 Chemical Oxygen Demand (COD)

COD measurement will be carried out by using spectrophotometer DR5000 and 8000-Reactor Digested Method according to the standard method provided by HACH(HACH, 2008). The DRB200-Reactor will be preheated to 150$^{o}$C. One ml of sample will be diluted at 1:100 of POME to distilled water ratio. Two mL of each diluted POME will be added to the corresponding high range COD Digestion Reagent vials. In the case of the "blank", 2 ml of distilled water will be added. Each vial will be mixed well and placed into the reactor block for two hours. After two hours, the vials will be removed to a cooling rack for 20 minutes before reading. The stored HACH program 435 COD HR was recalled for COD test. The blank vial will be placed in the cell holder with the light shield closed and set to zero. Then, the sample vial will be placed in the cell holder for the test with the reading of COD in mg/L will be displayed on the screen.

### 3.2.5 Biological Oxygen Demand (BOD)

Measurement of BOD with BOD track was carried out according to Standard Method provided by HACH(HACH, 2008). One ml of sample will be diluted at ratio 1:100 of POME to distilled water ratio. The sample (95ml) will be poured into the specialized 300 ml BOD track designed to allow full filling with no air space, and sample bottle will be sealed. Four samples will be prepared and 3.8 cm (1.5 in) magnetic stir bar will be placed in each sample bottle. BOD Nutrient Buffer Pillow 0.5 mL will be added to each sample and Lithium hydroxide Powder will be added to the seal cup of each sample bottle. The instrument will be placed in the incubator at

temperature of 20$^{o}$C. The stored Hach program for 5.25 days and 0-700mg/L will be selected for the BOD test. Then, the reading will be taken after 5 days with the reading BOD in mg/L displayed on the screen for each sample bottle.

### 3.2.6 Total Organic Carbon, Total Nitrogen (TOC & TN) and Oil and Grease

Measurement of TOC and TN will be carried out by using TOC Analyzer (TOC-VCSH SHIMADZU) and Oil and grease according to the APHA Standard Method (APHA, 2005).

Removal efficiencies of BOD, COD, TOC, TN and oil and grease were calculated using the following equation:

$$Removal\ efficiency\ (\%) = \frac{Ai - Af}{Ai} \times 100$$

where $A_i$ and $A_f$ are the initial and final parameter concentrations, respectively.

## 3.3 DETERMINATION OF CELL DENSITY AND DRY WEIGHT

### 3.3.1 Cell Density

The growth of microalgae will be measured through counting the number of cells by haemocytometer. On fixed days of alga growth, approximately 10µL sample will be removed by using capillary dropper. Sample will be then transferred to the filling slide chamber and examined under high power microscope (10 x 40 MAG).

### 3.3.2 Cell Dry Weight (DW)

One hundred ml of algal suspension will be filtered through a pre-dried and pre-weighed glass micro fibre filter (Whatman GF/C 0.47µ). The biomass will be washed with de-mineralized water and dried at 105$^{o}$C in oven overnight, cooled in a desiccator and dry weight measured. The formulations are as follows:

$$\text{Dry weight} = \frac{DW_A - DW_C}{V}$$

where $DW_A$ is the average dry weight retained on algal filter, $DW_C$ is average dry weight retained on control filter and V is volume used.

### 3.4 GANTT CHART OF ACTIVITIES

| Activities /Year /Month | 2014 | | | | |
|---|---|---|---|---|---|
| | J | J | A | S | O |
| Algal culture | | | | | |
| Wastewater analysis | | | | | |
| Development of immobilization techniques based on calcium alginate and polyvinyl alcohol foam | | | | | |
| Analysis of microalgae sp. for removal of pollutants from wastewater | | | | | |
| Analyses of cell growth | | | | | |

### 3.5 MILESTONE

| **Activities /Year /Month** | **2014** | | | | |
|---|---|---|---|---|---|
| | **J** | **J** | **A** | **S** | **O** |
| Completion of Algal culture and POME characterization | | | | | |
| Completion of freely-suspended microalgae | | | | | |
| Completion of wastewater analysis treatment. | | | | | |
| Completion of dissertation writing | | | | | |

# CHAPTER 4

# RESULT AND DISCUSSION

## 4.1 POME CHARACTERISTICS

Raw POME was considered as the mixtures of the effluents from sterilizer condensate, clarification sludge and hydrocyclone discharge. The determined parameters included pH, BOD, COD, TOC, TN, oil and grease, total solids and total suspended solids. The analyzed results are shown in Table 1.

**Table 1:** POME characterization.

| Parameters | Literature (mg/L) [173, 174] | This study (mg/L) |
|---|---|---|
| pH | 3.8 | 3-3.5±0.4 |
| Temperature ºC | 80-90 | 80$^{o}$C |
| Chemical Oxygen Demand (COD) | 69500 | 65272±105 |
| Biological Oxygen Demand (BOD) | 25000 | 24117±77 |
| Total Organic Carbon (TOC) | --- | 4671±91 |
| Total Nitrogen (TN) | 650 | 385±13 |
| Total Suspended Solid (TSS) | 28900 | 68367±278 |
| Oil and Grease (O&G) | 10540 | 3546±53 |
| Total solids (TS) | 55000 | 39600±153 |
| Total volatile solids (TVS) | 24000 | 32743±111 |

The characteristics of raw POME show that the pH was 3.5-5 with COD of 65772 mg/L, BOD of 24117 mg/L, TOC of 4746 mg/L, TN of 385 mg/L, TSS of 68367 mg/L and Oil and grease of 3546 mg/L, indicating high amount of organic matter. These are comparable to previously reported values (Subhash et al, 2007; Hee-Jeong et al, 2012).

## 4.2 ALGAL CELLS GROWTH AND LIPID CONTENT

Figure 1 and 2 shows the cell density and dry weight of *Nannochloropsis oculata* and *Isochrysis galbana*. The highest cell density and dry weight were achieved with *N.oculata* at 62.2 × $10^6$ cell $mL^{-1}$ and 0.65 g $L^{-1}$, while brown microalgae *Isochrysis galbana* 10.12 × $10^6$ cell $mL^{-1}$ and 0.69 g $L^{-1}$, respectively. These are comparable to reported maximum cell concentration for *Nannochloropsis* 65 × $10^6$ (Suzana et al, 2012). The microalgal cells from logarithmic, early stationary and stationary phase were extracted for lipid content. The highest lipid contents were recorded 27.5%, while showed slightly lower lipid contents of *Isochrysis galbana* of 22.1%. The reported lipid content of *N. oculata* is 14.92% growing at room temperature and continuous photon flux density of 70.0 $\mu Em^{-2}s^{-1}$ (Attilio et al, 2009).

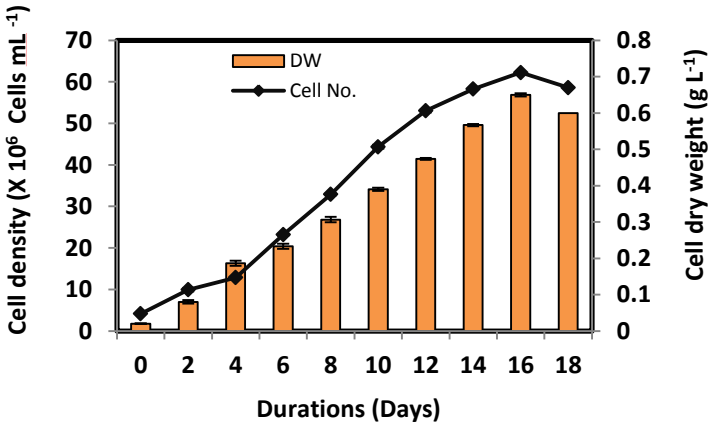


**Figure 3:** Profile of Cell density and Dry weight of Nannochloropsis oculata

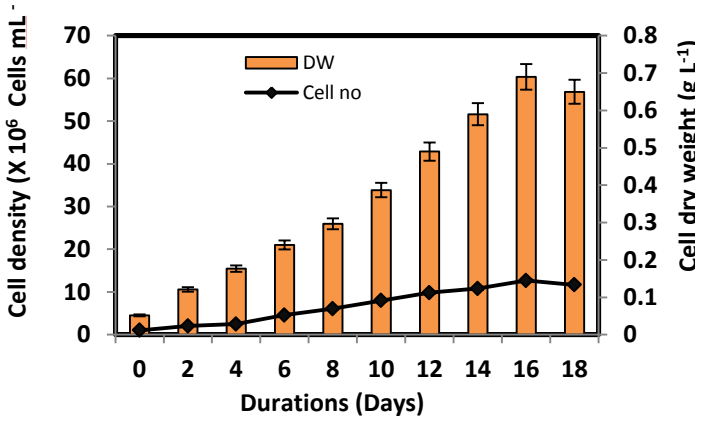


**Figure 4:** Profile of Cell density and Dry weight of Isochrysis galbana

## 4.3 BIOCHEMICAL OXYGEN DEMAND

Biochemical oxygen demand (BOD) is the amount of dissolved oxygen needed by biological organisms in a body of water to break down organic material present in a given water sample at certain temperature over a specific time period. Dilution method will be used to determine the value of BOD of each sample. This analysis is performed using 300 ml incubation bottles in which buffered dilution water is dosed with seed microorganisms and stored for 5 days in the dark room at 20 °C to prevent DO production via photosynthesis Since the American Public Health Association Standard Methods Committee adopted the 5-day biochemical oxygen demand ($BOD_5$) test, this method has been widely used as the standard method for determining the concentration of biodegradable organics in wastewater (In Seop Chang, 2003). There are 4 samples that were prepared. . These results are before and after treatment of microalgae.

| POME Level | Before treatment (mg/L) | After treatment (mg/L) | Removal efficiency |
|---|---|---|---|
| **1%** | 182 | 22 | 88% |
| **5%** | 842 | 32 | 96% |
| **10%** | 1641 | 44 | 97% |
| **15%** | 2448 | 94 | 95% |

**Table 2:** Biochemical Oxygen Demand before & after treatment using N. oculata

| POME Level | Before treatment (mg/L) | After treatment (mg/L) | Removal efficiency |
|---|---|---|---|
| **1%** | 182 | 34 | 81% |
| **5%** | 842 | 47 | 94% |
| **10%** | 1641 | 62 | 96% |
| **15%** | 2448 | 114 | 95% |

**Table 3:** Biochemical Oxygen Demand before & after treatment using Isochrysis galbana

The highest average BOD of 2448 mg $L^{-1}$ and l82 mg $L^{-1}$ was recorded at 15% and 1% POME, respectively, before the treatment of *N. oculata* and *I. galbana* (Table 2 & 3). The BOD removal of 88-97% were achieved for 1-15% POME after the addition of *N.oculata*, while the BOD removal 81-96% were achieved after the addition of *Isochrysis galbana*. The COD and TOC removal efficiencies also varied with different POME levels.

## 4.4 CHEMICAL OXYGEN DEMAND

The chemical oxygen demand (COD) is one of the most widely used analysis method as an indicator to identify the characteristics of wastewater. The dichromate reflux methods using other oxidants because of superior oxidizing ability, applicability of a wide variety of samples and ease of manipulation (Yun Whan Kang, 1998). Chemical Oxygen Demand (COD) measurement will be carried out by using spectrophotometer DR2800 and 5000-Reactor Digested Method. The following table is the result for my final year project. Below are the results that I had obtained through my experiment.

| POME Level | Before treatment (mg/L) | After treatment (mg/L) | Removal efficiency |
|---|---|---|---|
| **1%** | 627 | 63 | 90% |
| **5%** | 2974 | 145 | 95% |
| **10%** | 5839 | 375 | 94% |
| **15%** | 8947 | 558 | 94% |

**Table 4:** Chemical Oxygen Demand before & after treatment using N. oculata

| POME Level | Before treatment (mg/L) | After treatment (mg/L) | Removal efficiency |
|---|---|---|---|
| **1%** | 627 | 84 | 87% |
| **5%** | 2974 | 196 | 93% |
| **10%** | 5839 | 463 | 94% |
| **15%** | 8947 | 1638 | 93% |

**Table 5:** Chemical Oxygen Demand before & after treatment using Isochrysis galbana

Higher removals of COD (90-95%) were achieved for 1-15% POME after the addition of *N. oculata* while lower removals of COD (87-94%) were achieved after addition of *I. galbana*. The COD removal was enhanced when the POME level was increased to 5 % and 10 %. Different algal strain could utilize the different organic compounds as carbon sources at different efficiency depending on the nature or severity of the waste water conditions. The algal strain could utilize the amount of dissolved oxygen to break down organic material. The higher biological load, containing high amounts of organics, could enhance algal growth. However, if the loading rate had gone beyond a threshold level, the nutrient buildup could be lethal to the algae.

## 4.5 TOTAL ORGANIC CARBON

Organic carbon, representing the single largest constituent of organic matter, provides the most direct proxy for productivity (Pedersen and Calvert, 1990, Canfield, 1994, Tyson, 2005 and Zonneveld et al., 2010). Primary producers in the photic zone take up $CO_2$ from the atmosphere to form organic matter via photosynthesis. Below tables are the results that obtained from my experiment.

| POME Level | Before treatment (ppm) | After treatment (ppm) | Removal efficiency |
|---|---|---|---|
| **1%** | 33.4 | 12 | 64% |
| **5%** | 153.1 | 53 | 65% |
| **10%** | 285.5 | 87 | 70% |
| **15%** | 456.8 | 115 | 75% |

**Table 6:** Total Organic Carbon before & after treatment using N. oculata

| POME Level | Before treatment (ppm) | After treatment (ppm) | Removal efficiency |
|---|---|---|---|
| **1%** | 33.4 | 19 | 43% |
| **5%** | 153.1 | 67 | 56% |
| **10%** | 285.5 | 104 | 63% |
| **15%** | 456.8 | 132 | 71% |

**Table 7:** Total Organic Carbon before & after treatment using Isochrysis galbana.

TOC removal efficiencies also varied with different POME levels. High removal of TOC were achieved for 1-15% POME after the addition of *N. oculata* while lower removal of TOC (43-71 %) were achieved after the addition of *I. galbana*.

# CHAPTER 5

# CONCLUSION AND RECOMMENDATION

## 5.1 CONCLUSION

Microalgal cell immobilization techniques can be developed based on calcium alginate and polyvinyl alcohol for POME remediation and heavy metals removal in the future. Comparative studies of freely suspended for POME remediation will be conducted. The major challenges for wastewater treatment systems based on microalgae are the harvesting of the biomass at the end of the treatment process. There will be cost reduction of wastewater treatment with green energies as by-products and environmental protection. Immobilization of cells can be an alternative for cell harvesting as well as providing advantages such as an increase in the cell retention time within bioreactors and higher metabolic activity.

The influences of different composition of POME in sea water on microalgal cell growth were investigated. High removal efficiencies of COD, TOC, and BOD were achieved at different levels of POME. Cultivation of microalgae in POME may be a practical and economical alternative to efficiently enhance the nutrients removal from POME.

## 5.2 RECOMMENDATION

Suitable microalgal strain and biopolymers for microalgal cells immobilization could be developed and further improvement in both performance and costs could be expected in future. Furthermore, others immobilization techniques with different freshwater and marine microalgal strains for POME remediation can be carried out. To attract more usage of immobilization technology, some strategies have to be developed to solve microalgal harvesting problem and to convert harvested biomass into bioenergy generation.

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**APPENDICES**

**Calculation for the removal efficiency for COD, BOD and TOC**

$$Removal\ efficiency\ (\%) = \frac{Ai - Af}{Ai} \times 100$$

Where,

$Ai$ = Initial parameter of concentration

$Af$ = Final parameter of concentration

| **POME Level** | **Before treatment (mg/L)** | **After treatment (mg/L)** | **Removal efficiency** |
|---|---|---|---|
| **1%** | 182 | 22 | 88% |
| **5%** | 842 | 32 | 96% |
| **10%** | 1641 | 44 | 97% |
| **15%** | 2448 | 94 | 95% |

Table of BOD value using *Nannochloropsis oculata.*

$$\frac{182\ mg/L - 22\ mg/L}{182\ mg/L} \times 100 = 88\%$$

| POME Level | Before treatment (mg/L) | After treatment (mg/L) | Removal efficiency |
|---|---|---|---|
| **1%** | 627 | 63 | 90% |
| **5%** | 2974 | 145 | 95% |
| **10%** | 5839 | 375 | 94% |
| **15%** | 8947 | 558 | 94% |

Table of COD value using *Nannochloropsis oculata.*

$$\frac{182\ mg/L - 34\ mg/L}{182\ mg/L} \times 100 = 81\%$$

| POME Level | Before treatment (ppm) | After treatment (ppm) | Removal efficiency |
|---|---|---|---|
| **1%** | 33.4 | 12 | 64% |
| **5%** | 153.1 | 53 | 65% |
| **10%** | 285.5 | 87 | 70% |
| **15%** | 456.8 | 115 | 75% |

Table of TOC value using *Nannochloropsis oculata.*

$$\frac{33.4\ ppm - 12\ ppm}{33.4\ ppm} \times 100 = 64\%$$